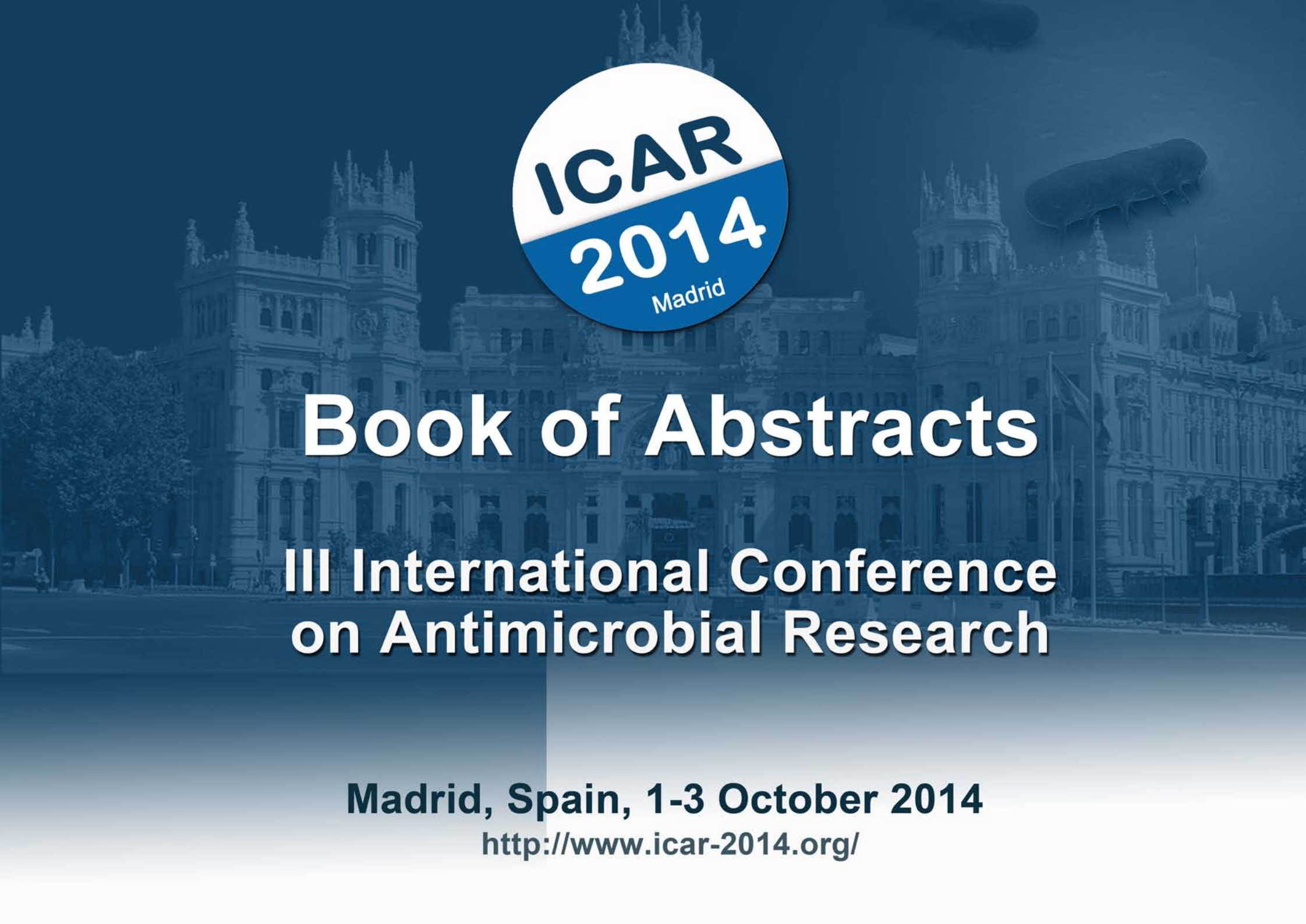

Book of Abstracts

III International Conference on Antimicrobial Research

Madrid, Spain, 1-3 October 2014

http://www.icar-2014.org/

Book of Abstracts

III International Conference on Antimicrobial Research - ICAR2014

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INTRODUCTION

This book contains a selection of the abstracts that were accepted for presentation at the III International Conference on Antimicrobial Research (ICAR2014), which was held at the Complutense University in Madrid, Spain, from October 1st to 3rd, 2014.

The ICAR conference series aims at establishing as a key forum in Europe for the presentation, exchange and dissemination of information and experiences on anti-microbe strategies. "Anti" is here taken in the broadest sense as "against cell cycle, adhesion, or communication", when harmful for the human health, industry or economy (infectious diseases, chemotherapy, food, biomedicine, agriculture, livestock, biotechnology, water systems...). It will also cover topics on antimicrobial resistance, (early) microbial and resistance detection, enhancement of innate defenses against pathogens, as well as methods and techniques.

This third edition of the ICAR conference gathered around 450 participants, coming from more than 60 countries. And more than 440 works were presented at the conference. This was a more than satisfactory level of attendance for this research forum, especially in the context of a global budget constraint.

The organization called for research papers dealing with the following topics (related on either bacteria, fungi, microbial parasites or viruses):

- **Antimicrobial natural products I - Peptides:**
  Antimicrobial peptides. cyclic lipopeptides…
- **Antimicrobial natural products I - Terrestrial and marine organisms:**
  Antimicrobial substances from terrestrial and marine organisms. Essential oils. Bioactive phytochemicals. Plant/Herbal extracts…
- **Biocontrol. Biosynthesis of antimicrobials:**
  Microbial-derived toxins. Bacteriocins (colicins, microcins, lantibiotics…). Archaeocins. Biocontrol approach to microbial invasions (probiotics, lactic acid bacteria…). Biosynthesis of antibiotics. Genetic and metabolic engineering. Gene regulation…
- **Bacteriophages:**
  Phage therapy and biocontrol in humans, animals (agriculture-farm animals, aquaculture), plants, food industry… Materials functionalization with bacteriophages. Using bacteriophages for microbiological detection...
- **Biofilms:**
  Biofilm formation, control and eradication. Microbial adhesion to surfaces. Novel characterization techniques. Biofouling. Biofilms susceptibility to antimicrobials. Antibiotic resistance of microorganisms in biofilms. Genomics and Proteomics...
- **Antimicrobial materials science and surface chemistry. Antimicrobials in consumer products:**
  Antimicrobial, anti-adhesive surfaces & coatings. Microbial adhesion to surfaces. Physical and chemical (inorganic (e.g. silver, copper compounds) and organic) surface modification. Cationic surfaces. Functionalization strategies for polymers, metals, metal oxides, ceramics. Drug-eluting concepts...
  Antimicrobials in consumer products: Textiles (hygienic clothing, active wear, medical textiles…). Paper industry. Active packaging (food industry…). Safety and toxicological aspects…
- **Antimicrobial chemistry:**
  Synthesis and screening of novel chemical compounds for antimicrobial action. Natural, synthetic and semi-synthetic antibiotics. Analogs. Structural determination. *In-silico/ab-initio/de-novo* antimicrobials discovery. New targets for antimicrobials. Rational design of antimicrobials.

Bioinformatics and comparative genomics for the identification of antimicrobial targets…

- **Non-antibiotic biocides:**
  Disinfectants, antiseptics, preservatives… Mechanism of action. Resistance to non-antibiotic biocides. Combination of physical and chemical treatments. Hygiene and Sterilizing. Sanitizers. Regulatory issues. Good practices…
- **Antimicrobial physics:**
  Exploitation of physical properties for killing/inactivating microbes: surface tension (nano-emulsions), radiation, ultrasounds, temperature, specific properties of nano-materials (nano-particles, nano-tubes/wires, nano-crystals, nano-grained materials…). Resistance to physical agents...
- **Clinical and medical microbiology, infectious diseases and antimicrobials. Public health**
- **Strengthening of innate immune system as antimicrobial strategy:**
  Immunotherapy, immunomodulating agents, cytokines (interleukins, colony-stimulating factors, interferons…), hormones… Novel vaccines for preventing or treating disease…
- **Antimicrobial resistance. Mechanisms of action of antimicrobial agents:**
  Microbial resistance to antibiotics and biocides. Resistance genes. Prevention of resistance. Surveillance & statistics. Genetics and Proteomics…
  Inhibitors of bacterial cell wall biosynthesis. Inhibition of protein biosynthesis. Inhibition of nucleic acid biosynthesis. Alteration of cell membrane function. Inhibition of cell metabolism (antimetabolites).
  Superbugs. Multi-resistant strains. Emerging and re-emerging bacteria and fungi in humans, animals, and plants.
  Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin Intermediate/Resistant *Staphylococcus aureus* (VISA/VRSA), *Clostridium difficile*, *Mycobacterium tuberculosis*, Vancomycin-resistant *Enterococcus* (VRE), *Cryptosporidium*, *Plasmodium* parasites, *Plasmodium falciparum*, Leishmania species, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, *Cryptococcus*, *Escherichia coli* O157:H7, *Helicobacter* spp., *Enterobacter sakazakii*, *Serratia* spp., *Pseudomonas aeruginosa*, Fluoroquinolone-Resistant *Pseudomonas aeruginosa* (FQRP)...
- **Attenuation of virulence as antimicrobial strategy:**
  Interfering microbe-microbe communication (quorum sensing). New strategies...
- **Techniques and Methods:**
  Susceptibility Testing. Rapid microbial and resistance detection. Detection of antibiotics in environmental samples. Microscopy, microanalysis & spectroscopy, single-cell studies, high-throughput studies, nanomechanical studies, microfluidics, lab-on-a-chip concepts, miniaturized science, analysis of microbial surfaces, heterogeneity, statistics. Interaction of antimicrobial drugs with model membranes. Analytical techniques…

The regular conference program was complemented with three keynote lectures:

- "Rapid Detection and Identification of Microbial Agents" presented by Prof. Ali Karami (Research Center of Molecular Biology, Baqiyatallah University of Medical Sciences, Iran)
- "Role of efflux pumps in bacterial resistance" presented by Dr Goutam Gupta (Los Alamos National Laboratory, USA)
- "Artilysins are a novel class of enzyme-based antibacterials that quickly kill (multidrug-resistant) *Pseudomonas aeruginosa* and their persisters: from concept to application" presented by Dr Yves Briers (University of Leuven, Belgium) and Dr Stefan Miller (Lisando GmbH, Germany)

The following people had a role in the conference design, preparation and celebration:

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Last but not least, we hereby acknowledge the support of the companies *Thüringisches Institut für Textil- und Kunststoff-Forschung e.V* (The **Thuringian Institute of Textile and Plastics Research)**, and **Garland Science (Taylor & Francis Group)**, for their support and for choosing the conference to promotr their products and services to the scientific community in the antimicrobial sector.

We hope readers will find the content of this third edition of the conference inspiring and stimulating and look forward to seeing another fruitful edition in 2016.

A. Méndez-Vilas
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Spain

TITK DIVERSITY IN POLYMERS

ABOUT TITK

Using the research and development work carried out by the "Institute for Man-Made Fibre Technology" as a basis, the TITK has advanced from an organisation offering research expertise for the application of fibres in the textile industry to an ultra-modern and world-renown institute for polymer materials research.

As an **industry-oriented research institute**, the TITK conducts both fundamental and applied research in fields of relevance to various industries.

Among other things, we support small and medium-sized enterprises with their innovation efforts by providing them with interdisciplinary expert knowledge, innovative ideas, specialised knowledge of the industries we work with, and state-of-the-art technical infrastructure.

The objective of such collaboration is to develop technological processes and competitive products that meet the individual requirements of customers, whereby the results of our activities can be used for the most diverse applications in the **fields of chemistry, plastics and textiles**.

A **team** of 150 scientists and laboratory, technical and commercial assistants are employed by the institute and its subsidiary company. Together, these staff members ensure rapid and competent implementation of **R&D tasks** and **material testing procedures**.

Qualified and dedicated specialists also work in project groups on the development of materials, processes and methods that help increase our clients' competitiveness.

The TITK also plays an important role in the professional training of young men and women. At the moment, four apprentices are obtaining their qualifications as a textile production mechanic, chemistry technician, process mechanic for plastics and rubber production, and physics lab assistant. Students of chemistry, physics, textile engineering, process engineering, materials engineering and other subjects are also supported through internships and assistance with their Master theses and Doctoral dissertations.

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DEPARTMENT OF PLASTICS RESEARCH

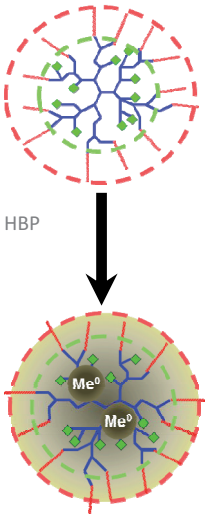
Activities at the Department of Plastics Research focus on the **modification of plastics** as a means of lending them new or improved characteristics. Such modification can be carried out either as early as the polymerisation phase or in subsequent processing steps such as extrusion and injection moulding.

The department focuses on the following research fields:

- Examination of polymerisation possibilities for polyamides, polyester, polycarbonates and biopolymers
- Fibre-reinforced plastics
- Flame-retardant plastics
- Electrically conductive plastics
- Electromagnetic shielding plastics
- Thermally conductive plastics
- Magnetic and magnetisable plastics
- **Biologically active plastics**
- **Thermal energy storage plastics**
- Modification cast polyamide
- Nanocomposites
- **Production in clean room (ISO 8-6) with medical grade materials**

BIOLOGICALLY ACTIVE PLASTICS

ANTIBACTERIAL, FUNGICIDAL AND ALGICIDAL MODIFICATION OF POLYMERS



THE TITK CONCEPT:

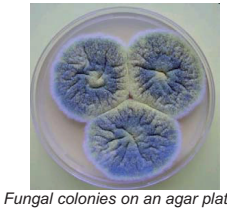
HBP as active substance carriers

- Good compatibility with various materials (plastic, wood, metal)
- High efficacy
- Good processability
- Good flowability
- Suitable for coatings and compounds

HIGHLY EFFECTIVE DISPERSIONS AND MASTERBATCHES

PolyXenia bioactive *sensitive Zinc*

EFFECTIVE AGAINST FUNGI!



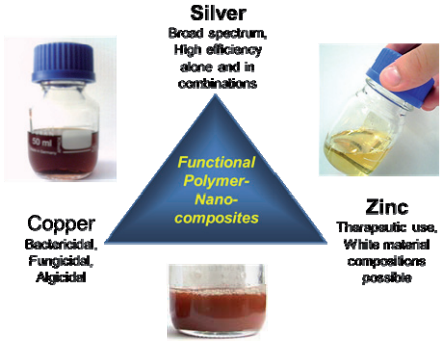
Fungal colonies on an agar plate[1)]

PolyXenia bioactive *power Silver*

HIGHLY EFFECTIVE AGAINST BACTERIA!

S. aureus on a blood agar plate[1)]

Due to the embedding of silver, copper or zinc in dendritic hyperbranched polymers (HBP), it is possible to incorporate them in a simple way as antimicrobial additives in polymeric matrix materials for plastics and coating applications. Furthermore, a high antimicrobial activity can be obtained at relatively low concentrations (usually at <0.5% Ag, Cu or Zn),



[1)]: Source: Carl von Ossietzky Universität Oldenburg (mikrobiologischer-garten.de)

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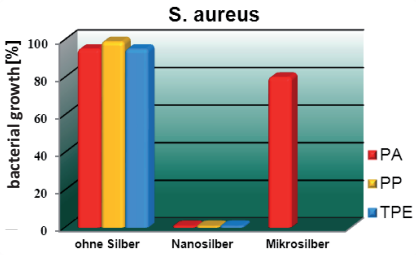
INNOVATIVE MATERIALS FOR MEDICAL APPLICATIONS

ANTISEPTIC CATHETER SURFACES

Within the framework of a cooperation project with the Christoph Miethke GmbH & co. KG (Germany), innovative solutions for antiseptic functionalized catheters were developed. Postoperative infections can be minimized by the anti-microbial surface modification of catheters. The bioactive functionalization is based on a process / technology developed at the TITK and takes advantage of the wide- band bactericidal effect of silver. Its embedding in a dendritic carrier-polymer creates an active addition agent (Ag-hybrid) that combines a high level of compatibility with easy formulation and dispersion in many plastic materials (compounds, coatings). Moreover, a controlled release of bioactive silver ions and a high biocompatibility is enabled by this approach.

PROPERTIES:

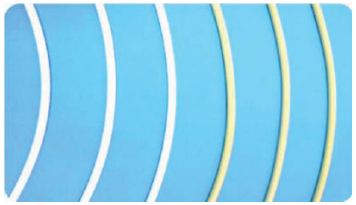
- **only low concentration of silver necessary**
- **adjustment according to the particular application**
- **without toxical educts**
- **surface and / or bulk functionalization possible**
- **reduction of the risk of infections**
- **enhancement of safety and hygiene of medical plastic grades**



Antibacterial properties of various compounds acc. to ISO 22196 containing < 0,7% Ag-Hybrid and 2% Silver (Micro), respectively

FIELD OF APPLICATIONS:

- **Antimicrobial functionalization of catheters made from silicone and other plastics**
- **Biocompatible modified thermoplastic compounds with antimicrobial properties for catheters and other medico-technical products**



Catheters made from silicone: not functionalized (left) and with antiseptic (antimicrobial) properties (right)

IN-HOUSE BIOLOGY LABORATORY

BIOACTIVITY TESTS

ANTIBACTERIAL TESTS ACC. TO ISO 20743 AND ISO 22196 ON:

- **Staphylococcus aureus (gram +)**
- **Klebsiella pneumoniae (gram -)**
- **Escherichia coli (gram -)**
- **Pseudomonas aeruginosa (gram -)**

ANTIFUNGAL TESTS ON:

- **Candida albicans (human-pathogenic yeast)**

BACTERIA LABORATORY

ALGICIDAL ACTIVITY TESTS ON:

- **Desmodesmus subspicatus**

GENETICS LABORATORY

BIOCOMPATIBILITY TESTS

IN VITRO CYTOTOXICITY TESTS ACC. TO ISO 10993-5:

- **Mouse fibroblasts L929**
- **Human HaCaT keratinocytes**

DEAD/LIFE STAINING:

- **According to DIAZ et. al., 2003**

CELL CULTURE LABORATORY

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Gneupel, C. *et al*.: Antibacterial und fungicidal plastics by dendritic hyperbranched polymer-copper-hybrids (Code 229)

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Antimicrobial natural products I - Peptides

A frog skin-derived antimicrobial peptide against *Pseudomonas aeruginosa*-induced infections

Maria Luisa Mangoni[1], Satya S Kolar[2], Vincenzo Luca[1],HasnaBaidouri[2], Antonio Di Grazia[1], Alessandro Pini[3]and Alison M McDermott[2]

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2 The Ocular Surface Institute, University of Houston College of Optometry, Houston, TX, USA
3. University of Siena, Department of Medical Biotechnology, Siena-Italy

P. aeruginosa is the most prevalent bacterium causing mucosal surface infections such as those found in the lungs of cystic fibrosis sufferers or associated with contact lens wear resulting in bacterial keratitis [1]. The growing emergence of multidrug-resistant strains calls for the discovery of new antibiotics with new modes of action. Naturally occurring antimicrobial peptides (AMPs) hold promise as new therapeutics [2]. They are produced by almost all forms of life as key components of the innate immune response [3]. Unlike conventional antibiotics, most AMPs interact with and increase the permeability of the microbial membrane as part of their killing mechanism [4]. Amphibian skin secretions are one of the richest sources for AMPs, which are synthesized and stored within granules of holocrine-type serous glands and released upon stimulation [6-8].

Here we investigated the anti-*Pseudomonal* efficacy of a frog skin-derived AMP, Esculentin(1-21) [Esc(1-21)], *in vitro* and in mouse models of lung/ocular *Pseudomonas* infections [5]. Our results revealed that Esc(1-21) has (i) a rapid anti-*Pseudomonal* activity against both free-living and biofilm forms of this pathogen, with a membrane-perturbing activity as a plausible mode of action. This limits the emergence of resistance; (ii) the capability to preserve its bactericidal activity under physiological conditions that better mimic the lung/ocular surface milieu (i.e. in the presence of high salt concentration and/or tears); (iii) the ability to neutralize the toxic effect of bacterial lipopolysaccharide and (iv) the ability to induce migration of epithelial cells in a wound healing assay. Regarding *in vivo* studies, Esc(1-21) has been found to promote survival in mouse models of *P. aeruginosa*-induced pulmonary infections by reducing the number of colony counts within the lungs, after a single intratracheal administration. In addition, it has been shown to significantly reduce the level of ocular infection in murine models of *P. aeruginosa* keratitis, upon topical treatment, three times/day for up to 5 days post-infection and to reduce the amount of viable bacterial cells and neutrophil infiltration within the cornea, compared to PBS treated animals. Overall, Esc(1-21) has great potential for development as a novel pharmaceutical for the treatment of *Pseudomonas*-induced pneumonia or keratitis upon local application to the site of infection.

1. Kolar SS and McDermott AM. Cell Mol Life Sci (2011); 68:2201-13
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3. Boman H.G. Annu. Rev. Immunol. (1995); 13:61-92.
4.Shai, Y. Biochim. Biophys. Acta(1999); 1462, 55–70
5. Luca V et al. Cell Mol Life Sci(2013); 70:2773-86

Keywords: Esculentin; *Pseudomonas aeruginosa*; bacterial keratitis, cystic fibrosis

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A novel cyclic pentadepsipeptide, neoN-methylsansalvamide exhibiting a synergistic effect with paclitaxel on multidrug resistance cells

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NeoN-methylsansalvamide is a novel low-molecular-weight cyclic pentadepsipeptide that exerts cytotoxic effects. Its structural analysis using liquid chromatography mass/mass spectrometry showed the cyclic structure sequence -phenylalanine-leucine-valine-N-methylleucine-leucic acid-. The full-scan of neoN-methylsansalvamide showed the protonated molecular ion at m/z 601.59 along with an ammonium adduct at m/z 618.5, followed by the protonated fragmentation ions at m/z 573.59, 454.51, 340.92, and 214.25. Further information on the fragmentation in ESI–MS/MS analysis showed clear serial loss of the subunits through cleavage across the ester and amide bonds from the ion at 573.59, which was produced by the loss of –CO after opening of the cyclic molecule. The next fragment ions in the spectra were created by the sequential loss of Phe, Leu, and Val at m/z 454.51 (M+H– Phe), 340.92 (M+H– Phe – Leu), and 214.25 (M+H– Phe – Leu – Val – CO), respectively. The intrinsic cytotoxic and multidrug resistance reversal effects of neoN-methylsansalvamide were evaluated on the cell lines MES-SA and HCT15 as well as on their multidrug resistance sublines (MES-SA/DX5 and HCT15/CL05, respectively) using the sulforhodamine B assay. The EC50 values of paclitaxel for MES-SA, HCT15, and for the multidrug resistance sublines MES-SA/DX5 and HCT15/CL05 were 1.00±0.20, 0.85±0.63, 10.00±0.53, and >1000 nmol/l, respectively. However, the EC50 values for paclitaxel including 3 μmol/l neoN-methylsansalvamide for MES-SA/DX5, HCT15, and HCT15/CL02 were 1.58±0.12, 0.10±0.02, and 288.40±21.02 nmol/l, respectively. The in-vitro multidrug resistance reversal activity of neoN-methylsansalvamide was similar to that of the control verapamil. These finding suggests that a novel cyclic pentadepsipeptide, neoN-methylsansalvamide, is effective in reversing multidrug resistance in vitro, and this activity may be a major applicable biological function of this compound.

Keywords: cyclic pentadepsipeptide, neoN-methylsansalvamide, multidrug resistance

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[2] Lee HS, Lee C. Structural analysis of a new cytotoxic demethylated analogue of neoN-methylsansalvamide with different peptide sequence produced by Fusarium solani isolated from potato. J Agri Food Chem 2012; 60:4342–4347.

A novel natural product, humidimycin (MDN-0010), that potentiates the antifungal activity of caspofungin and itraconazole

Francisca Vicente[a], Maria Cândida Monteiro[c], Jesús Martín[a], Vito Valiante[b], Emilia Mellado[c], Noureddine El Aouad[a], José R. Tormo[a], Ignacio Pérez-Victoria[a], Nuria de Pedro[a], Ignacio Gonzalez[a], Olaf Kniemeyer[b,d,e], Karine Guilloux[f], Jean-Paul Latgé[f], Anne Beauvais[f], Robert Altwasser[g], Axel A. Brakhage[b,d] , Olga Genilloud[a], Fernando Reyes[a]

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Aspergillus fumigatus is one of the most important opportunistic fungal pathogens, which causes life-threatening invasive aspergillosis with high mortality rates. The increase of these fatal fungal infections, coupled with the emergence of antifungal drug resistant strains, makes it an urgent task to search for new therapeutic strategies. A drug applied to antifungal therapy is the echinocandin derivative caspofungin (CAS). However, there is increased resistance and a so-called paradoxical effect leading to attenuated activity of echinocandin antifungals at high concentrations. Therefore, we aimed at identifying novel compounds enhancing the CAS effect by inhibition of putative salvage pathways. As shown here, the discovery of novel compounds displaying synergism with commercially available antifungals represents a promising strategy. By screening 20,000 microbial natural extracts we identified a novel siamycin-like peptide, named as humidimycin, which was isolated from liquid culture broths of the actinomycete *Streptomyces humidus* using a bioassay to detect compounds potentiating the antifungal activity. The compound displayed a strong potentiation of the growth inhibitory effect of caspofungin and itraconazole against *Aspergillus fumigatus* and *Candida albicans*. Humidimycin showed no antifungal effect when used alone. Humidimycin alone and in combination with CAS did not show any toxicity in the human hepatocyte cell line Fa2N-4 and the human ether-a-go-go-related gene (hERG) channel inhibition test making them excellent candidates for their combined use. Parallel studies indicated that humidimycin mostly activates the HOG pathway, thereby increasing CAS activity when used in combination.

Keywords: Humidimycin, peptide isolation, antifungal, caspofungin and itraconazole potentiation, HOG pathway, *Aspergillus fumigatus*, *Candida albicans*

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Antichlamydial activity of recombinant human peptidoglycan recognition proteins

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All macroorganisms contact with external environment and can be attacked by different microorganisms. Skin, mucous membranes and other natural barriers protect tissues from invasion of pathogenic, non-pathogenic transient, but normal flora. These tissues form not only mechanical barrier, but also they synthesize and secrete different antimicrobial proteins and peptides. In medicine today antimicrobial proteins and peptides are the most promising anti-infective agents. Peptidoglycan recognition proteins (PGLYRP, PGRP) are innate immunity components that recognize peptidoglycan and lipopolysaccharides of bacteria and show antibacterial activity. We cloned human PGLYRP1, PGLYRP3 and PGLYRP4 genes for homologous expression in human cell lines. We obtained stable transfected HeLa cells, and transiently transfected Expi293F^{TM} cells, which synthesize recombinant human PGLYRP1, PGLYRP3 and PGLYRP4 to the cultural medium. We also obtained transiently transfected Expi293F^{TM} cells which synthesize 6His-tagged PGLYRPs to confirm their presence in cultural medium. Target proteins with 6His-tags were detected in cultural medium of Expi293F^{TM} cells transfected with pcDNA3.4-PGLYRP-6His plasmids. His-tagged proteins showed the same activity as non-tagged. These recombinant proteins expressed in homologous expression systems reveal antibacterial activity against Gram-positive bacteria *B.subtilis* and *S.epidermidis*.

For the first time we showed that recombinant peptidoglycan recognition proteins reveal antichlamydial activity, which characterizes in reducing the amount of chlamydial inclusions in infected HeLa cells after incubation of *C.trachomatis* EBs with cultural medium containing recombinant PGLYRPs. We showed that chlamydial progeny titer decreases 2 logs after contact of PGLYRPs with parental *Chlamydia trachomatis* EBs.

It was shown that binding of PGLYRPs to peptidoglycan or lipopolysaccharides of bacteria, activates two component stress-response system [1]. *C. trachomatis* also have such system of stress defense (CtcB-CtcC). We report that contact of PGLYRPs with *C.trachomatis* EBs provokes activation of two-component stress response system genes expression, which may result in activation of CtcB-CtcC system and lead to an inhibition of chlamydial infection. Maximum ctcB-ctcC mRNA levels were observed after 2h from contact of EBs with PGLYRPs.

In summary, our results demonstrate existence of antichlamydial activity of recombinant human peptidoglycan recognition proteins, which is connected with activation of chlamydial two-component stress response system genes.

Keywords: *Chlamydia trachomatis*; peptidoglycan recognition proteins; two-component system

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Antimicrobial activity of a chelatable cyclic lipopeptide amphisin produced by *Pseudomonas fluorescens* DSS73

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Lipopeptides constitute a structurally diverse group of metabolites produced by various bacterial and fungal genera via non-ribosomal pathways [1]. Structurally they are amphiphilic molecules that comprise both hydrophobic and hydrophilic moieties, being the apolar component usually a alkyl chain, whereas the polar part, more variable, can be ionic (anionic or cationic) or non-ionic. In the past decades, research on lipopeptides has been focused on their antimicrobial, antitumor and surfactant activities. Amphisin is a lipoundecapeptide with an N-terminal β-hydroxydecanoyl fatty acid side chain and a nonapeptide lactone core resulting from the cyclization of the Thr-3 hydroxyl group onto the C-terminal carboxylate [2].

In the present study, the antimicrobial activity of amphisin obtained by cultivation of *Pseudomonas fluorescens* DSS73 was investigated against multidrug-resistant microorganism such as *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus epidermidis, Proteus mirabilis* and *Candida albicans* found in gastrointestinal and urinary tracts. Amphisin antimicrobial activity was tested in presence or absence of metal ions. The results demonstrated that amphisin in the presence or absence of metal ions has a broad spectrum of action, including antimicrobial activity against microorganisms with multidrug-resistant profiles. Binding to amphisin of Cu^{2+} and Zn^{2+} ions increase the antimicrobial activity against *E. coli* and *E. faecalis.* The interaction of lipopeptide with lipid bilayer of bacterial membranes may be facilitated by metal ions, both by neutralizing the anionic charges and by favoring association with the membrane head groups.

The results obtained suggest the possible use of this biosurfactant as an alternative antimicrobial agent in the medical field for applications against microorganisms responsible for diseases and infections, making it a suitable alternative to conventional antibiotics.

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Keywords: biosurfactant; *Pseudomonas fluorescens*; lipopeptides; antimicrobial activity

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Antimicrobial combination therapies: a network perspective

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The growing number of resistant strains and biofilm-related infections emerging in healthcare settings and in the general community is a major biomedical concern. Currently, antimicrobial studies are revisiting the potential of old products and looking for new products with alternative modes of action. Most notably, antimicrobial peptides (AMPs) are receiving a lot of attention because of the widespread availability, multiple mechanisms of action, non-specific molecular targets, and anti-biofilm capabilities [1], [2].

Considering that most of the results obtained in these studies lay in scientific literature, and manual curation is time and resource consuming, the development of bioinformatics approaches for the systematic screening of the literature is of obvious interest. In particular, the reconstruction of drug interaction networks reflecting *in vitro* and *in vivo* results is considered useful to identify the most promising candidates for the development of alternative antimicrobial therapies, such as antimicrobial combinations [3], [4]. Such networks can aid in profiling and interpreting the activity of AMPs and the added value of antimicrobial combinations, and thus, help exploit their potential.

As a first contribution to this line of analysis, this work presents a novel network reconstruction for results obtained by AMP-drug combinations in fighting *Pseudomonas aeruginosa* infections [5]. This network contains information about strains, combination methodologies, mode of growth, compound description (with drug and AMP database cross-linking) and quantification values (MICs, FICs, log reduction, etc.). So far, the network comprises 239 combinations, such that 83 % of the interactions pair an AMP with a non-AMP compound (antibiotics, enzymes, etc.), mainly traditional antibiotics. The majority (82 %) of the studies focused on the use of combinations on planktonic cells, and surprisingly enough, only 3 % of the studies tested the combination in biofilms. Furthermore, the network is dominated by a small number of highly connected nodes, namely the peptides colistin and polimyxin B. These are the products that are more often tested in antimicrobial combinations.

The network is publicly available, and may be further explore using graph-based analysis tools. Hopefully, this will be a valuable resource to the design of new experiments, unveiling different mechanisms of action and helping in the prediction of new combinations.

Keywords: antimicrobial peptides (AMPs); *Pseudomonas aeruginosa*; biofilms; network; bioinformatics

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Antimicrobial properties of membrane-active dodecapeptides derived from MSI-78

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Antimicrobial peptides (AMP) are a class of broad-spectrum antibiotics known by their ability to disrupt bacterial membranes and their low tendency to induce bacterial resistance, arising as excellent candidates to fight bacterial infections. In this study we aimed at designing short 12-mer AMP, derived from MSI-78 (22 residues), a highly effective and broad spectrum synthetic AMP, by truncating the peptide at the *N*- and/or *C*-termini while spanning the MSI-78 sequence with 1 amino acid (aa) shifts [1,2]. These designed peptides were evaluated regarding antimicrobial activity against selected gram-positive *Staphylococcus* strains and the gram-negative *Pseudomonas aeruginosa (P. aeruginosa)*.

In general most of the short 12-mer peptides tested showed a reduction in antimicrobial activity, an effect that was more pronounced for gram-positive *Staphylococcus* strains. However, the 12-mer peptide CEM1 (GIGKFLKKAKKF) arose as a good candidate to fight *P. aeruginosa* infections, as it displays antimicrobial activity against this strain and selectivity, with negligible toxicity to mammalian cells even at high concentrations. Interestingly, CEM1 and a highly similar peptide differing by only one aa-shift (CEM2: IGKFLKKAKKFG), showed a remarkably contrasting AMP activity. These two peptides were chosen for a more detailed study regarding their mechanism of action, using several biophysical assays and simple membrane models that mimic the mammalian and bacterial lipid composition.

We found a direct correlation between peptide helicity and antimicrobial activity and propose a mechanism of action that supports an antibacterial action based on bacterial membrane lysis and consequent bacterial cell death.

Keywords: Antimicrobial peptides, MSI-78, pexiganan, antibiotic resistance, cytotoxicity and membrane models

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Application of lactoferricin B to control microbial spoilage in cold stored fresh foods

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Psychrotrophic *Pseudomonas* spp. were proved to cause dramatic quality losses in fresh foods like leafy greens and dairy products resulting in the shortening of their shelf-life. These spoilage microorganisms are resistant to most disinfection treatments. This work was addressed to assay the antimicrobial efficacy of the food-grade bovine lactoferrin (BLF) and its hydrolysates (LFH), obtained after digestion with pepsin or the enzymatic cardoon extract. The inhibition assays were carried out *in vitro* on 8 selected *Pseudomonas* spp. strains involved in off-color development in ready-to-eat vegetables (RTE) and in mozzarella cheese.

A higher antimicrobial activity against most of the tested strains was found for pepsin digested BLF in comparison with BLF digested with the enzymatic cardoon extract. A solution of the antimicrobial peptide lactoferricin B (LFcinB), purified from the pepsin-LFH, was applied to both cold stored RTE lettuce leaves and mozzarella cheeses, inoculated with the spoilage strains. A significant reduced tissue browning of RTE lettuce leaves, as well as absence of mozzarella cheese discoloration applying LFcinB. The results obtained show the possibility to use milk-derived antimicrobial peptides in the control of food spoilage caused by *Pseudomonas* strains.

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Keywords: *Pseudomonas* spp.; bovine lactoferrin; antimicrobial peptides; shelf-life;

AvBD9: The importance of cysteine and tryptophan amino acids for anti microbial activity

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Avian Beta Defensins (AvBDs) are a group of cysteine-rich peptides characterised structurally by three di-sulphide bonds, which are proposed play a role in the host innate immune response (1,2,3). This study focussed on AvBD 9 and investigated the roles of single amino acid changes, targeting specifically the cysteine and tryptophan amino acids, on its bacterial killing capabilities.

The antimicrobial properties of AvBD 9 peptide were determined using a time-kill antimicrobial assay focussing on two avian clinical isolates, *Escherichia coli* and *Enterococcus faecalis.* To test the premise that cysteine amino acids are important in AvBD 9 function, variants were prepared in which the cysteine amino acids were mutagenised to either Alanines or Glycines and the anti-microbial activities (AMAs) of the mutagenised AvBD 9 peptides, against both Gram negative and positive bacteria, determined. Additionally, the C-terminal tryptophan was mutagenised to a glycine and the effects of this change on AvBD 9 AMA determined.

Kill curves indicated that the antimicrobial activities of wild-type (6C) and 3C AvBD9 peptides were not significantly different. Using 25 µg/ml of each peptide, 29 % and 39% of *E.coli* and 21% and 59% of *E faecalis,* respectively, were killed. At 50 µg/ml, 49% and 60% killing of *E coli,* and 53% and 69% killing of *E faecalis* were observed. Following mutagenisation of all the AvBD9 cysteines, the peptide was no longer active against *E.coli*; no killing was detected at either 25 or 50 µg/ml, although 63 % of *E faecalis* was killed using 50 µg/ml. Substituting the C-terminal tryptophan of wild-type AvBD9 for a glycine also resulted in a loss of AMA with no *E coli* killing and only 17% *E faecalis* killing detected using 50µg/ml.

These data suggest that AvBD9 has microbial killing properties and plays an important role in chicken innate immunity. The cysteines and tryptophan amino acids also appear functionally important in the AvBD9 killing mechanism against both Gram negative and positive clinical isolates.

Key words: Antimicrobial peptides; avian defensin; *E coli; E facalis*; cysteine; tryptophan; *in vitro* synthesis; and mutagenesis

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Cathelicidins in the Tasmanian devil (*Sarcophilus harrissii*)

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Antimicrobial peptides are a primitive component of the innate immune system. Cathelicidins are a predominant family within mammals, contributing to host immunity through antimicrobial and immunomodulatory functions. They have been studied extensively in eutherian mammals but marsupials are relatively unexplored. Marsupials have a short gestation and give birth to altricial young which are immunologically naive. During development the young are protected from infection by cathelicidins which are expressed in the mother's milk and in the epithelium of the pouch as well as in the skin of the young themselves. This unique reproductive physiology has encourgaed lineage specific expansion of the cathelicidin gene family within marsupials, resulting in numerous diverse peptides.

The Tasmanian devil (*Sarcophilus harrisii*) is the largest remaining carnivorous marsupial and is found only on the island state of Tasmania. The future of the Tasmanian devil is currently under threat from a contagious cancer, Devil Facial Tumour Disease (DFTD), which has already decimated over 85% of the population. Interestingly, young devils do not catch DFTD, and we hypothesize that, given human and bovine cathelicidins can exhibit anti-tumour activity against a number of cancers, antimicrobial peptides in devil milk may protect young devils against cancer.

We identified six cathelicidins in the Tasmanian devil genome and have synthesized the mature peptides. These will be tested against a range of bacterial and fungal pathogens, and DFTD cells. Thus far only two cathelicidins have been tested against fungi. One peptide was capable of killing *Candida krusei, Candida parapsilosis* and was more effective at killing *Cryptococcus gattii* and *Cryptococcus neoformans* than the antifungal drug fluconazole. Cytotoxic and haemolytic activity of all six cathelicidins against human cells has also been determined. Four Tasmanian devil cathelicidins did not kill human lung epithelial cells or red blood cells, and only two showed moderate cytototoxic and haemolytic activity. This study highlights the potential for marsupials such as the Tasmanian devil to provide new drugs to treat human and animal disease.

Keywords: Cathelicidin; Tasmanian devil

Chemistry and antimicrobial potential of BuMAP-34, a novel buffalo myeloid cathelicidin

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A novel putative cathelicidin antimicrobial peptide of 34 amino acid residues was deduced from buffalo myeloid gene sequences and named as BuMAP-34. Structure-function relationship of the custom synthesized peptide was evaluated both *in vitro* and *in vivo*. Highly cationic, amphipathic peptide showed a net charge of +6 and predicted hydrophobic ratio of 38 %. Ramachandran plot analysis showed that 96.9% and 3.1% of phi and psi angles of the amino acids were in favoured and allowed regions of the plot respectively, indicating high structural stability. Secondary structure analysis revealed an α-helix with sharp turns at both ends of the helix. The peptide showed potent antimicrobial activity against a wide spectrum of microorganisms including Gram-negative and Gram-positive bacteria, fungi, spirochetes and virus. Minimum inhibitory concentration (MIC) on various strains of bacteria, *E. coli, S. aureus, P. multocida* (DP1), *S. Typhimurium, K. Pneumonia, R. anatipestifer* and fungus, *C albicans,* ranged from 1.1 to 1.5 μM. Interestingly, the MIC of the peptide was >100 μM for *P. multocida* (HS). Morphological examination of the peptide treated *E. coli* and *S. aureus* indicated the ability of the peptide to bind with anionic components of the cell such as, DNA, further confirmed by DNA binding assay. Bactericidal and fungicidal effect of the peptide was examined by SEM analysis, which revealed cell wall and membrane lysis. Antispirochete activity on four serovars of *Leptospira interrogans*, showed a dose dependent effect and the MIC ranged from 5 to 20 μM. Among the various serovars, lowest MIC was observed with *L.* Autumnalis. Antiviral activity was tested against Newcastle disease virus, which showed an inverse relationship with increasing peptide concentration and highest activity was observed at 25 μM of the peptide. Cytotoxicity studies showed absence of haemolysis in human RBCs at 12.5 μM and in sheep RBCs even at 100μM concentration of the peptide. Mice infected with an intra-peritoneal injection of lethal Duck *pasteurella* (DP1), when treated with the peptide protected 80% and 100% of the animals at 6.25 μM and 12.5 μM respectively. Phylogenetic analysis revealed that the peptide, BuMAP-34 has an evolutionary relationship with BMAP-34 of cattle, MAP-34 of Goat and SMAP-34 of sheep. The present study suggests that the novel cathelicidin, BuMAP-34 has strong antimicrobial activity and could be developed as a promising broad spectrum antimicrobial agent.

Keywords: Antimicrobial peptide; BuMAP

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Detailed genetic characterization and expression analysis of protegrin like sequences in the pig genome

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Protegrins (PGs) are potent antimicrobial peptides that act on a broad spectrum of microorganisms, including bacteria, fungi and some enveloped viruses. We analyzed the expression pattern of protegrins in seventeen different pig tissues by RT-PCR, and developed an anti-PG-1 polyclonal antibody. Western blot analysis using the antibody showed that protegrins are mainly present as prepropeptide forms in normal tissues, rather than as mature peptides. Immunohistochemical analysis showed that protegrin expression was specific to a few cell types, including neutrophils, epithelial, and Leydig cells. Genetic analyses of the five previously reported protegrin sequences showed that they are encoded at a single locus, rather than from multiple paralogous genes. By genotyping 28 animals across five breeds, we identified nine different alleles of the PGs. In addition, we showed that *PMAP-37* has a high nucleotide similarity to the protegrins.

Keywords: protegrin; antimicrobial peptide; cathelicidin; swine; antibody

Detection of antimicrobial activity in the venom of the spider *Lasiodora* sp.

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Spiders are the most diverse and successful terrestrial invertebrates, excluding the insects. They are ecologically important predators and produce complex venoms that enable them capturing specific preys. The Brazilian spider *Lasiodora* sp., whose trivial names are "caranguejeira" or tarantula, is widely distributed in northeastern Brazil, in the rainforest. In arthropods, antimicrobial peptides participate in the cellular defenses, while humoral defense usually involves components released from hemocytes. Venoms of arthropods contain peptides that act as antimicrobials and thus *Lasiodora* sp. spider venom may interfere on survival and growth of microbial pathogens. This work evaluated the venom for protein concentration, profile on polyacrylamide gel electrophoresis (SDS-PAGE) and antimicrobial activity against bacteria and fungi. The venom was diluted 1:2 in nutrient broth (NB) and a series of 10 double dilutions was performed into a microtitre plate well; all wells were inoculated with the bacterial or fungal culture. Optical density was measured at 490 nm using a microplate reader after incubation at 37°C by 24 h. Negative control wells contained NB medium and the microorganism. Minimal inhibitory concentration (MIC) was determined as the lowest protein concentration at which there was 50% reduction in optical density relative to the control well; the lowest protein concentration showing no bacterial or fungal growth was recorded as the minimal bactericide (MBC) or minimal fungicidal (MFC) concentration. *Lasiodora* sp. venom contained 4.53±0.38 µg/µL of protein and showed polypeptides with molecular masses ranging from 10 to 100 kDa. Different profile was detected when SDS-PAGE was performed under denaturing and reducing conditions revealing that several polypeptides contain disulfide bridges. The venom inhibited the growth and killed all tested microorganisms, with MIC, MBC and MFC values ranging from 0.58 to 100 µg. According to the MBC/MIC and MFC/MIC ratios, the venom can be classified as a bactericide agent against the Gram-positive bacteria *Bacillus subtillis* (MIC: 12.5 µg, MBC: 12.5 µg) and *Micrococcus luteus* (MIC: 1.56 µg, MBC: 1.56 µg) and the Gram-negative bacterium *Aeromonas* sp. (MIC: 12.5 µg, MBC: 12.5 µg). Also, it can be classified as a fungicide agent on *Candida parapsilosis* (MIC: 6.25 µg, MFC: 6.25 µg) and *Candida albicans* (MIC: 25 µg, MFC: 50 µg). The venom acted as a bacteriostatic drug against the Gram-positive species *Staphylococcus aureus* (MIC: 1.56 µg, MBC: 100 µg), the Gram-negative bacteria *Pseudomonas aeruginosa* (MIC: 6.25 µg, MBC: 50 µg) and *Klebsiella pneumoniae* (MIC: 3.1 µg, MBC: 50 µg) as well as fungistatic agent on the yeasts *Candida tropicalis* (MIC: 0.78 µg, MFC: 12.5 µg) and *Candida krusei* (MIC: 1.5 µg, MFC: 12.5 µg). In conclusion, *Lasiodora* sp. venom is source of antimicrobial agents with clinical relevance.

Keywords: *Lasiodora*, antibacterial activity, antifungal activity, spider venom.

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Detection of genes encoding antimicrobial peptides in the coleopteran insect *Tribolium castaneum* and potential therapeutic application

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Insect immunity comprises a suit of constitutive responses that rely on insect haemocytes and several rapid activated enzyme cascades such as phenoloxidase, as well as inducible components such as antimicrobial peptides. The systemic immune response of *Tribolium castaneum* (Tc) larve after exposure to different *Bacillus thuringiensis* (Bt) spore-toxin mixtures was evaluated using qRT-PCR by comparing the expression of two genes encoding the antimicrobial peptides Defensin1 and Defensin2. The Cry1Ac spore-toxin mixture that is not active against Tc larvae showed no induction of any of the genes analyzed relative to non-intoxicated larvae. In contrast, the Cry3Aa and Cry3Ba spore-toxin mixtures that displayed differential toxicities against Tc larvae, induced gene expression in a strain specific manner, suggesting a possible association between the host immune response and larval susceptibility to Bt. Up-regulation of the antimicrobial peptide Defensin1 was the highest following Cry3Ba spore-toxin intoxication of the larvae. A peptide fragment of Tc Defensin1 corresponding to residues 53-82 of the protein was used to assess its antimicrobial activity against three human microbial pathogens, *E. coli, S. aureus* and *C. albicans*. The peptide was effective against the three pathogens being *S. aureus* the most susceptible to its antimicrobial action. These results support Tc antimicrobial peptides might have a potential use in clinical medicine, representing an alternative means to antibiotics to combat infections.

Genome mining of beneficial soil bacteria for novel antimicrobials and enzymes

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Natural ecosystems represent an enormous untapped resource for discovering novel microorganisms, traits, genes and bioactive compounds. Here we focus on the diversity and metabolic potential of microorganisms in the rhizosphere of plants grown in soils that are naturally suppressive to specific fungal plant pathogens. These disease suppressive soils harbor a relatively high abundance of beneficial microorganisms that guard plants against fungal infections [1]. By metataxonomic analyses we identified different groups of bacteria, including Actinobacteria, Bacilli, Burkholderias, Planctomycetes and Pseudomonads, that were enriched on or inside the roots of plants grown in disease suppressive soils. For each target group, a collection of isolates was established and analyzed for their antimicrobial and enzymatic activities.

For Actinobacteria, strains belonging to the genera *Streptomyces* and *Microbacterium* were tested for the production of volatile organic compounds (VOCs) with antifungal activities or with effects on plant growth and development. GC-MS analysis and *in vitro* bioassays led to the idenitification of terpenes in *Streptomyces* and azole-like compounds in *Microbacterium*. For the Bacilli, new *B. pumilus* and *B. simplex* strains were identified for which no antifungal compounds have been reported to date. The secondary metabolites from these *Bacillus* strains were identified by mass spectrometry and the genes encoding these metabolites are being targeted by genome sequencing and mutagenesis. For the Burkholderias, five species clasified as *B. caledonica*, *B. graminis*, *B. hospita*, *B. pyrrocinia* and *B. terricola* were isolated from the plant rhizosphere. VOCs produced by *B. graminis* strongly inhibited growth of fungal pathogens and GC-MS analysis is being conducted to identify the compounds involved in this antifungal activity. The other four *Burkholderia* species exibited different enzymatic activities, including protease, pectinase, xilanase, glucanase and lipase, but also displayed antimicrobial activities against fungal and bacterial pathogens. Screening a collection of approximately 100 Planctomycete strains revealed at least 30 strains with interesting enzyme activities, including protease and glucanase activity. The genomes of four selected Planctomycete strains were sequenced and are now being analyzed for new antimicrobials by bioinformatic and chemical analyses. The last group that is being targeted are the Pseudomonads. For one of the isolated *Pseudomonas* strains, three new lipopeptide compounds were identified with activities against various bacterial, fungal and oomycete pathogens of plants and fish. The identified lipopeptides include the chlorinated 9-amino-acid lipopeptide thanamycin [1], the 2-amino-acid brabantamide [2] and a yet unknown 8-aminoacid lipopeptide. For each of these three lipopeptides, the corresponding gene clusters were cloned and sequenced.

In conclusion, this inventory demonstrates that natural ecosystems represent a rich resource for the discovery of new microorganisms and antimicrobial compounds.

Keywords: antibiotic discovery; plant microbiome; Planctomycetes, Actinobacteria, Bacillus, Burkholderia, Pseudomonas

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Hepcidin function in fish: two sides of the same coin

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Background: Due to the nature of their surrounding environment, fishes have developed several defense mechanisms against waterborne pathogens, with one of the first lines of defense being a number of relatively small peptides with antimicrobial properties. Among these antimicrobial peptides is hepcidin, a small cysteine-rich molecule discovered in the turn of the century, which also came to be considered the long sought key regulator of iron metabolism. Unlike most mammals, which have only one hepcidin gene, with a dual function as an antimicrobial peptide and an iron metabolism regulator, fishes can present many isoforms, which are generally attributed to genome duplications and positive Darwinian selection, influenced by host-pathogen interactions. It has been suggested that different hepcidin isoforms may assume different functions in fish, and its antimicrobial activity may be very important in fish. **Results:** To study the role of the different hepcidin genes in teleost fishes, we have isolated and characterized several hepcidin genes for a teleost fish, the European sea bass *Dicentrarchus labrax*, and evaluated variations in their expression when fish were subjected to different experimental conditions, including iron overload, anemia, bacterial infection and hypoxia. Although several isoforms were found, they could be clustered in two groups: hamp1-like, with a single isoform similar to mammalian hepcidins, and hamp2-like, with several isoforms. Under experimental *in vivo* conditions, *hamp1* was found to be up-regulated in response to iron overload and infection and down-regulated during anemia and hypoxic conditions. *Hamp2*, on the other hand, did not respond to either iron overload or anemia, but it was highly up-regulated during infection and hypoxia. In *in vitro* experiments with spleen derived leukocytes, *hamp1* was again up-regulated during iron overload and early stages of infection, whereas *hamp2* was only highly up-regulated during infection. Furthermore, one Hamp1 and three different Hamp2 peptides were commercially synthesized and tested against several pathogens. Hamp1 peptide showed no effect against any of the tested pathogens, whereas the Hamp2 peptides presented variable activity against several fish and human pathogenic Gram-negative and Gram-positive bacteria. **Conclusions**: In teleost fishes that present two hepcidin types, there seems to have been a subfunctionalization of hepcidin's functions, with hamp1 seemingly more involved in the regulation of iron levels, whereas the various hamp2 are mostly performing an antimicrobial function, with the peptides presenting different affinities. With this study, we provide an integrative insight on the functions of the different hepcidin genes in a teleost fish and demonstrate their antimicrobial potential not only for fishes, but also for other species.

Keywords: teleosts; European sea bass (*Dicentrarchus labrax*); hepcidin; infection; iron;

High throughput activity screening of secreted peptides

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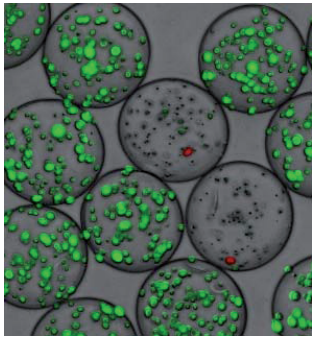

We apply microcapsules (aka nanoliter-reactors or "NLRs") as reaction carriers for high throughput screening in order to identify novel antimicrobial peptides at rates of up to several 100'000 analyzed variants per day.

Specifically tailored microbial biosensor and library cells are poisson diluted in highly monodisperes NLRs (see picture, translucent spheres). The NLRs are highly overpopulated with regard to the sensors (> 100 cells per NLR, expressing a green fluorescent protein) but contain only one library cell (expressing a green fluorescent protein) on average. The thus inoculated NLRs are soaked with a customized growth medium and incubated in a hydrophobic carrier solvent required in order to prevent cross-talk and keep substrates and secreted substances immobilized. In the course of the incubation, both sensor and (see picture, green fluorescent colonies) and library cells (see picture, red fluorescent colonies) form microcolonies. The size of the sensor colonies is then used as an indicator for an estimation of the potency of the peptides secreted by the sensors: While atrophied sensor colonies indicate high potency larger ones point towards rampant sensors growth and therefore to the absence of bioactive substances. The isolation of putative positive candidates by flow-cytometric approaches is straightforward as the ratio of green-over-red fluorescence can be easily used as triggers for NLR isolation.

We will present results on the screening of ~10^5 peptide variants all obtained by site-saturation mutagenesis or synthetic biology approaches (see poster) and while relying on natural antimicrobial peptides as blueprint used as design template. Both, throughput and assay accuracy are high which substantiates the value of the approach for the identification of novel interesting natural products.

Keywords: Lantibiotica, high throughput screening, nano-liter reactors, bioassay

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Honey glycoproteins containing antimicrobial peptides, Jelleins of the Major Royal Jelly Protein 1, are responsible for the lytic and bactericidal activities of honeys

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We recently reported on a presence of a dirigent- like protein in buckwheat honey that showed an extensive similarity with plant disease resistance proteins [1]. To investigate whether other members of innate immune system, specialized in fighting microbial pathogens could also be found in honey, we employed Concavalin A chromatography to isolate glycoproteins, based upon the premise that many disease resistance proteins are glycosylated. The glycoprotein fractions (glps), but not flow-through fractions from affinity chromatography, exhibited strong growth inhibitory and bactericidal properties. The MIC/MBC values of glps isolated from different honeys ranged from 13.8µg/ml to 2.5µg/ml against *Escherichia coli* to 13.8µg/ml to 1.24µg/ml against *Bacillus subtilis*. Due to high-mannose moiety in their structure, glps agglutinated both bacterial species but did not agglutinated red blood cells. Light and scanning electron microscopy demonstrated that glps caused membrane permeabilization and shrinkage of red blood cells, while in bacteria they caused cell wall degradation, formation of filaments and spheroplasts and with time, led to cell lysis. Glps operated by two distinct functionalities; via the high-mannose structure that imparted binding selectivity to bacterial targets and their agglutination, and a non-specific pore-formation/ membrane permeabilization activity observed in both bacterial cells and erythrocytes. Time-kill kinetics in combination with microscopic observations revealed that the fate of cell–wall deficient forms depended of glps concentration and bacterial growth phase. At concentration of 1xMBC, glps irreversibly led to cell lysis of exponentially growing cells, reducing bacterial counts by >5-log10 within 15 min incubation. In contrast, sub-optimal glps concentrations allowed some cell-wall deficient forms to revert to mature cells. MALDI-TOF and electrospray electrospray quadrupole time of flight mass spectrometry, (ESI-Q-TOF-MS/MS) analysis of the main 61kDa band shared by all isolated glps showed sequence identity with the the Major Royal Jelly Protein 1(MRJP1) precursor that harbors three antimicrobial peptides; Jelleins 1, 2 and 4 [2-4]. The presence of Jelleins in glps explained membrane damaging effects caused by MRJP1[4] while the high-mannose moiety in the MRJP 1 explained the lectin-like activity. Together, these finding suggest that the MRJP1, in addition to other functions, is a disease defense protein and an inherent part of the mechanism of honey bactericidal activity. Its antibacterial action is significantly, if not completely, correlated with the overall honey antibacterial activity.

Keywords: antimicrobial peptides, Jelleins, Major Royal Jelly Protein 1 precursor, antibacterial activity, glycoproteins, honey, *E. coli*, *B. subtilis*, cell lysis, spheroplasts

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Interaction of OP-145, a derivative of human cathelicidin LL-37, with bacterial plasma and cell wall components: impact of secondary structure and aggregation status

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OP-145, also known as P60.4Ac, a synthetic antimicrobial peptide derived from the human cathelicidin LL-37, has been successfully used as treatment of chronic otitis media before [1]. OP-145 displayed strong antimicrobial activity [2] but is also lytic to human cells at higher concentrations. Its mechanism(s) of action is (are) still unclear.

In the present study, we investigated the interactions between OP-145 and bacterial and mammalian model membranes to obtain better insight into peptide's specificity and its mode of action. Thermodynamic and structural studies using liposomes composed of phosphatidylcholine (PC) mimicking mammalian cell membranes showed that OP-145 induces disintegration of PC liposomes into disk-like micelles and bilayer sheets, suggesting a detergent-like action. The same studies using liposomes composed of phosphatidylglycerol (PG) mimicking Gram-positive bacterial membranes revealed formation of quasi-interdigitated lipid-peptide structures in the gel and membrane thinning in the fluid phase. Although, OP-145 interacted with both PG and PC systems, the extent of perturbation of bacterial cell membrane was shown to be higher. The presence of bacterial cell wall components lipoteichoic acid (LTA) and peptidoglycan (PGN) did not interfere with the activity of the peptide towards PG membranes. Indeed, OP-145 was capable of displacing LTA as well as PGN from DPPG membranes.

CD spectroscopy revealed α-helical structures of the peptide for both model systems, but the different oligomerisation grades of the peptide in these systems may explain the higher activity of OP-145 towards bacterial as against mammalian membranes.

Keywords: aggregation; disk-like micelles; phosphatidylcholine; phosphatidylglycerol; lipoteichoic acid; peptidoglycan; quasi-interdigitation

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Mechanism of action of lipopeptide biosurfactants on *Candida albicans*

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Candida albicans is a human opportunistic pathogen and can often lead to serious fungemias in immunocompromised hosts [1]. *C. albicans* shows a growing resistance to currently used antifungal drugs. This requires prospecting for new therapeutic compounds and lipopeptide biosurfactants can be such novel agents. Biosurfactants are surface active compounds of microbial origin. Biosurfactants have often antimicrobial and antiadhesive properties but the knowledge about the mechanism of their action is scarce [2]. In this work we demonstrate that lipopeptides pseudofactin II (PFII) [2] and surfactin (SU) effectively decrease cell surface hydrophobicity (CSH) of *C. albicans* cells, but only in the case of hydrophobic strains. Tested lipopeptides also have activity against *C. albicans* adhesion to polystyrene plates and the effect was concentration dependent. Our results indicate that the antiadhesive activity of lipopeptides occurs not only through a change of CSH of cells but also by other mechanisms. Our investigations have excluded the impact of biosurfactants on the expression of *CSH1* gene, which deletion reduces *C. albicans* CSH by 75% [3]. In contrast, after incubation of *C. albicans* at the presence of the PFII or SU, the proteins of masses in the range of 10-40 kDa have been found in supernatants in the contrary to control samples. Therefore, modification of the *Candida* cell wall can be one of the mechanisms for the impact of lipopeptides on cells. To our knowledge this is the first time such an effect has been shown.

Keywords: biosurfactant; lipopeptide; CSH; adhesion; CWP; *Candida*

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Mechanism of LL-37 pore formation

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Mammals protect themselves from inflammation triggered by microorganisms through secretion of antimicrobial peptides (AMPs). These peptides carry out broad spectrum antibiotic activity against fungi, bacteria and viruses [1,2]. To understand the mechanisms by which cathelicidin derived peptide LL-37 actively disrupts bacterial membranes, we performed structural and functional studies. The crystal structures of LL-37 in the absence and presence of detergents resemble a dimeric arrangement of anti-parallel helices with a charged surface on one side and a hydrophobic surface on the opposite side. The positive global charge is compensated by anions located at the dimer interface. The membrane conformation of the peptide structure shows a significant rearrangement of the N-terminus leading to the exposure of hydrophobic residues occluded in the solution structure. Moreover we report co-crystal structures of detergent molecules and LL-37 transmembrane peptides. Specific mutations at the dimer interface and terminal truncations alter peptide stability and antimicrobial activity. Higher oligomeric states and channel formation can be induced *in vitro* by lipophilic molecules and defined conductance channels in artificial planar membranes can be determined. Oligomeric peptide structures are also important *in vivo* to introduce discontinuities of defined size in bacterial outer and inner membranes followed by cell death. A synergistic effect in LL-37 activity was observed against *Staphylococcus aureus*, with daptomycin, a drug altering membrane integrity. Collectively, this study offers deep insights into AMP-membrane interactions and traces structural changes to support rational drug development.

Keywords: antimicrobial peptides, cathelicidines

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Molecular characterization mammocytes defensin (Exon-2) for exploring its potency for synthesis of novel antimicrobial agents

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Antibiotic resistant is a serious concern for both veterinary and medical practices and to trounce these problems there is a need to search alternate group of drugs for prevention and treatment of different diseases. Mammalian defensin and cathelicidin are the two broad classes of antimicrobial peptides expressed by different epithelial lining of the living organisms. Present study was designed to characterize the buffalo mammocytes defensin to find out the template for synthesis of novel antimicrobial agents. Genomic DNA was isolated from buffalo mammocyte. The amplified PCR product was purified cloned and sequenced. The size of the PCR product was 263 bp and cloned DNA after sequencing revealed that exon-2 of the mammocyte defensin is comprised of 129 bases. The total number of predicted amino acids was 42 and the aligned amino acid sequences of buffalo mammocyte defensin with other defensin peptides showed six conserved cysteine residue at different position. The mature peptide had six strongly basic and eleven hydrophobic amino acids including one tryptophan in all most at middle of the peptide. From the present study, it can be concluded that the buffalo mammocyte defensin can act as blue print for synthesis of new antimicrobial agents.

Key Words: antimicrobial peptide, mammocyte, cationic peptide, defensin

Mytilus galloprovincialis immunity and Myticin C

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Ancient and rapid defence mechanisms allow marine bivalves to fight their potential pathogens. Today, only a few genomes of bivalve molluscs are publicly available but the increasing amounts of transcript datasets still offer a great chance of discovery, with the focus on genes centrally involved in the innate immune systems. Gene-encoded antimicrobial peptides (AMPs) are humoral components of the innate immunity, effective as broad-spectrum antibiotics and regarded with great interest for the development of new preventive and therapeutic agents.

The Mediterranean mussel *Mytilus galloprovincialis* differs in antimicrobial resistance from other marketable bivalves and, therefore, its repertoire of host-defence peptides requires structure-activity investigations. The class of Myticin C (Myt C) has been discovered as highly polymorphic group of AMP precursor transcripts in mussels injected with Gram positive, Gram negative and viral-like molecular fragments [1]. Myt C has a cysteine array typical of cystein-rich αβ antimicrobial peptides, it is expressed in the main mussel tissues and all along the development, with individual patterns of variant expression [2]. Expression patterns and allelic-variant diversity, possibly sustained by positive selection, confirm the central role of Myt C in the host-pathogen interactions [3,4]. More recently, the antimicrobial activity as well as the antiviral and immunomodulatory behaviour of Myt C have been investigated [5,6].

Using all available Myt C ESTs to identify the most common peptide sequence, we investigated the antimicrobial and antifungal activity of synthetic Myticin C and some derivatives. We also achieved the oxidative refolding of one of this derivatives and confirmed it by micellar ^{1}H NMR. As suggested by other authors [7] the results indicated the importance of the β-sheet peptide portion in antimicrobial activity. Based on these findings we are now focussing on the minimal active peptide, as advisable for potential commercial uses. Finally, the oxidative refolding of synthetic Myticin C is giving us encouraging preliminary data and opens the way to more detailed activity-structure studies.

Keywords: Immunity; Myticin; mussel; antimicrobial; antifungal; peptide

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New antimicrobial marine cyclolipopeptides

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Multidrug-resistant Gram-negative bacteria (*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) are a serious threat to human health and need the development of new drugs to combat microbial infection. The antimicrobial peptides (AMPs) have been proposed as an alternative. [1]

Novel antibacterial cyclic lipopeptides have recently been characterized from marine micro-organisms [2][3]. They contain unnatural amino acids such as didehydro aminobutyric acid (Dhb), α, γ - diamino butyric acid (Dab) and β-hydroxy Dab (β-OH Dab). The latter are commercially unavailable and need to be synthesized to allow solid phase peptide synthesis.

We will present the synthesis of β-hydroxy Dab (β-OH Dab) conveniently substituted to realize the preparation of new cyclo lipopeptides as well as new analogs of didehydro aminobutyric acid, α, γ - diamino butyric acid (Dab) and β-hydroxy Dab. All these new synthetic monomers could be subsequently introduced in peptide synthesis by SPPS using a Fmoc/t-Bu strategy.

A set of synthetic analogs has been assayed for antimicrobial activity to clarify structure activity relationships. The crucial role of Dhb and β-OH Dab in antibacterial activity was highlighted.

Key words (Lipopetides, cyclopseudopeptides, antimicrobial, amino acid analogs, dehydro amino acids)

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Pinensins A and B, the first lantibiotics isolated from a Gram-negative bacterium, *Chitinophaga pinensis*, are also the first antifungals of their class

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Lantibiotics (lanthionine containing antibiotics) are ribosomally synthesized and post-translationally modified peptides, a rapidly growing category of natural products comprising more than twenty distinct compound classes and until now exclusively isolated from Gram positive bacteria i.e. firmicutes and actinobacteria. Members of the Gram negative genera Chitinophaga and Flexibacter are known producers of secondary metabolites with antimicrobial activity, although the number of bioactive substances is far from those isolated from actinobacteria, myxobacteria, or bacillus-species. Two lantibiotic peptides, pinensins A and B, were now isolated from gliding *Chitinophaga pinensis*. The structures of the peptides were established by HRESIMS and NMR spectroscopy. The results revealed that pinensins A and B are closely related lantibiotics, and only differ in an alanine residue (A) or hydroxyl group (B), respectively. Pinensins A and B are the first lantibiotics isolated from a Gram-negative bacterium. Against expectation, pinensins A and B were found active against many fungi and yeasts with minimal inhibitory concentrations between 2.1 and 33.3 $\mu g/mL^{-1}$ while they show only weak antibacterial activity.

Keywords: lantibiotics; antifungals

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Production of diverse β-amino fatty acid containing lipopeptides by soil cyanobacteria of genus *Cylindrospermum*

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Besides many other secondary metabolites, cyanobacteria produce large variety of chemical structures possessing diverse bioactivities. Some of these are cyclic lipopeptides comprising of 4-15 amino acid residues in the cycle with intercalated β-amino fatty acid. Within this group compounds with anti-bacterial, anti-fungal and cytotoxic effect can be found. In our laboratory we have previously isolated and characterized cyclic lipopeptides puwainphycins F and G (PUW F/G) exhibiting membrane disruption activity against human cells and slight antifungal activity. Interestingly, the puwainphycin structure and its biosynthesis share similarities to antibiotics of iturin family. Puwainphycin F was isolated as major component in Cylindrospermum C24 extract, however, based on the HPLC-HRMS analyses, we have predicted more lipopeptide congeners to be produced by the cyanobacterium. Approximately 18 molecular ions corresponding to putative puwainaphycin analogs were found in Cylindrospermum C24 extract. Fragmentation experiments confirmed identical amino acid composition to PUW F and G variants and so the non-identical part of the molecule can be attributed to the fatty acid chain moiety. By combination of HRMS and NMR techniques we were able to determine the second major lipopeptide in Cylindrospermum extract as 4-methyl-Ahdoa PUW F with tetradecanoic acid replaced by dodecanoic acid. As the NMR measurement and HRMS results were congruent, we have identified other puwainaphycin analogs solely by manual HRMS measurement. Finally we were able to detect PUW congeners of fatty acid chain length in range C10-C16. The retention time of these congeners on C18 reversed phase columns is only partially dependent on the amino acid composition but strongly depends on the length of the fatty acid chain, within the linear gradient the relationship of retention time on the length of the fatty acid chain is almost perfectly linear. In addition, some variants were found to be functionalized by hydroxy or chlorine groups on the fatty acid chain. This study is pointing high structural diversity of cyanobacterial lipopeptides. Since it is highly probable that the bioactivity of these structures is connected with the fatty acid chain, it brings also new possibilities for isolation of novel compounds with interesting bioactivities.

Keywords: lipopeptides; cyanobacteria; LC-HPLC

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S20 - a synthetic peptide - as an antimicrobial and anticancerous agent

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A peptide containing 20 amino acids, S20, was designed based on the combinatorial chemistry and analysed using suitable software. Analysis revealed a total net charge of +8, isoelectric point (pI) of 12.70, hydrophobic moment (μH) of 0.508, Boman index value of 3.86 kcal/mol and a hydrophobic ratio of 40%. Helical wheel projection analysis revealed amphipathic property and in Ramachandran plot, Phi and Psi angles of all the amino acids were in the favoured region showing greater structural stability of the molecule. The peptide was custom synthesized with C-terminal amidation and confirmed the primary structure by NMR analysis. Helix-turn-helix secondary structure prediction was further confirmed by CD spectroscopy. The peptide was then tested for its structure-function relationship, as an antimicrobial and anti-cancerous agent. Antimicrobial activity was explored both *in vitro* and *in vivo* whereas, anticancerous activity was assessed *in vitro.* Potent antimicrobial activity was exhibited on all the tested bacteria (both Gram-negative and Gram-positive), fungi and virus. However, the peptide did not inhibit the growth of *Pasteurella multocida* subsp. *Multocida* even at 100 μM concentration. The MIC on Gram negative bacteria, and fungi ranged from 0.6 to1.2 μM. Compared to Gram positive bacteria (MIC, 4 to 5 μM) the peptide showed selectivity towards Gram negative bacteria. Morphological changes, such as filamentation and clumping, were observed in peptide treated bacteria and fungi both in Gram's staining and SEM analysis. DNA binding assay revealed the potential of the peptide to bind and disintegrate DNA. *In vivo* antibacterial activity study on lethal duck *pasteurella* (DP1) challenged mice (i.p injection) proved to be effective from 3.1 μM onwards, i.e from 70% survival to 90 and 100% survival with 6.25 and 12.5 μM concentrations of the peptide respectively. *In vivo* antiviral activity in chick embryo infected with Newcastle disease virus showed an inverse relation to the peptide concentration, with highest activity at 25 μM. Anticancerous property was tested in HeLa cell lines, displayed an IC_{50} value of 0.78 μM. Haemolytic assay using sheep and human RBCs revealed absence of haemolysis upto a peptide concentration of 100 and 50 μM respectively. Besides, a very low per cent (1.5%) of haemolysis was observed in human RBCs even at 100 μM. The study suggests that the synthetic peptide, S20 have high therapeutic index and is a promising candidate to be developed as a therapeutic drug.

Keywords: Antimicrobial peptide; Synthetic peptide

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Salivary gland cells transcriptome of a medicinal leech

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Intensive usage of antibiotics in medical practice leads to inevitable emergence of resistant bacterial strains. To overcome this problem, regular development and adoption of new antibiotics are required. Among other candidates for novel antimicrobial agents are small polypeptide molecules, the so-called antimicrobial peptides (AMPs).

Gene encoded AMPs are widely distributed among living organisms including plants, invertebrates and vertebrates. In invertebrates, most of the data describe the structure and the function of arthropods AMPs. While antimicrobial peptides in annelids remains largely unknown [1]. Among annelids leeches are vast natural pharmacopoeias selected by evolution. Ingredients of leech saliva may suppress inflammation, reduce the intensity of pain, inhibit blood coagulation in the host and enrich the ingested blood with anti-microbial substances for long-term storage [2].

Despite the broad focus of research on leech saliva, little has been done at the genomic and transcriptomic levels. For solving the problem, we analyzed the salivary gland cells transcriptome of medicinal leeches. It is known that different medicinal leech species produce distinct suites of bioactive compounds in their salivary secretions. We performed whole transcriptome profiling of three leech species (*Hirudo medicinalis, H. verbana, H. orientalis*).

For precision isolation of the salivary glands cells from fixed sections we used ad hoc laser microdissection protocol with gravity transfer. Total RNA was isolated from dissected i) salivary gland cells and ii) muscle cells of medicinal leeches after starvation for no less than four months and normalized cDNA libraries for each leech species were constructed. Using Ion Torrent PGM sequencing platform a total of about 1,000,000 reads were generated for each of libraries and primary bioinformatics analysis was made. Several potential antimicrobial sequences were selected for further analysis.

Thus for the first time using the NGS data we developed transcriptomics resources for salivary gland cells of different medicinal leech species.

Keywords: antimicrobial peptides; medicinal leech; transcriptome; salivary gland cells

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Structure-function analysis of the peptaibol Harzianin HK-VI

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Peptaibols are naturally occurring helical peptides isolated from fungal sources (mainly *Trichoderma*). The peptides are characterized by an unusual composition: they contain aminoisobutyric acid (Aib), carry an amino alcohol at their *C*-terminus, and are *N*-terminally acetylated/alkylated. Peptaibols are bacteriolytic – they destroy bacterial membranes by forming pores. Due to their abundance in nature, high proteolytic stability, low immunogenicity and small size, their potential as pharmacological drug leads for developing novel antibiotics is high. However, a detailed understanding of the molecular mechanisms underlying membranolytic activity is still lacking.

The aim of our study is to determine the structure and membrane alignment of the short 11-residue peptaibol harzianin HK-VI (HZ, Ac-Aib-Asn-Ile-Ile-Aib-Pro-Leu-Leu-Aib-Pro-Leu-ol) from *T. pseuodkoningii*, which can permeabilize lipid bilayers [1]. Its small size gives rise to the mechanistic question as to how the short peptide is able to span the much thicker lipid membrane, needed for pore formation. There are several hypotheses which could resolve the problem of matching peptide length with membrane thickness: the peptide could either oligomerize, change its conformation, or modulate the membrane thickness. We set out to find the most plausible scenario by analysing the HZ structure and orientation, when embedded in lipid bilayers, using solid-state ^{19}F-NMR and circular dichroism (CD) spectroscopy [2]. In particular, we use tailor-made α-trifluoromethylated amino acids (TfmAla [2] and Tfm-Bpg [3]) as isosteric replacements of Aib and Leu/Ile to provide site-specific ^{19}F-NMR information. Despite the synthetical challenge, altogether ten peptide analogues were successfully produced by Fmoc solid-phase peptide synthesis using oligopeptidyl assembly and extended coupling times. The peptides were isolated by HPLC without any fluorine background.

All analogues display the same low antimicrobial activity against Gram-positive *S. aureus* (minimum inhibitory concentration = 128 μg/ml), whereas Gram-negative strains are not affected. Conformational analysis by CD in solution indicates that all mutants are structurally indistinguishable from the parent peptide. In aqueous environments HZ shows no defined structure due to the low solubility of this intrinsically hydrophobic sequence. On the other hand, all analogues adopt a 3_{10}-helix conformation in lipid membrane-mimicking environments. This structure is preserved in the oriented membranes as confirmed by oriented CD. When the peptides are reconstituted in proteobilayers, this structure is neither influenced by the lipid charge, phase state, or temperature. The TfmAla-substituted peptides are found to be suitable for oriented solid-state NMR analysis, as seen by defined ^{19}F-NMR dipolar splittings and by an unperturbed lamellar bilayer structure (^{31}P-NMR). Solid-state ^{19}F-NMR in different lipid compositions suggests that the structurally invariant 3_{10}-helix of HZ re-orients in lipid bilayers depending on the membrane properties such as chain length and degree of saturation. We were able to identify three sample compositions with distinct HZ alignments. In conclusion we observed a conformational stretching of the peptide in the form of a 3_{10}-helix, instead of the expected α-helix; and we are currently investigating the influence of HZ on the degree of membrane thinning.

Keywords: peptaibols; harzianin; peptide-membrane interaction; solid-state ^{19}F-NMR; 3_{10}-helix;

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The impact of hLF1-11 antimicrobial peptide immobilization on its antimicrobial activity

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Introduction

Antimicrobial peptides are recognized as a promising new class of antimicrobial agentes. They are known for their broad spectrum of activity, even against antibiotic-resistant bacteria, and their low tendency to induce resistance [1]. hLF1-11 is a AMP derived from human Lactoferrin with such characteristics, whose clinical value was tested as the antimicrobial agent in a calcium-phosphate cement delivery system on an implant application [2]. Although, some activity was found, the burst release, and rapid clearance of the peptide from the local, suggested that a different administration strategy should be pursued. Covalent immobilization of AMP has been suggested as a way to locally enhance the activity period of time, while avoiding some of the peptide free form issues: fast degradation, peptide aggregation and/or peptide toxicity when used at high titers [3]. The aim of this study was to evaluate if hLF1-11 tethering could solve its application problems. To this end, two different immobilizations were tested: direct immobilization onto a polymer and spacer-derived immobilization [4].

Experimental Methods

Chitosan solution was spinned onto Gold substrates to obtain ultrathin films, that were then further functionalized through carbodiimide chemistry with N-acetyl cysteine or a spacer ($SHCH_2$-$[OCH_2CH_2]_7$-OCH_2CH_2COOH), in order to assess direct vs flexible immobilization of the peptide. hLF1-11 was then immobilized onto these functionalized surfaces establishing a disulfide bridge between the free –SH of modified chitosan and the –SH of the hLF1-11 cysteine, under oxidative conditions (DMSO). Full surface characterization was obtained through X-ray Photoelectron Spectroscopy, Infrared reflection absorption spectroscopy, ellipsometry, water contact angle, Atomic Force Microscopy and surface peptide quantification. The antimicrobial activity of the hLF1-11 was firstly assessed in its soluble form through a Minimal Inhibitory Concentration assay with Methicillin-resistant *Staphylococcus aureus* ATCC 33591. Then the modified surfaces were tested with 10^7 CFU for 4h at 37ºC. At the end of this incubation period, different sample batchs undertook different assays: the supernantants were serially diluted and plated, for total adhered bacteria counts the surfaces were stainned with VECTASHIELD® Mounting Medium with DAPI, and for viable adhered bacteria counts, samples were sonicated, serially diluted and plated.

Conclusions

hLF1–11 covalent immobilization was successfully performed using specific orientation through its C-terminal cysteine, with N-acetylcysteine and spacer in similar amounts. Chitosan thin films by themselves decreased bacterial adhesion. The functionalization with hLF1–11 increased significantly bacterial adhesion to chitosan films, particularly when the peptide was covalently coupled without a spacer. However, when a spacer is used, hLF1–11 maintained part of its antimicrobial activity.

Keywords: Antimicrobial Peptide; Surface immobilization; Bacterial adhesion

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The X marks the spot: investigations on the site of action of a cyclic antimicrobial peptide

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Since the number of infections caused by multi-resistant bacteria is increasing over the last years, there is a pressing need for the investigation of antimicrobial peptides as new class of antibiotic agents. Structural characterization and thorough understanding of their mechanism of action will aid to design novel compounds with enhanced antimicrobial features and improve future pharmacological applications.

Our work focusses on a cyclic synthetic hexapeptide which is highly active against Gram positive and Gram negative bacteria while no toxicity towards eukaryotic cells was detected [1]. We could demonstrate rapid cell killing kinetics and strong binding to model lipid membrane systems. However, membrane permeabilisation, which is the most common mode of action of antimicrobial peptides in general, could not be observed [2].

Using laser scanning microscopy (CLSM) and HPLC, further investigations concentrated on translocation as antibacterial mechanism whereupon the peptide might disturb cytoplasmic processes, such as protein synthesis and DNA replication. Unexpectedly, our results indicate a mode of action that is not based on translocation and distribution into the cytoplasm but rather on peptide interaction with inner membrane components. Distinct sites of peptide accumulation suggest interference with specific cell structures and might result in demixing of lipids and/or disturbance of functional protein complexes. In order to investigate binding of the cyclic hexapeptide to specific phospholipids we apply isothermal titration calorimetry (ITC) and co-localisation studies with labelled membrane components. In addition, the influence of our antimicrobial peptide on cell division is studied in different bacterial strains expressing mutations that affect proteins of the divisome complex.

Keywords: cyclic hexapeptide; mechanism of action; membrane accumulation; cell division; lipid interaction

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Antimicrobial natural products II

Terrestrial and marine organisms

A hybrid NRPS/PKS containing fatty-acyl ligase synthesizes the cytotoxic antifungal β-amino fatty acid lipopeptides puwainaphycins in the cyanobacterium *Cylindrospermum alatosporum*.

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Analogous to other bacterial groups, cyanobacteria possess a unique biosynthetic apparatus capable of generating enormous structural diversity in various secondary metabolites [1,2]. Large multidomain enzymes of non-ribosomal peptide synthetase (NRPS) machinery and polyketide synthetases (PKS) can be reassembled to generate an almost infinite number of chemical structures. Some of the end-products of this machinery have been found to be important pharmaceuticals or toxins. As one of the less explored cyanobacterial secondary metabolites, cyclic lipopeptides may be important due to reported bioactivity in various organisms, which also raises questions on their possible toxicity to humans [3, 4]. The cyanobacterial lipopeptides show some similarity to the best known bacterial lipopeptides isolated from *Bacillus subtillis* (iturin family antibiotics), which display a broad range of biological effects including antibiotic and antifungal activity [5]. Nevertheless, the genetic background of cyanobacterial lipopeptide biosynthesis remains largely unexplored.

Here we investigated a putative operon encoding the biosynthetic pathway for the cytotoxic and antifungal cyanobacterial lipopeptides puwainphycins in the cyanobacterium *Cylindrospermum alatosporum* [6]. Bioinformatics analysis of whole genome sequencing data enabled sequential prediction of puwainaphycin biosynthesis. This process is initiated by the activation of a fatty acid residue via fatty acyl-AMP ligase (FAAL) and continued by a multidomain non-ribosomal peptide synthetase/polyketide synthetase. High-resolution mass spectrometry and nuclear magnetic resonance spectroscopy measurements proved the production of puwainaphycin F/G congeners differing in the fatty acid chain length. Because only one puwainaphycin operon was recovered in the genome, we suggest that the fatty acyl-AMP ligase and one of the amino acid adenylation domains (Asn/Gln) show extended substrate specificity. Our results provide the first insight into the biosynthesis of frequently occurring β-amino fatty acid lipopeptides in cyanobacteria, which may facilitate analytical assessment and development of monitoring tools for cytotoxic cyanobacterial lipopeptides in general. As a starting point for such efforts, we provide a preliminary comparison of related lipopeptide biosynthetic pathways and their components.

Keywords: biosynthetic pathway; cyanobacteria; *Cylindrospermum*; cytotoxicity; lipopetide

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A study of two medicinally important plant extracts of the genus *Lippia* against two predominant uropathogens

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Medicinal plants are generating an ever increasing interest due to their effectiveness, low cost and minimum side effects associated with drugs derived from them. In the present investigation, two common medicinal plants of the genus *Lippia*, namely *L. nodiflora* and *L. geminata* were selected to check their antibacterial activity against two epidemiologically studied most prevalent urinary tract infecting bacteria of Eastern India – *E.coli* and *Klebsiella pneumoniae*. To check the antibacterial effect, the leaves of above plants were collected, washed properly with water and were dried in shade for 3 weeks. The leaves were grinded in a motorized grinder. Two grams of powdered leaves were suspended in 10 ml ethanol, 70% methanol, acetone and hot water separately and kept in a rotary shaker for 24 hours at 37^{o}C. Finally the extracts were filtered and were checked for their antimicrobial activity following standard disc diffusion method. The ethanolic extract of both the leaves exhibited significant antimicrobial effect against both the clinical isolates. Synergistic effect of these leaf extracts with different solvents and a broad spectrum antibiotic (streptomycin) were also checked. Minimum Inhibitory Concentrations (MIC) was measured for each leaf extract with the ethanol. Silver nanoparticles were prepared with the ethanolic plant extract and their antimicrobial activity was also checked.

Further purification of the extracts will be needed for the isolation of the pure compounds which can be helpful for the better understanding of their pharmacological potentialities. These compounds can be utilized for drug designing or drug remodeling.

Keywords: antimicrobial activity; plant extract; urinary tract infection.

Antagonistic activity of lactic acid bacteria against selected pathogenic and spoilage bacteria

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Over past decades, lactic acid bacteria have been extensively studied for their health-promoting effects and have been successfully used to control gastro-intestinal diseases. Lactic acid bacteria can produce numerous antimicrobial components such as organic acids, hydrogen peroxide, carbon peroxide, diacetyl, bacteriocins, as well as adhesion inhibitors, which strongly affect microflora.

The aim of this study was to determine the antibacterial properties of three probiotic bacteria: *Lactococcus lactis 12, Lactobacillus acidophilus G14* and *Lactobacillus bulgaricus P1* against pathogenic and food-contaminating bacteria.

Lactococcus lactis strain denoted as 12 was isolated from cherry blossom, *Lactobacillus acidophilus G14* from apple blossom and *Lactobacillus bulgaricus P1* from kefir following the traditional methods. The inhibitory activity of these bacteria against *Bacillus subtilis, Escherichia coli, Mycobacterium citreum, Mycobacterium rubrum, Sarsina flava* and *Staphylococcus aureus* was studied.

To compare the antagonistic activity of lactic acid bacteria against the pathogenic cultures, the index of antagonistic activity (IAA) was determined. Titratable acidity of lactic acid bacteria was measured according to Alonso-Calleja et al. (2002).

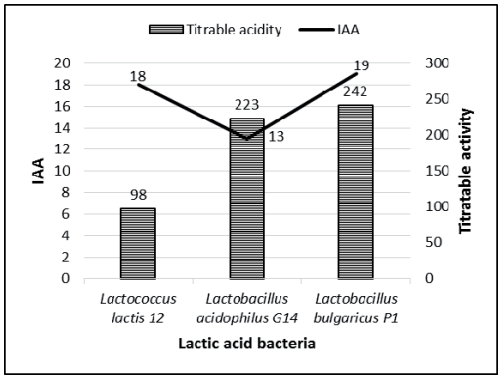

Figure 1. Antagonistic and acidifying activity of lactic acid bacteria

All lactic acid bacteria have showed the inhibition ability in relation to pathogens and food-contaminating bacteria.

Lactococcus lactis 12 and *Lactobacillus bulgaricus P1* inhibited the germination of pathogenic bacteria, especially *Bacillus spp.* spores. However, *Lactobacillus acidophilus G14* showing relatively high acidifying activity had a lower inhibitory activity than *Lactococcus lactis 12* that had lower titratable acidity.

According to obtained results, the level of TAA and titratable acidity were different among isolated probiotic bacteria. It is explained with that lactic acid bacteria inhibit selectively the growth of many pathogenic and spoilage microbial species and their activity depend not only on the species and strain, but also on various factors such as temperature, pH and the chemical composition of the medium.

Keywords: lactic acid bacteria; antagonistic activity; acidifying activity; pathogenic bacteria; food-contaminating bacteria

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Anti-staphylococcal activity of *Callistemon lanceolatus* (Sm.) Sweet. leaf extract

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Staphylococcus aureus is a critical pathogen causing hospital and community acquired infections which are widely involved in minor to severe infections. The pathogen has a wide range of virulence factors and can cause infection at many anatomical sites. A major problem is the increasing of antibiotic resistant strains resulting in the difficulty for treatment. Therefore, alternative approaches are necessary and natural products especially plants may provide a promising remedy. *Callistemon lanceolatus* leaves were collected from Songkla Province, Thailand. The leaves were dried in an oven at 60°C for 48 h. The ground leaves were extracted with 95% ethanol for 7 days and evaporated using rotary evaporator. Percentage yield of the ethanol extract of *Callistemon lanceolatus* was 5.2%. The ethanol extract was evaluated for its antibacterial activity against *S. aureus* ATCC 25923 and methicillin resistant-*S. aureus* (MRSA). The extract exhibited very good antibacterial activity against tested *S. aureus*. The inhibition zones on *S. aureus* ATCC 25923 and MRSA NPRC 1001 were 17 and 12 mm, respectively. The minimal inhibitory concentration (MIC) of the extract against both strains was 16 µg/ml with similar values of the minimum bactericidal concentration (MBC). Time-kill study was further assessed at 0.5MIC, MIC, 2MIC, 4MIC, and 8MIC by counting viable bacterial cells after time intervals of treatment. At 4MIC, the viability of treated *S. aureus* ATCC 25923 and MRSA NPRC 1001 declined by at least 3 log fold within 12 and 8 h, respectively. This finding challenges the use of the extract from *Callistemon lanceolatus* as an alternative agent for *S. aureus* infection. More research on antibacterial mechanisms and toxicity should be thoroughly investigated for drug development.

Keywords: *Staphylococcus aureus*; *Callistemon lanceolatus*; antibacterial activity

Anti-tuberculous activity of treponemycin produced by *Streptomyces* strain MS-6-6 isolated from Saudi Arabia

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A *Streptomyces* strain MS-6-6 with promising anti-tuberculous activity was isolated from soil sample in Saudi Arabia. In addition, it has promising broad spectrum of antimicrobial activity (antibacterial and antifungal). According to the cultural and physiological properties, the isolate was identified as a member of the genus *Streptomyces*. The nucleotide sequence of the 16S rRNA gene (1,426 bp) was aligned with all presently available 16S rDNA gene sequences in the GeneBank databases (Accession no. NR 044139.1). Phylogenetic analysis using the 16S DNA gene sequence suggests the strain is similar to *Streptomyces mutabilis.* A bioactivity-guided approach was applied to isolate and elucidate the bioactive constituent responsible for anti-tuberculous activity from the tested strain. According to the obtained results, only one compound coded MYA-3 showed anti-tuberculosis activity. The structure of the isolated compound MYA-3 was determined by comprehensive analyses of its 1D and 2D NMR as well as HRESI-MS. The compound is belonging to polyketide macrolide group and was identified as treponemycin**.** Evaluation of the anti-tuberculous activity of the isolated compound MYA-3 was carried out by Resazurin Microtiter Plate Assay against *Mycobacterium tuberculosis* ATCC 25177. The results confirmed the promising activity of the isolated compound (treponemycin) with MIC value of 4.15 ug/ml.

Key words: *Streptomyces*; Anti-tuberculous activity.

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Antibacterial activity of Algerian *Punica granatum* Linn. extracts (Juice, pericarp and seed) against clinical isolates of *β*-lactamase producing methicillin resistant *Staphylococcus aureus* and extended-spectrum beta-lactamase ESBL-producing Enterobacteriaceae

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Punica granatum is ethnomedicinal important plant and depicted ameliorating medicinal value which used for the treatment of various diseases. It is an important medicinal plant in Algeria. The present study deals with the evaluation of the *in vitro* antimicrobial potential of four different extracts of Algerian *Punica granatum* Linn by using agar diffusion methods and minimum inhibitory concentration (MIC)-determination. Moreover, the extracts were investigated for their polyphenolic content. The quantitative assays of total polyphenols by the Folin-Ciocalteu test revealed the richness of the different pomegranate extract by polyphenol, content varies from 18.69 to 116.1mg GAE/100g of fruit this value. The phenolic compounds were abundant in acetone, methanolic and aqueous extracts. It's depends on the polarity of the solvent and the extraction method. According to the results of the aromatogramme, all the phenolic extracts showed antibacterial effect against all extended-spectrum beta-lactamase ESBL-producing Enterobacteriaceae with a strong inhibitor with MIC <500µg/ml power. This effect is especially marked by the pericarp extract against *Escherichia coli, Citrobacter freundii* and *Klebseilla pneumoniae* . On the other hand the extract *Punica granatum* (pericarp and juice) showed a broad-spectrum of antibacterial activity with an inhibition zone size of 11 mm to 29 mm, against *β*-lactamase producing methicillin resistant *Staphylococcus aureus* strains.

Keywords: *Punica granatum,*, Enterobacteriaceae producing β-lactamase, polyphenols, Antimicrobial activity, *Staphylococcus aureus*, MRSA, extended-spectrum beta-lactamase ESBL-producing Enterobacteriaceae.

Antibacterial activities and GC-MS analysis of phytocomponents of *Ehretia abyssinica* R.Br. ex Fresen

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The *Ehretia abyssinica*, a small tree, belong to *Boraginaceae* family is traditionally known for its medicinal properties. The present study was therefore carried out to investigate the antibacterial activities and to unveil the Phytochemicals of the bioactive components in the leaves extract of this plant species. The antimicrobial activities of leaves extract was investigated against 7 medically important bacterial strains, namely *Bacillus subtilius*, MRSA, *Micrococcus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsella pneumoniae*. The antibacterial activity was determined using agar well diffusion method. The most susceptible bacteria to this extract was *Pseudomonas aeruginosa,* followed by *Staphylococcus aureus*, while the most resistant bacteria was *Micrococcus*. GC-MS analysis revealed that the ethanol leave extract of *Ehretia abyssinica* contained mainly Octadecenamide (5.77%); Lucenin 2 (5.46%); Docosane and Nonacosane (3.75%); Cyclopropene (3.50%); Hematoporphyrin (2.68%); Tetratetracontane (2.36%); Dotriacontane (1.57); Acetic acid (1.53); Nmethylglycine (1.49%); Propyne antimicrobial (1.41%). All identified compounds are known to have antimicrobial activity.

Keywords: *Ehretia abyssinica*, antimicrobial activities, phytochemicals, GC-MS analysis.

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Antibacterial activities of low molecular weight chitin prepared from shrimp shell waste

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Chitin is a long-chain polymère of N-acetyl glucosamine found in many places throught the world. It has proven useful for several medecinal and industrial purposes. In the present study, chitin was chemically extracted from shrimp shells obtained from a seafood restaurant. It was confirmed that all shells were from a single species of shrimp *Parapenaeus longirostris* (Lucas, 1846). The obtained chitin was depolymerized by HCl to prepare low molecular weight chitin. Then, low molecular weight chitin was characterized. Further, antibacterial activities of low molecular weight chitin were tested with four Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 43300, *Bacillus subtilis* and *Bacillus cereus*) and seven Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium*, *Vibrio cholerae*, *Shigella dysenteriae*, *Prevotella melaninogenica* and *Bacteroides fragilis*). The results indicate that low molecular weightchitin exhibited a bacteriocid effect on all bacteria tested.

Further studies are necessary to determine the *in vivo* activities and applications of chitin and derivatives, in particular, in the design of new lines of drugs for use in the treatment of bacterial diseases and hopefully eradication.

Keywords: antibacterial activity; low molecular weight chitin, shrimp shell waste.

Antibacterial activity of ethanolic extracts of *Astronium* sp loaded or not loaded into nanostructured systems

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Medicinal plants have great relevance in view of the use of active substances as prototypes for the development of drugs and as a source of pharmaceutical raw material[1]. In this context, researchers have evaluated the biological potential of various plant species from the Brazilian Cerrado. Among these plants *Astronium* sp presents the species *A. urundeuva* (aroeira-do-sertão), *A. graveolens* (guaritá) and *A. fraxinifolium* (Gonçalo-alves), popularly used as anti-inflammatory, antimicrobial, anti-ulcerogenic and healing agent[2]. The use of nanotechnology systems, such as microemulsions, are considered the ultimate tool to reduce the toxicity and increase the bioavailability of drugs. The minimum inhibitory concentration (MIC) of the plant extracts (1000 to 7.81 µg/mL) was determined by microdilution technique against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella setubal* ATCC 19196 at a concentration of 10^7 UFC/mL[3]; ampicillin was used as a control (50 µg/mL) and the visual reading was performed with the developer resazurin (0.01%). The extracts were loaded into two systems: system 1 [10% oil phase (cholesterol), 10% surfactant (Brij 98® and soybean phosphatidylcholine - 2:1) and 80% aqueous phase (phosphate buffer pH=7.4)]; system 2 [10% oil phase (cholesterol), 10% surfactant (Brij 58® and soybean phosphatidylcholine - 2:1) and 80% aqueous phase (phosphate buffer pH=7.4 and Polaxamer 470® - 0.5%), both prepared by sonication (amplitude of 10 and 15%, respectively; power 700 watts). The results showed that after loading the leaves of *A. graveolens* into the system 2, the antibacterial activity against *S. aureus* was enhanced (MIC 250 µg/mL for free extract and MIC 7.81 µg/mL for loaded extract into the system 2). In addition, the leaves of *A. urundeuva* when loaded into the system 2 showed a MIC of 1.95 µg/mL, while it had showed a MIC of 125 µg/mL before loading the extract into the system. This study can conclude that the incorporation of these plant extracts into the system 2 are able to enhance the antibacterial activity against *S. aureus* and suggests that the use of nanotechnology can be used as an effective alternative therapy with lower doses.

Keywords: Antibacterial activity; *Astronium* sp; Nanostructured systems.

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Antibacterial activity of *Manilkara rufula* (Miq.) H. J. Lam. (Sapotaceae): an endemic specie of Northeastern Brazilian flora

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The increase in bacterial resistance to commercial antibiotics resulted in a growing search for new antibacterial compounds active against pathogenic bacteria. Brazilian plants deserve special attention because of their high level of endemism, taxonomic diversity and biomolecular richness, however they have not been studied for their pharmacological potential. Among the Brazilian species, we highlight the Massaranduba tree (*Manilkara rufula*), which is popularly known as "Massaranduba do sertão" belonging to the Sapotaceae family, widely distributed in the semi-arid regions of Northeast of Brazil, which comprises part of the Minas Gerais, Bahia, Sergipe, Alagoas, Pernambuco, Paraíba, Rio Grande do Norte, Ceará and Piauí states. This plant is popularly used in the ethnobotany as vermifuge, antipyretic and cicatrizant. There have been a number of reports that demonstrate the antibacterial activity of Sapotaceae, and only limited information were available from Sapotaceae of northeastern from Brazil. The objective of this study is to investigate the antibacterial properties of extracts (chloroform, ethyl acetate, methanol and aqueous) of *M. rufula* leaves against strains of Gram-positive pathogenic bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa). *M. rufula* leaves were collected at Catimbau National Park, state of Pernambuco, Brazil, in March 2013. The samples were deposited in the Herbarium of Instituto Agronômico de Pernambuco (IPA) and registered under the numbers 86769 and 86770. The leaves were briefly washed with distilled water and left to dry at 37 ^{o}C for 3 days, and after were powdered. Hundred grams of the powered samples were packed in muslin cloth and used for obtainment of extracts into soxhlet apparatus using solvents in eluotropics series chloroform, ethyl acetate, methanol and aqueous at a temperature below the boiling temperature of each solvent. All samples were refluxed until saturation (24 h), filtered and solvents were pulled out separately concentrated to dryness in a rotary evaporator at 45 ºC under reduced pressure. The residues were dissolved in sterile dimethylsulfoxide (DMSO-9:1) in 50 mg/ml concentration. The extracts were filtered using 0.22 micro filter (Type GV- Millipore) and used for antimicrobial activity study. All bacteria were isolated from medical material and provided by Departamento de Antibióticos (DA), Universidade Federal de Pernambuco (UFPE). Gram-positive bacterial strains: *Bacillus subtilis* (UFPEDA-86), *Staphylococcus aureus* (UFPEDA-02) and *Micrococcus luteus* (UFPEDA-100) and Gram-negative (*Escherichia coli* (UFPEDA-224), *Pseudomonas aeruginosa* (UFPEDA-416) were maintained in Difco- Nutrient Agar (NA) and stored at 4 ºC. Minimum inhibitory concentration (MIC) was determined by the broth dilution methods. Dilutions were prepared in a 96-well microtiter plates to get final concentrations ranging from 0 to 50 mg/ml, and Chloraphenicol was used as positive control. The results showed that methanol extract showed the highest inhibitory activity against *M. luteus* (MIC 0.78 mg/mL) and *S. aureus* (MIC 1.56 mg/mL) followed by ethyl acetate against *M. luteus* (MIC 0.78 mg/mL) and *S. aureus* (MIC 3.12 mg/mL). Chloroform and aqueous extracts were found totally inactive against all the pathogens tested. The beneficial effects of plant materials typically result from the secondary metabolites present in plants; even it is usually not attributed to a single compound but to a combination of the metabolites. *M. rufula* leaf extracts have great potential as antimicrobial agents, of low cost, suggesting possible applications for pharmacological purposes. Thus, it leads to the establishment of new compounds which are used to formulate more potent antimicrobial drugs from nature with fewer side effects. However, further studies are required to develop new pharmaceutical formulations based on these extracts efficient against these pathogens.

Keywords: Antibacterial; extracts ; *M. rufula.*

Antibacterial activity of multiple plant essential oils and their potential use as food preservatives

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It is widely understood, that plant essential oils (EO) exhibit inhibitory potential against foodborne pathogen bacteria, which has put them into focus of intensive research regarding food preservation and safety. Nonetheless there is still very fragmentary information on the antimicrobial potency of essential oils from various plant genera. Due to the variety of EO containing plants, the use of inconsistent methods for antimicrobial susceptibility testing and the fact that information on non-antimicrobial EOs often remains unpublished, it is challenging to adjust penetrative microbiological research on less investigated EOs. Therefore it is necessary to extend data on antimicrobial activity of plant EOs considering standardized microbiological examination as well as chemical characterization. This study should provide additional data in means of minimal inhibitory concentrations (MIC) of a multitude of plant EOs against food borne pathogen bacteria (i.e. *Escherichia coli, Staphylococcus aureus*) acquired by an accepted microdilution assay for antimicrobial susceptibility testing (CLSI standard), without the influence of organic solvents or emulsifying agents. Building a consistent database, aggregating information on antibacterial active and non-active plant essential oils, will help to identify the most promising EOs regarding further investigations on their use as plant-derived natural food preservatives.

Keywords: antimicrobial activity; essential oils; foodborne pathogen bacteria; food safety; food preservation

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Antibacterial activity of *Thymus vulgaris* against different strains of antibiotic resistant *Staphylococcus aureus*

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Staphylococcus aureus is a facultative anaerobic Gram-positive coccal bacterium which is one of the major resistant pathogens. It found on the mucous membranes and the human skin. It is extremely adaptable to antibiotic pressure, also the wide spread use of antibiotics is playing a significant role in the emergence of resistant bacteria. The present study aimed to investigate antibacterial activity of thyme against 8 resistant *Staphylococcus aureus* isolates. Five strains were methicillin-resistant, while and all strains were resistant to vancomycin, oxacillin and ceftazidime. The minimum inhibitory concentration (MIC) of ethanolic extract of *Thymus vulgaris* was measured using well diffusion and macro dilution broth method. The result indicated that MIC of ethanolic extract of *Thymus vulgaris* was different and 3 strains showed more susceptibility to ethanolic extract and the obtained MIC for them was lower than the others. The Mic of *Thymus vulgaris* ethanolic extract was detected 0.125 mg/ml of medium.

From our data we concluded that *Thymus vulgaris* has a good activity against antibiotic resistant *Staphylococcus aureus* strains.

Keywords: Staphylococcus aureus**,** Thymus vulgaris**,** Antibiotic resistance

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Antibacterial and anti-biofilm forming capacity of ophiobolin-A produced by *Bipolaris* species

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Ophiobolins are secondary metabolites produced mainly by phytopathogenic fungi. These sesterterpenes are characterized with an unusual tricyclic skeleton. More than 25 ophiobolin species have been described in the literature possessing a broad inhibitory activity against viruses, bacteria, fungi and nematodes [1, 2, 3]. In this study the antibacterial effect of ophiobolin-A on planctonic cells and biofilms of Gram negative and positive bacteria was investigated.

Ophiobolin-A (OPA) was extracted from *Bipolaris oryzae* SZMC 13003 (Szeged Microbiological Collection). Fungal culture after 12 days cultivation at 28 ^{o}C was filtered then the filtrate was extracted two times with equal volumes of ethyl acetate (EtOAc). The pooled material was evaporated to dryness and the crude extract was fractionated by semipreparative silica gel column chromatography. The resulting sub fractions were combined and further purified to 95.3% purity. The obtained OPA was used to determine antibacterial action against *Bacillus subtilis*, *Bacillus cereus, E. coli* and *Pseudomonas putida.*

The bacteria were maintained on LB or TGE medium at optimum temperatures (37 ^{o}C for *E. coli*; 30 ^{o}C for the bacilli and 25 ^{o}C for *P. putida*). Antibacterial effect of ophiobolin-A was observed in microtiter plates containing ophiobolin-A in the concentration range of 3.75 µg/ml to 120 µg/ml. The wells were inoculated with a bacterial suspension of 10^5 CFU/ml and absorbance was measured after 24 h cultivation. MIC values were determined as the concentration where the absorbance of the treated culture was ≤ 10% of the non-treated culture. For biofilm inactivation, wells containing the appropriate medium were inoculated with a bacterial suspension of 10^8 CFU/ml. The cells were allowed to attach to the surface for 4 h. Non-adherent cells were then removed and fresh medium was added with ophiobolin-A in MIC/2 concentration. After 24 hours, biofilms were stained with crystal violet and absorbance was measured at 590 nm. Inhibition of biofilm formation was determined by the equation: Inhibition (%) = $(1- (A_{treated}/A_{non\text{-}treated})) \times 100$. The experiments were repeated at least two times and six parallel measurements were made each time.

MIC values for the Gram positive *B. cereus* and *B. subtilis* were 30 and 45 µg/ml. Although according to literature data ophiobolins have limited or no effect on Gram negative bacteria, the MIC value for *P. putida*, 32 µg/ml, was in the range of the MICs of Gram positive ones. *E. coli* was the most insensitive strain with a MIC of 120 µg/ml. Biofilms of the two Bacillus strains were only slightly affected: inhibition of biofilm formation was less than 10% in both cases. Surprisingly, *E. coli* biofilm was more sensitive than the *Bacillus* biofilms; OPA caused 24.7% inactivation. Biofilms of *P. putida* were not affected.

Concluding our results, OPA had good growth inhibitory effect against Gram positive and also against Gram negative bacteria, but had very limited or no effect on biofilm formation of the investigated species.

Keywords: ophiobolin A; Bipolaris oryzae; biofilm; antibacterial effect.

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Antibacterial and antibiofilm activity of Antarctic lichens against species of fish pathogen *Vibrio*

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Vibrio plays an important role in the marine ecosystems biodegrading the organic matter; nevertheless, also can act as opportunistic pathogens for aquatic organisms causing diseases like vibriosis [1], which is treated and controlled through the use antibiotic; however treatments are limited by the emergence of strains resistant to the main antimicrobial used in aquaculture and by the ability of bacteria to grow as biofilm, decreasing the effectiveness of antibiotics [2]. Nowadays due to the shortage of active molecules, there is an urgent necessity to find new natural compounds with antibacterial and antibiofilm activity against these pathogen microorganisms. Lichens have been recognized capable of producing a large amount of secondary metabolites with biological activity [3], many of them are active against Gram-positive and Gram-negative bacteria [4]. For this reason in this work the antibacterial and antibiofilm activity of total extracts of 3 species of Antarctic lichens was assayed against different species of Genus *Vibrio*.

Antarctic lichens *Himantormia lugubris* (HL)*, Usnea auriantiaco-atra* (UAA) and *Usnea antarctica* (UA) collected from Fildes Peninsula, King George Island, were used to prepare the methanolic extracts. Antimicrobial activity was measured by an agar diffusion method upon strains of *V. anguillarum* (2)*, V. ordalii* (1)*,* and *V. splendidus* (3). Additionally, minimal inhibitory (MIC) and minimal bactericidal concentrations (MBC) of pure compounds (usnic acid, atranol and a b-orcinol depsidone) purified from lichen extracts were also determined. The specific biofilm formation (SBF) activity of methanolic, acetonic and aqueous extracts of lichens was investigated againt a strain of *V. anguillarum* 3276 isolated from ill fishes in the South of Chile.

Methanolic extracts of HL and UAA were active against all strains assayed; however extracts of HL were significantly more actives upon *V. splendidus* and *V. ordalii*. Extract of UA was only active upon *V. ordalii*. The MIC of atranol and a b-orcinol depsidone ranged between 50 – 100 mg/L, and their MBC was 100 and > 100 mg/L, respectively, upon all strains of *Vibrio* spp. assayed. The MIC of usnic acid varied between 12.5 – 100 mg/L, and the MBC between 50 - >100 mg/L, being a little more active against the strain of *V. ordalii.* On the other hand, the results also revealed that methanolic extracts of HL and UA had the best antibiofilm activity diminishing the biofilms formation in 90 % and 86 %, respectively.

We conclude that extracts of Antarctic lichens and their secondary metabolites could be used to develop new antibacterial and antibiofilm compounds useful to control the growth and the biofilm formation of the fish pathogen *Vibrio* spp.

Keywords: vibriosis, antibiotic, biofilm, lichens, Antarctica

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Antibacterial and antibiofilm properties of tryptoquivalines and meroditerpenes isolated from marine-derived and soil fungi of the genus *Neosartorya*

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In a time that new effective antibiotics are needed, natural sources are becoming more and more explored for the search of antibacterial compounds [1]. Both terrestrial and marine-derived fungi of the genus *Neosartorya* have been reported as a very interesting source of many bioactive compounds [2]. In this study, a new meroditerpene, sartorypyrone C (**5**), was isolated, together with the known tryptoquivalines L (**1a**), H (**1b**), F (**1c**), 3′-(4-oxoquinazolin-3-yl) spiro [1*H*-indole-3,5′]-2,2′-dione (**2**) and 4(3*H*)-quinazolinone (**3**), from the culture of the marine sponge-associated fungus *Neosartorya paulistensis* (KUFC 7897). Fractions from a previous study of the culture of the diseased coral-derived fungus *N. laciniosa* (KUFC 7896) were reexamined leading to the isolation of a new tryptoquivaline derivative tryptoquivaline T (**1d**). Compounds **1a**–**d**, **2**, **3**, and **5**, together with aszonapyrones A (**4a**) and B (**4b**), chevalones B (**6**) and C (**7a**), sartorypyrones B (**7b**) and A (**8**), were tested for antibacterial activity against Gram-positive and Gram-negative bacteria, including environmental multidrug-resistant isolates. Aszonapyrone A (**4a**) and sartorypyrone A (**8**) exhibited significant antibacterial activity as well as synergism with antibiotics against the Gram-positive multidrug-resistant strains. Antibiofilm assays of aszonapyrone A (**4a**) and sartorypyrone A (**8**) showed that practically no biofilm was formed when these compounds were present at their MIC. In conclusion, the meroditerpenes aszonapyrone A (**4a**) and sartorypyrone A (**8**) are two promising antibacterial agents, especially against Gram-positive bacteria, with the potential to become a new class of antibiotics.

Keywords: antibacterial; antibiofilm; tryptoquivalines; meroditerpenes; *Neosartorya*; marine-derived fungi

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Antibacterial and cytotoxicity evaluation of alkyl gallates and a possible mechanism of action

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Gallic acid is an intermediate component of plant metabolism and together with its analogs, has been associated with a wide variety of biological actions including antibacterial [1], antioxidant [2], antifungal [3], and antiviral [4]. The antibacterial activity in *Xanthomonas citri* subsp *citri* (Xac, Gram negative) a plant pathogen and the causal agent of citrus canker, a severe disease that affects the most important species of citrus worldwide, and *Bacillus subtilis* (Gram positive) were evaluated and the artificial inoculation of citrus with Xac pretreated with alkyl gallates showed that the bacterium loses the ability to colonize its host, which indicates the potential of these esters to protect citrus plants against Xac infection. Alkyl gallates induced altered cell morphology in Xac such as cell length increase, and investigations of the possible intracellular targets using Xac strains labeled for septum and centromere pointed to a common target involved with chromosome segregation and cell division [5]. Here, we present data on citotoxic potential of alkyl gallates as their possible mechanism of action in *B. subtilis*.

Alkyl gallates were also active against *B. subtilis* and the FtsZ localization (cell division protein) is rapidly perturbed after the treatment, which suggested that the compounds may target the cell division machinery. When studied *in vitro*, the alkyl gallates affected FtsZ by forming structures that could easily be spun down at high velocity, independent of the presence of nucleotide. These structures seem to be specific since the BSA (bovine serum albumin) protein did not sediment in the presence of the alkyl gallates. Also, GTP hydrolysis, an indicator of FtsZ dynamics, was inhibited by the alkyl gallates. The cytotoxic potential of the drugs were verified using a hepatocarcinoma cell line HepG2. Combined, these data indicate that alkyl gallates are a good candidate to treat bacterial infections and probably have the cell division machinery as a target.

Keywords: alkyl gallates, *Xanthomonas citri* subsp. *citri, Bacillus subtilis*, cell division,

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Antibacterial effect of 7α-acetoxy-6β-hidroxyroyleanone from *Plectranthus grandidentatus*

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In the last years a large number of phytochemical studies were carried out in *Plectranthus* species due to the presence of significant amounts of metabolites with bioactivity [1]. *Plectranthus* species are rich in diterpene compounds that demonstrated antibacterial activity, namely against multi-resistant strains (MRSA) of *Staphylococcus aureus* [2]. Royleanones are diterpenes with an abietane scaffold, which are associated with potent antibacterial activity against Gram-positive bacterial strains with MIC values between 3.12-15.63 μg/mL [3]. The goal of the present work was to optimize the extraction of 7α-acetoxy-6β-hydroxyroyleanone in *P. grandidentatus,* through the quantification of this compound using different extraction methods. In parallel, an exploratory study of the antibacterial mechanism of 7α-acetoxy-6β-hydroxyroyleanone on MRSA bacteria strains was undertaken.

Air dried and powdered plants of *P. grandidentatus* were subjected to several extraction methods (decoction, infusion, microwave and ultrasonic irradiation) to obtain different aqueous extracts. Acetone extracts through maceration, ultrasonic methods and supercritical fluid (SCF) extraction were also prepared. The quantification of 7α-acetoxy-6β-hydroxyroyleanone in all the extracts was performed by HPLC-DAD. The antibacterial effect of this royleanone was studied by analysis of the bacterial growth of *S. aureus* MRSA CIP 106760, subject to three different concentrations of the compound (2×MIC, MIC and MIC/2) and, by observation of the morphology of the bacteria using scanning electron microscopy (SEM).

The quantification of 7α-acetoxy-6β-hydroxyroyleanone by HPLC showed that the highest yield was obtained for the SCF extract (57.35 $\mu g.mg^{-1}$ of extract). Acetonic extracts (ultrasonic and maceration) resulted in a lower amount of 7α-acetoxy-6β-hydroxyroyleanone with 8.04 $\mu g\ mg^{-1}$ and 9.77 $\mu g\ mg^{-1}$, respectively. In all aqueous extracts a lesser quantity of 7α-acetoxy-6β-hydroxyroyleanone ($\leq 2\ \mu g\ mg^{-1}$ of extract) was found. The antibacterial activity of 7α-acetoxy-6β-hydroxyroyleanone was evaluated by monitoring the bacterial growth curve for 200 min from the addition of the compound to the growth medium. Bacterial growth was arrested in the presence of the diterpene at the different tested concentrations. Bacteria treated with 7α-acetoxy-6β-hydroxyroyleanone showed morphological changes when compared with the untreated control.

In conclusion, the SCF extraction is the best method to obtain the highest amount of 7α-acetoxy-6β-hydroxyroyleanone. Other methodologies are under study to unveil the mode of action of 7α-acetoxy-6β-hydroxyroyleanone against resistant bacteria strains. These studies are essential to improve the antibacterial activity of this diterpene through the preparation of new royleanone-derivates by a hemi-synthetic approach.

Keywords: *Plectranthus grandidentatus*; SEM; antibacterial activity; 7α-acetoxy-6β-hydroxyroyleanone.

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Antibacterial Effects of Extracts of Two Types of Red Sea Algae

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The aim of this study was to detect some of these compounds and examine their impact on the enteric bacteria; *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*. Bacteria were tested with methanol, ethanol, petroleum ether, or dimethyl formamide extracts of *Turbinaria triquetra* and *Halimeda opuntia*. Bacterial growth inhibition, the minimal inhibitory concentrations (MICs), and potassium leakage, and analyzed the bacterial cells with scanning electron microscopy and energy-dispersive X-ray spectroscopy were measured. The *T. triquetra* extract produced with methanol strongly affected the bacteria tested. When the results for *T. triquetra* and *H. opuntia* were compared with those of omacillin, the *T. triquetra* and *H. opuntia* extracts in most solvents were more effective than the antibiotic. Differences in the bacterial growth inhibition and MICs depended on the type of alga and the solvent used. At the end of the incubation period, potassium leakage had increased by 62.98% for *E. coli*, 61.24% for *S. typhi*, 61.32% for *S. dysenteriae*, 64.02% for *K. pneumoniae*, and 63.10% for *E. aerogenes* when treated *T. triquetra*. *Turbinaria triquetra* extracted with methanol strongly affected the growth of the bacteria tested. Therefore, it is a potential source of natural antibacterial compounds.

Keywords: Bacterial growth, Inhibition, *Turbinaria triquetra*, *Halimeda opuntia*, omacillin.

Antibacteria properties of *Aframomum danielli* fractions on two food borne pathogens

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Introduction: *Aframomum danielli* spice belongs to the Zingiberace family, also called African cardmon is known for its antimicrobial properties. The objective of this study was to assess the antimicrobial activities of the fractionated components of the spice on S. *aureus* and B.cereus.

Materials and methods: *A .danielli* seeds was extracted with petroleum ether and further with ethanol by soxhelt extraction and fractionated by vaccum liquid chromatography. Through thin layer chromatography, eight fractions were identified. The antibacterial properties of the fractions on two pathogenic microorganism was determined by agar diffusion method. Incubation of plates was 37^0C for 24 hours. Zones of Inhibition and Minimum Inhibitory concentrations for the microorganisms were also determined (APHA, 2001).

Results: Fractions of *A .daniiell* showed antimicrobial activity from 100ug/ml-800ug/ml on *S. aureus* and 400-800ug/ml on *B. cereus*. Zones of inhibition were in the range of 25-28mm for *S. aureus*. Fractions F5, F6 and F7 had a MIC of 100ug/ml *for S. aureus* and Fractions F4 to F8 had MIC of 400 from *B. cereus*.

Conclusion: With the potency of *A. danielli o*n some food borne pathogen, the spice will find application in food preservation.

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Antibacterial ,antioxidant activity and phytochemical screening of Rhus Leptoditya plant

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Introduction: Many medicinal plants produce a variety of compounds of knows therapeutic properties. Higher plants are still regarded as potential source of new medicinal compounds. They offer a wide range of natural antioxidants due to the structural diversity of their secondary metabolites. Secondary metabolites are often involved in key interactions between plants and their antibiotic environments that influence them(Survewaran et al, 2006).

Rhus species is a genus that consists of 250 species. It is commonly known as sumac or sumac. These plants are found to be individual species of flowering plants in the family *Anacardiceae*. Some of them were used as herbal medicines. Rhus is deciduous or evergreen shrubs and shrubby trees. The leaves of sumac or rhus species are three leaflet and they have thin texture (Kuhnlein and Receveur, 1996).

Methods: A: extraction: The fine powdered materials of *Rhus* l*eptodictya* were extracted with five different solvents of increasing polarity.
B:Antioxidant activity ;The antioxidant assay or free radical scavenging was done using the 2,2- diphenyl-picrylhydrazyl (DPPH).
C:Phytochemical screening:
Chemical tests for the screening and identification of bioactive chemical constituents were carried out using extracts as well as powder by implementing the standard procedures described by Savithramma, et.al 2011. The test were done to determine the presence or absence of active constituents such as carbohydrates, protein, phenols, flavonoids, saponins and tannins as given below. The tests were done in triplicates.

Results:

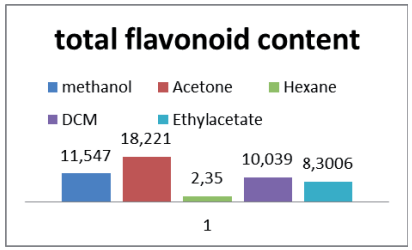

Figure1:total flavonoid content

Table 1: Relative allocation and amount of resources in research

	Amount of Resources	Allocation Freedom
Industry	high	constrained
Academic	Low	unconstrained

Discussion & Conclusions: the results of the present study showed that the acetone extract contain the high amount of flavonoids and it exhibit the great antioxidant activity, all extract in this research exhibit different extent of antioxidant activity. (Pourmorad F, 2006) .

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Antifungal activity of aromatic waters distilled from thyme and sage

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Aromatic waters result from the aqueous phase obtained during the extraction of essential oils from medicinal plants. They are known for the content of water-soluble volatile components that confer particular properties, such as antimicrobial activity. In this study two aromatic waters, resulting from distillation of thyme and sage, were assayed for their antifungal activity against plant pathogens *Rhizoctonia solani* and *Sclerotinia minor*. To evaluate the ability of these co-products to inhibit growth of fungal plant pathogens, *in vitro* plate tests were performed. Thyme and sage aromatic waters were separately tested by submerging a plug (5mm in diameter) removed from the edge of the growing mycelia, in aromatic waters. After overnight incubation at 25 °C, the plug was transferred in the centre of a PDA Petri plates (diameter 90 mm). Control plates were prepared without the addition of the tested aromatic waters. For each treatment, plates were inoculated in triplicate and incubated at 25 °C. The diameter of the mycelia was measured daily until fungi reached the edge of control plates; data were expressed as growth rate (mm d^{-1}). Assays evidenced antimicrobial activity of aromatic waters that reduced the *in vitro* growth rate of the pathogens. Sage water showed highest inhibition than those of thyme. These results are promising for sustainable application in the field of plant disease control.

Keywords: Plant Pathogens; Plant-derived antimicrobials

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Antifungal activity of essential oils from *Mangifera indica* L. cultivars against strains of *Candida* spp.

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Mangifera indica L. (Anacardeaceae) leaves are widely used in folk medicine for many purposes as stomachic, anti-diarrheic and against genito-urinary inflammations, bronchitis and asthmas and in external use, in baths or washes against scabies and syphilis. This work reports the chemical study, and anticandidal activity of leaf essential oil from *Mangifera indica* cultivars Moscatel, Jasmine, Tommy Atkins and Rosa. The essential oils were obtained by hydro-distillation and analyzed by gas chromatography/mass spectroscopy. The anti-*Candida* activity was evaluated against strains isolated from dogs by the agar-well diffusion method and the minimum inhibitory concentration (MIC) by the broth microdilution method. Tommy Atkins cultivar presented *β*–selinene (29.49%), caryophyllene oxide (12.40%) and humulene II epoxide (8.66%) as main constituents, while the main constituents of Rosa, Moscatel and Jasmin varieties are caryophyllene oxide (23.62, 48.42 and 30.77%, respectively) and humulene II epoxide (11.56, 23.45, 16.27%, respectively). The means of inhibition zones were 11 ± 0.71, 13.5 ± 3.54, 10.5 ± 0.71 and 13.5 ± 0.71mm to Tommy Atkins, Rosa, Moscatel and Jasmim varieties, respectively. For Tommy Atkins, the MIC ranged from 0.62 to 1.25 mg/mL; for Rosa, ranged from 0.31 to 1.25 mg/mL; for Jasmim ranged from 0.31 to 0.62 mg/mL; and for Moscatel, the MIC value was the same for all strains of *Candida* spp. Essential oils of four *M. indica* cultivars are active *in vitro* against *Candida* spp. demonstrating good antifungal activity and can be a useful source of antifungal compounds for veterinary medicine.

Keywords: *Mangifera indica*, Essential oil, *Candida* spp., *β*–Selinene, Caryophyllene

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Antifungal activity of essential oils from *Lavandula luisieri* and cineole against *Rhizopus* sp. isolated from strawberry

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The microbiological contamination of strawberry, caused by filamentous fungi, leads to high fruit stock losses during his transport and storage period. *Lavandula luisieri* (Rozeira) Rivas-Martínez is an endemic plant of the southwestern Iberian Peninsula [1] and previous studies showed that *L. luisieri* oil contained several compounds such as 1,8-cineole, lavandulol, linalool and their acetates, also present in other *Lavandula* species, in addition to a series of compounds with a 1,2,2,3,4-pentamethylcyclopentane (necrodane) structure [2]. The use of essential oils from *L. luisieri* to control fungi of spoilage may be a powerful solution. So, the main goal of this work was to test the antifungal activity of essential oils (EO) of *L. luisieri* and cineole against *Rhizopus* sp. isolated from strawberry.

In order to test the antifungal activity of essential oils against *Rhizopus* sp. the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) were determined, respectively, by the broth microdilution method [2] and by the drop plate method onto solid medium [3]. The celular suspensions, $0.4x10^4$ to $5.0x10^4$ spores/ml, were performed according NCCLS [4] and Alizadeh-Salteh [5]. The media used were Potato Dextrose Agar and Sabouraud Dextrose Broth, at a pH value of 3.0. Cineole and six essential oils of *L. luisieri* with different origins were tested.

The MIC values of the six *L. luisieri* essential oils against *Rhizopus* sp. were 0.19% (v/v) for all the six oils, and 0.38% (v/v) for cineole. Concerning the MFC values were the same as those obtained for the MIC, ie 0.19% (v/v) and 0.38% (v/v), respectively for all the six oils and cineole. Essential oils of *L. luisieri* were more effectives in *Rhizopus* sp. inhibition than the cineole. The results showed an effective antifungal activity of essential oils of *L. luisieri*, suggesting that they may be used in the developing formulations of edible films to control strawberry spoilage.

Keywords: Essential oils; *Lavandula luisieri*; *Rhizopus* sp.; Antifungal activity.

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Antifungal activity of methanol extract from mycelium of *Dacryopinax* sp. FB KCCM11084P

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A methanol extract was prepared from mycelium of *Dacryopinax* sp. FB KCCM 11084P (DME) and its antifungal activity was investigated. The minimum inhibitory concentration (MIC) was studied against various fungal stains *including Aspergillus, Fusarium, and Candida species*. DME inhibited successfully the growth of most test fungal strains with relatively higher MIC value than amphotericin B which was used as an antifungal drug. *Asp. flavus* was the most sensitive species with the lowest value of MIC(19 ug/ml) and the other fungi such as *Rizopus oryzae* showed similar growth inhibition pattern by treatment of DME. DME did not inhibit the growth of *C. tropicalis*. Antifungal mechanism of DME was investigated on *F. oxysporum, C. albican* and *Asp. flavus* using time-kill test. Mean colony count data (log_{10} CFU/ml) were plotted as a function of time for each isolate at each concentration of antifungal test. Time-kill data were characterized as fungicidal or fungistatic. Fungicidal activity was defined as a $\geq 3log_{10}$ (99.9%) reduction in the number of CFU per milliliter from the starting inoculum count, and fungistatic activity was defined as a $<3log_{10}$ (99.9%) reduction in growth from the starting inoculum count. DME and amphotericin B displayed dose-dependent fungicidal or fungistatic activity. DME and amphotericin B exhibited fungicidal activity against *F. oxysporum* at high concentration (4X and 16X MIC, respectively). However, They showed fungicidal activity against *Asp. flavus* at below MIC. Similary, DME showed fungicidal activity against *C. albican* at high concentration (4X MIC), whereas amphotericin B has fungicidal activity on *C. albican* at below MIC

Keywords: Antifungal activity, *Dacryopinax*

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Antifungal activity of phenolic extracts of microalgae *Spirulina* sp. LEB18 and *Nannochloropsis oculata* against strains of *Fusarium* complex

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A promising strategy to limit fungal damage in crop ceral would be employ natural compounds with antifungal activity, considering that the application of synthetic fungicides can be toxic to humans and animals, plus the environmental impact [1]. Phenolic compounds present in *Spirulina* sp. have demonstrated the ability to inhibit fungal growth and difficult the production of their toxins [2], however, other species of microalgae, like *Nannochloropsis oculata* have not been studied for their ability antifungal. The resistance of this microalga the abiotic conditions makes it attractive to explore its potential antifungal [3]. As yet this work evaluated the antifungal capacity of phenolic extracts from microalgae *S.* sp. and *N. oculata* against different strains of *Fusarium*, evaluating the halo as indicative of the fungal development.

The extracts were obtained by method adapted [2,4], which consisted of extraction with methanol, evaporation of the solvent and resuspension in sterile water, clarified with barium hydroxide 0.1 M and zinc sulfate 5%, the final extract was filtered vacuum with sterile membrane to flask autoclaved. The extracts were analyzed for the phenolic acid profile employing high performance liquid chromatography with ultraviolet detector [5]. Three strains belonging to *Fusarium* complex were used to evaluate the antifungal activities of the extracts, each derived from a cereal grain (rice, wheat and barley). The phenolic extracts were applied at different concentrations into the culture media (agar BDA) and the with strains of *Fusarium* was inoculated, which were submitted photo period of 12 h at 25 ° C. The measure of the of halo fungal development was conducted for 8 days to every 24 h. The inhibitory concentration (IC50) was taken as the extract concentration required to inhibit 50% of fungal growth compared with the control experiments.

The profile of phenolic acids indicated predominance of the chlorogenic acid in both microalgae, comprising 93 to 76% of total phenols in *S.* sp. and *N. oculata*, respectively. Both microalgae extracts have high antifungal effect, reaching the complete inhibition of growth after 8 days in the case of *S.* sp. against the complex of *Fusarium*. We estimated IC50 values (Table 1), only in the case of the extract of *N. oculata* against barley complex did not show any inhibitory action. The IC50 value demonstrated the high antifungal activity.

Table 1. IC50 (μg/ml) of phenolic extracts of *S.* sp. and *N. oculata* after 8 days against strains of *Fusarium*.

Fusarium	*S.* sp.	*N. oculata*
Barley	36,2	-
Wheat	33,8	42,8
Rice	31,7	41,5

Keywords: phenolic compounds; inhibiting fungal.

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Antifungal activity of wild *Capsicum* foliar extracts containing polyphenols against phytopathogenic fungi *Alternaria alternata*, *Rhizoctonia solani*, *Sclerotinia minor* and *Verticillium dahliae*

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Natural plant-derived antifungal substances can be explored as valid alternative to conventional chemical fungicides for to control plant pathogens development. In this study, thirteen aqueous crude foliar extracts of *Capsicum* genotypes were examined for their antifungal activity. Grinded 70 °C-dried leaves were autoclaved, at a dose of 0.5 g ml^{-1}, in potato dextrose agar medium and assayed for the growth of phytopathogenic fungi on Petri dishes. The employed fungal pathogens were *Alternaria alternata*, *Rhizoctonia solani*, *Sclerotinia minor* and *Verticillium dahilae*. Growth rate of all tested fungi were significantly suppressed by extracts obtained from wild *Capsicum annuum*, *C. annuum* var. *glabriumsculum*, *C. baccatum* var. *baccatum*, *C. chinense* and *C. tovarii*. Microscopic analysis revealed remarkable morphological alterations in hyphal structures of samples exposed to the most bioactive leaf extract. UPLC–MS/MS analysis was performed to obtain polyphenolic profiles of extracts and quantify the individual known components, including gallic acid, chlorogenic acid, catechin, caffeic acid, epicatechin, coumaric acid, rutin, ferulic acid, hyperoside, luteolin, and quercetin. The role of antimicrobial activity of polyphenols have been discussed.

Keywords: Plant Pathogens; Plant-derived antimicrobials

Antileishmanial Activity of Low Molecular Weight Chitin Prepared from Shrimp Shell Waste

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Chitin is a tough, protective, semitransparent substance, primarily a nitrogen-containing polysaccharide, forming the principal component of the shell of crustacean, cuticles of insects and cell walls of fungi. The waste of this natural polymer is a major source of surface pollution in coastal areas. It has been proved to be biologically renewable, biodegradable, biocompatible, non-antigenic, non-toxic and biofunctional. In the present study, chitin was chemically extracted from shrimp shells. The obtained chitin was depolymerized by HCl to prepare low molecular weight chitin. Then, chitin and low molecular weight chitin were characterized. Further, antileishmanial activity of low molecular weight chitin was evaluated using *Leishmania infantum* LIPA 137 and *Leishmania infantum* LIPA 155/10, two reference strains isolated from patients in Pasteur institute from Algeria. The results showed effective antileishmanial activity of low molecular weight chitin against *Leishmania infantum* LIPA 137, but no antileishmanial activity of low molecular weight chitin against *Leishmania infantum* LIPA 155/10. It was also demonstrate that *Leishmania infantum* LIPA 155/10 is resistant to leishmaniasis drug glucantime® and *Leishmania infantum* LIPA 137 is sensitive to glucantime®. Further studies are necessary to determine the in vivo activities and applications of chitin and derivatives, in particular, in the design of new lines of drugs for use in the treatment of leishmaniasis and hopefully eradication.

Keywords: antileishmanial, chitin, *Leishmania infantum*, shrimp shell waste.

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Antimicrobial activities of crude solvent extracts of *Nauclea latifolia* leaves, a Nigerian traditional medicinal plant

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A study to determine the in vitro antimicrobial activities of various solvent extracts of leaves of *Nauclea latifolia*, a Nigerian traditional medicinal plant against some microorganisms of food and clinical importance was conducted. The antimicrobial activities of the crude solvent extracts of the leaves were determined using well in agar diffusion method against *E. coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae* and *Kluyveromyces sp*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined to establish the antimicrobial potential of extracts. The antimicrobial results revealed that ethanol extract produced maximum zone of inhibition (23.78mm) against *E. coli*. All the extracts had no inhibitory activity on *S. typhi* and *P. aeruginosa* at the lowest concentration tested (3.2 mg/ml). The least MIC (4.72 mg/ml) was produced by the crude ethanol extract on *Kluyveromyces sp*. while the least MBC (25 mg/ml) was produced by ethanol and methanol extracts on Kluyveromyces sp., *E. coli, S. aureus*. Ethanol and chloroform extracts had the highest and least inhibitory effects on the isolates respectively. The present study has revealed that the solvent extracts of *N. latifolia* leaves possess potent antimicrobial activity that can be harnessed. It may also be a new source of antimicrobial compounds that could be used to combat drug resistance which has become a global challenge.

Keywords: Antimicrobial activity; *Nauclea latifolia* extracts; agar diffusion; drug resistance; inhibition

Antimicrobial Activities of Essential Oils

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Although the antimicrobial qualities of aromatic and medicinal plants are known since antiquity, it was necessary to await the beginning of the 20th century so that the scientists start to be interested in this area. The use of natural essential oils in various matrixes and the development of new applications have been the subject of many researches in the world these last years. Moreover, the problem raised by the resistance of bacteria to antibiotics has encouraged the search for alternative natural (biological) molecules for therapeutic use.

The anti-infectious effectiveness of the empirically use of essential oils has been scientifically established *in vitro* and *in vivo* by studying their effects on diverse micro-organisms.

Keywords: Essential oil1; Natural preservative 2; Food safety 3; Antifungal activity 4; Vapour phase 5

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Antimicrobial activities of *Spirulina platensis*

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The biochemical contents of *Spirulina platensis* were analyzed, results indicated that it strangely has abundant supply of proteins, pigments, vitamins along with growth factor. *Spirulina platensis* contains high concentration of protein 60%, while carbohydrate and lipid, 14 and 3.6 % respectively and β-carotene 5.68 g/kg dry weight (DW). *S. platensis* water and ethanolic extracts were tested against some microorganisms such as two Gram positive and Gram negative bacteria and fungi. Whole powder was effective against all the tested microorganisms at concentration 15%(w/v), the ethanolic extract was active against all the tested microorganisms, while water extract was not. The minimum concentration of the extract varied with the organism where *Staphylococcus aureus* NIOF001 was the most susceptible isolate.

Key words: *Siprulina platensis* , biochemical and antimicrobial activities

Antimicrobial activity of an anthocyanin rich blueberry extract, purified using SPE

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There is a wide recognition of the biological potential of phenolic compounds, with their potential as antimicrobials gaining a particular interest when taking into account the emergence of microbial resistance and the need for alternative sources of antimicrobial compounds. Anthocyanins are one of the families of flavonoids that gather most of the attention for their biological potential therefore they are one of the main focus whenever blueberry extracts are concerned. As such, the present work aimed to evaluate the antimicrobial potential of a purified anthocyanin fraction (ca. 85% of total phenolics are anthocyanins) against a plethora of microorganims: multiresistant clinical isolates of *Escherichia coli*, *Proteus mirabilis*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, a Methicillin resistant (MRSA) and a methicillin sensitive (MSSA) *Staphylococcus aureus*, *Pseudomonas aeruginosa* 10145, *Escherichia coli* 25922 and MSSA ATCC 25923.

Firstly, the antimicrobial activity of the extract against each microorganism was screened by drawing inactivation curves. The analysis of these results showed that the most sensitive microorganisms was *S. aureus,* for which all strains studied were unable to grow in the presence of 500 µg/mL of powdered extract. *P. mirabilis* and *A. baumanii* suffered no inhibition. All other microorganisms, while not fully inhibited showed some signs of biological activity by the extract with delayed start of the exponential stage, lower growth rates and/or lower microbial loads after 24 and 48h (when compared to the control). As an example, the *E. coli* clinical isolate registered a microbial load 22 to 25% (after 24 and 48 h respectively) lower than the positive control when incubated with 500 µg/mL of powdered extract, while the collection strain registered reductions of 47 to 69% (after 24 and 48 h respectively) when under the same conditions. As for *P. aeruginosa* both strains portrayed longer *lag* stages when exposed to 500 µg/mL, with the clinical isolate exhibiting a 9 h longer lag stage than that of the control.

Secondly, the extracts capacity to inhibit biofilm formation was screened. In this test, all microorganims except *P. mirabilis* demonstrated some sensitivity toward the extracts, with inhibition percentages ranging from 75 to 45% when exposed to 500 µg/mL, 75-23% when exposed to a 250 µg/mL concentration and in some cases, the extracts remained active when used in concentrations as low as 50 µg/mL (35-21%). In this assay, the collection strain of *P. aeruginosa* appeared to be the most sensitive to the extracts activity with concentrations as low as 50 µg/mL still being able to cause an inhibition of 47 ± 12 % of biomass. It is interesting to note that, while the growth of *A. baumanii* was not affected by the extracts its biofilm formation was with a range of inhibition percentages from 74 to 35 %. For *P. mirabilis*, the presence of the extracts appears to strongly promote biofilm formation. Additionally, the extracts capacity to inhibit short term adhesion, a 3h adhesion test was performed with only the clinical isolates of *S. aureus* and the collection strain of *P. aeruginosa* exhibiting inhibition of bacterial adherence in this time frame.

Overall, the present work demostrated that anthocyanin rich fractions possess significant antimicrobial and antibiofilm activity against several multiresistant and culture collection microorganisms, thus showing their potential as possible active ingredients in future pharmacological solutions for the treatment of bacterial infections.

Keywords: anthocyanins; antibiofilm; antimicrobial; adhesion

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Antimicrobial activity of ethanolic extract and essential oil of *Cymbopogon nardus* on pathogenic bacteria

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Knowledge about the therapeutic potential of plants has attracted scientific interest, indicating new alternatives for the control and treatment of various diseases including bacterial infections. The irrational use of antibacterial determines the emergence of resistant microorganisms, causing a decrease of therapeutic. The plant extracts are important sources in the search for new drugs due to their secondary metabolites. *Cymbopogon nardus* (L.) Rendle (citronella) belongs to the family Poaceae, native to Ceylon and cultivated in subtropical and tropical areas of Asia, Africa and America, including Brazil. Citronella essential oil is obtained from the leaves and is used as an insect repellent, stimulant and in perfumery. In Thailand, the infusion of the leaves of citronella is used for flatulence, indigestion and abdominal cramps [1]. In this sense, the aim of this study was to evaluate the antibacterial activity of ethanolic extract (EtE) and essential oil (EO) of *Cymbopogon nardus* against bacterial strains: *Helicobacter pylori* (ATCC 43504), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). The minimal inhibitory concentration (MIC) was determined by dilution in microplate [2]. The plants extracts were evaluated with concentrations ranging from 1000 to 7, 81 ug/mL. As control was used ampicillin with concentration ranging from 50-0, 19 ug/mL (*S. aureus* e *E. coli*) and amoxicillin, 500-1,95 ug/mL (*H. pylori*). After incubation at 37ºC/24 hours ((*S. aureus* e *E. coli)* and 37ºC/72 hours (*H. pylori*), the MIC of sample was detected following the addition of 0, 01% resazurin. The results are presented in Table 1. We highlight the MIC of EO against *H. pylori* (125 ug/mL). Data are promising considering the clinical importance of this microorganism in gastric and duodenal ulcers and are consistent with the popular use of infusion of the leaves of citronella in gastric disorders.

Table 1: MIC of ethanolic extract (EtE) and essential oil (EO) of *C. nardus*.

	Minimal inhibitory concentration (µg/mL)		
Samples	***H. pylori***	***S. aureus***	***E. coli***
EO	125	500	>1000
EtE	1000	>1000	>1000
Ampicillin	-	0,35	12,5
Amoxicillin	15	-	-

Keywords: Cymbopogon nardus; antibacterial activity; Helicobacter pylori; Escherichia coli; Staphylococcus aureus;

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Antimicrobial activity of extracts from agroindustrial subproducts

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Microbial growth is a process usually associated with disease and food spoilage. Therefore, some solutions have been searched but, during a long period of time, these solutions were almost focused exclusively in antibiotic therapies. Considering the increase of resistance of microorganisms against this conventional therapy, new alternatives have been studied and so, natural compounds with antimicrobial activity seem to be a great solution. Several authors have found antimicrobial properties in certain classes of polyphenols, which have been proposed to be included in the innovative approaches of new preservatives in foods. These compounds are widely distributed in the most extensive range of vegetable materials, as is the case of lignocellulosic materials (LCMs). These materials are mainly made up of cellulose, hemicelluloses and lignin, and they are the most abundant biomass, comprising nearly 70% of the total plant biomass produced by photosynthesis. Agroindustrial activities are an inexhaustible source of LCMs.

In this work, four extracts obtained from agroindustrial subproducts were used: corn cobs (CC), eucalypt wood (EW), almond shells (AS) and grape pomace (GP). To evaluate the antimicrobial activity of these extracts, five foodborne pathogenic bacteria were used: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Salmonella* spp. Four concentrations (1, 2, 3 and 4 % (w/v)) were tested for the antimicrobial activity evaluation of the extracts. Although the extracts did not show a selective action upon either Gram-positive or Gram-negative bacteria, they displayed antimicrobial activity. The EW extract was the most active, whereas the AS and CC extracts were the least active, but in all cases, this effect was not immediate; in the majority of cases it occurred only after approximately 24h.

Keywords: Antimicrobial activity; Pathogenic bacteria, Extracts, Agroindustrial sub-products, Lignocellulosic materials

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Antimicrobial Activity of Native Plants of Caatinga Biome: *Buchenavia tetraphylla, Pityrocarpa monilifomis*, *Anadenanthera colubrina* and *Libidibia ferrea*

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In recent years, resistance of pathogenic micro-organisms to multiple drugs has increased due to the indiscriminate use of antimicrobials. In this context, the search for new antimicrobial agents from plants is intense, mainly because of the lower search costs, probable less risk of side effects and a great potential of synergistic effects. Caatinga medicinal plants have become the focus of intense study recently in terms of conservation and as to whether their traditional uses are supported by actual pharmacological effects or merely folklore. In this region, people commonly treat diseases by means of drinking teas or infusions from plant extracts. In this work we evaluated the antimicrobial activity of *Buchenavia tetraphylla* and *Pityrocarpa moniliformis* and the synergetic potential of *Pityrocarpa moniliformis Anadenanthera colubrina, Libidibia ferrea* with antibiotics. Cyclohexane (BTLCF, PMLCF), ethyl acetate (BTLEF, PMLEF), and n-butanol (BTLB, PMLB) and aqueous (BTLAF, PMLAF) fractions were obtained from a liquid-liquid partition of *B. tetraphylla* and *P. moniliformis* leaves hydroalcoholic extracts (BTLHE, PMLHE), respectively, and their antimicrobial activity was analyzed. The combinatory effects of hydroalcoholic fruits extracts of *A. colubrina* (ACFHE), *L. ferrea* (LFFHE) and *P. moniliformis* (PMFHE) were evaluated with erythromycin. On the other hand, PMLHE, PMLCF and PLMB were combined with chloramphenicol, erythromycin, streptomycin and tetracycline. The combinatory effects of extracts and antibiotics were determined using the fractional inhibitory concentration indices (ΣFIC). In relation to *B. tetraphylla,* BTLHE inhibited the growth (minimum inhibitory concentration, MIC) of *Micrococcus luteus* (0.10 mg/mL), *Pseudomonas aeruginosa* (0.20 mg/mL), *Mycobacterium smegmatis* (0.39 mg/mL), *Proteus vulgaris,* and *Staphylococcus aureus* (0.78 mg/mL for both). The most active fractions (BTLCF, BTLB. BTLCF) showed better potential to inhibit *M. luteus*, *P. aeruginosa*, *S. enteritidis*, and *S. aureus*. In the case of *P. moniliformis*, PMLHE only inhibited *S. aureus* and *M. luteus* (MIC of 1.56 and 0.39 mg/mL, respectively), whereas its fractions (PMLEF, PMLCF, PMLB, PMLAF) also showed activity against Gram negative bacteria (MICs: 1.56 to 25), except to *E. coli*. Furthermore, only PMLB showed synergistic/additive effects with all tested antibiotcs, which may be explained by saponin presence, which can cause membrane permeabilization (it has a great hemolytic capacity). Regarding the combinatory effects of hydroalcoholic extracts, PMFHE demonstrated synergistic effects against *S. aureus* in six ratios (ΣFIC: 0.20 to 0.46). ACFHE in five (ΣFIC: 0.18 to 0.33) and LFFHE in four (ΣFIC: 0.20 to 0.47). *P. moniliformis* showed the highest synergetic potential and antagonistic effect was not found. This study showed at first time the antimicrobial activity extract/fractions of leaves from of *B. tetraphylla and P. moniliformis* and the capacity of *P. moniliformis* leaf extracts *A. colubrina, L. ferrea* and *P. moniliformis* fruit extracts to enhance the anti-*S. aureus* activity of some protein inhibitors drugs, creating a new perspective on the interesting association of traditional and scientific knowledge.

Keywords: Antimicrobial activity; Synergistic effects; Caatinga biome.

Antimicrobial activity of peptides from Sardinia dairy products

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The protection of the traditional agricultural foodstuff is a key point for the preservation and enhancement of the Italian productive and socio-cultural environments, mainly in some regions, such as Sardinia, where it represents a valuable resource for the regional economy. Unfortunately, some products, such as cheese, are now at risk of being marginalized partly because of the crisis that is affecting the Italian food industry. Therefore, a series of plans are need to safeguard and enhance the market of the traditional products, also through a study for the identification and exploitation of their functional properties, such antimicrobial activity against different pathogens. Aim of our work was to investigate the biological potential of some dairy products typical of the Sardinia region. The work was performed on three organic cheeses: the "Saboriu" Pecorino cheese (seasoning 3 months), obtained from raw milk and raw pasta with live autochthonous lactic acid bacteria; the "Callau axeddu" soft cheese, made with live autochthonous lactic acid bacteria; "Gioddu", a typical natural Sardinian yogurt produced with raw sheep milk fermented with live autochthonous lactic acid bacteria. The study was focused on the identification of peptides with antimicrobial activity, obtained after the *in vitro* gastrointestinal transit of products, treatment with acetonitrile to remove the protein fraction, and drying of samples to remove the organic eluent. The antimicrobial activity was assessed by the inhibition halo test using *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterobacter sakazakii* as pathogen strains; the antifungal activity was assessed *versus Penicillium expansum*, *Penicillium griseofulvus*, *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium digitatum*, *Penicillium citrinum*. The most effective antimicrobial activity was exhibited by extracts of yogurt Gioddu and Pecorino cheese, which were effective in inhibiting the growth of the toxigenic *E. coli*, *P. aeruginosa* and the emerging pathogen *C. sakazakii*. The extract of Callau axeddu inhibited the growth of *E. coli* and *C. sakazakii*. On the contrary, the extract of Gioddu did not give significant results on any of the fungal strains used in this experiment; on the other hand, extract from Callau was the most effective in inhibiting the growth of *A.niger* and *A.versicolor*, over that of *P. digitatum* and *P. citrinum*. The extract of Saboriu demonstrated inhibitory activity versus the two strains of *Aspergillus* and on *P. digitatum*.

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Antimicrobial activity of some novel benzimidazole derivatives

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In this study, a series of 6-fluoro-2-(4-sübstitüte-phenyl)-5- morpholino- 1H –benzimidazole derivatives were screened for their in vitro antimicrobial activities. The compounds were tested for their in vitro antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* , *Escherichia coli* and for their antifungal activity against *Candida albicans* using a disc diffusion method, which measures the diameter of the inhibition zone around a paper disc soaked in a solution of the test compounds.

All of the compounds were inactive against *S. aureus, E.coli* but compounds 6, 7 (6-fluoro-2-(4-klorofenil)-5-morfolino-1H-benzimidazol ve 6-fluoro-2-(3,4-dimetoksifenil)-5-morfolino- 1H –benzimidazol showed good activity against *B.subtilis.* The diameter of the inhibition zone of this compounds were found nearly the standart compounds ampicillin.

All of the tested compounds showed good inhibition zone against *Candida albicans.*

Antimicrobial activity with seasonal lectins identified in serum of Tambaqui Amazonian fish

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The tambaqui (*Colossoma macropomum*) is one of the most important fishes in Brazilian aquaculture and is the second largest scale fish found in the Solimões river in the Amazon Region. The tambaqui is of special interest to fish culturists in many South American countries since it has rustic nature and excellent meat quality been able to reach a length of 1 m and a body weight of 30 kg in its natural environment. Aquaculture operations strive to produce a large number of healthy fishes through procedures that are economically and biologically efficient. Therefore, prevention of disease is very important for both, small fish farmers as well as big industry. Lectins are proteins that specifically recognize carbohydrates which have important biological functions. The physiological role of lectins is not clearly defined, however studies suggest that lectins are defense proteins that can protect against viruses, fungi and bacteria attacks. In the present work, lectins from tambaqui serum (ComaSeL), were identified, biochemically characterized, conducted through a seasonal study and tested against antibacterial activity [1]. Blood samples from the caudal vein of an adult tambaqui were collected for each month of the year covering the major seasons in North and Northeast Brazil (summer and winter). After serum obtention [1] lectin activities [2] and protein concentrations were performed on the same day as the blood collections. Antibacterial activity assays against the pathogenic bacteria from freshwater fishes, *Aeromonas hydrophila* (IOC/FDA 11036) cultured in nutritive broth (NB, 30 ºC, 24 h), *A. sobria* (ATCC 43979) in trypticase soy broth (TSB, 30 ºC, 24 h), and *Edwardsiella. tarda* (A TCC 15947) in NB (37 ºC, 24 h), respectively. ComaSeL (200 - 3.125 µg/ml, 180 µl) containing TBS (20 mM Tris–HCl, 20 mM CaCl2, 15 mM NaCl, pH 8.0) were placed in NB or TSB medium in wells of a 96-plate microtiter plate. Following, 20 µl of the bacterial suspension (1.5 x 10^9 cells) was added to each well. TSB or NB containing only TBS buffer was the negative control for bacterial growth (Control 1); also, TSB or NB with bacterial suspension was used as the positive control (Control 2). The inhibition assay of antibacterial activity was performed with ComaSeL inhibited by the specific carbohydrate D-fucose. Control 1 with D-fucose was the negative control for bacterial growth; control 2 was applied following the antibacterial activity assay. All treatments were incubated and optical density at 490 nm was measured in a microplate photometer. Maximum growth of bacteria in Control 2 was taken as 100 % of bacterial viability, the baseline for calculating antibacterial activity and antibacterial activity inhibition. There were significant differences of lectin activity and protein concentration between summer and winter (lectin activity was 85% smaller during winter than during summer) in which there are sudden changes in temperature in the regions (North and Northeast of Brazil). The lectin showed antibacterial activity against *A. hydrophila*, *A. sobria*, and *E. tarda*, with a minimum inhibitory concentration (MIC) of 200, 12.5 and 50 µg/ml, respectively. Differences in activity according to seasonality corroborates the observation that tambaqui during winter becomes more susceptible to mortality from diseases caused by bacteria and fungi. It should be an interesting challenge to devise strategies to avoid handling these fishes during the winter. The identification of lectins in tambaqui serum and its potential application to antimicrobial activity can contribute to unraveling the role of these lectins in the fish immune system. It can also improve the management of tambaqui by pisciculturists from the North and Northeast regions of Brazil and other countries in South America.

Keywords: Tambaqui; lectin; antibacterial activity.

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Antimicrobial and Antioxidant Activities of *Ajuga genevensis* L. Extract

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In flora of Turkey Ajuga represented by 14 species and 27 taxa [1]. Some Ajuga species have been widely used for their diuretic, antipyretic, tonic, diaphoretic, astringent properties in the Turkish folk medicine [2].

In this study, antimicrobial and antioxidant effects of herbal methanol 70 % extract of *Ajuga genevensis* L. were investigated. $DPPH^{\bullet}$ radical scavenging activity IC_{50} values were calculated as 0.44 mg/ ml for extract, respectively and 0.06 mg/ml for BHT.

Phytochemical constituents were determined by LC-MS/MS where: luteolin and apigenin (with their derivatives), caffeic acid, coumaroyl glucose, galactonic acid were determined.

Antimicrobial actvity was evaluated 7 different pathogenic bacteria and yeast strains againts *Escherichia coli* NRRL B-3008; *Staphylococcus aureus* ATCC 6538; *Salmonella typhimurium* ATCC 13311; *Bacillus cereus* NRRL B-3711; *Candida albicans* ATCC 90028; *Candida tropicalis* ATCC 1369; *Candida parapsilosis* ATCC 22019, using broth microdilution CSLI methods [3,4]. Tetracycline, ampicilline, oxiconazole and ketoconazole were used as standart antimicrobial agents. Methanol extract of *Ajuga genevensis* was studied between [1250-2.44 µg/ml] for minimum inhibitory concentrations. The results of the antimicrobial assay showed that the extract have antibacterial effect at MIC= 312.5 µg/ml for bacteria strains and MIC= 156.25 µg/ml for yeast strains.

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Antimicrobial effect of commercial propolis sample from Turkey

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Propolis is a multifunctional natural honey bee (*Apis mellifera* L.) product which has been used for its medical benefits, including antimicrobial effect, since ancient times. Studies show that its antimicrobial activity comes from mainly flavonoids, aromatic acids and their esters. With the increase in consumption of natural products for the treatment of diseases and wellness, number of commercial propolis preparations in pure or combined forms are become available. But effectiveness of propolis can vary based on floral properties of the region collected. The aim of this study was to evaluate the effectiveness of commercial purified (wax have been removed) propolis sample which said to be a mixture of different regions of Turkey. Disc diffusion method was applied on 11 bacteria (6 Gram positive, 5 Gram negative) and *Candida albicans* (ATCC 033). The results indicate that propolis sample is most effective on *Staphylococcus aureus* (ATCC 29213) strain. Results are given in Table 1.

Keywords: antimicrobial effect; propolis

Table 1
Antimicrobial effect of ethanolic extract of propolis sample at different concentrations by disc diffusion test

Strain	Mean values of the diameters of inhibition zones (in mm)			
	1 mg/ml	1,5 mg/ml	2mg/ml	2,5 mg/ml
Bacillus subtilis (ATCC 6633)	-	-	12,5	13
Candida albicans (ATCC 033)	-	12	12	12
Enterococcus faecalis (ATCC 29212)	11	11,5	11,5	12
Enterococcus faecalis (ATCC 19433)	9	12	12	12
Escherichia coli (ATCC 25922)	10	12	12	12
Escherichia coli (ATCC 35218)	11	11,5	13	13
Klebsiella pneumoniae (RSKK 574)	-	12	12	12,5
Pseudomonas aeruginosa (ATCC 27853)	10	10,5	11	12
Pseudomonas aeruginosa (ATCC 9027)	11,5	12,5	12,5	12,5
Staphylococcus aureus (ATCC 25923)	10	11	11	11
Staphylococcus aureus (ATCC 29213)	11	12,5	13	14
Staphylococcus aureus (ATCC 43300)	12	12	12	13

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Antimicrobial effects of a silken web produced by the larvae of *Plodia interpunctella*

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The Indian meal moth, *Plodia interpunctella* (Hübner 1813), especially larval stage of insect, is a major pest of stored products, found all over the world. The larvae produce a silken web which has the protective role, primarly to the cocoon (Mohandass *et al*., 2007).

The silken web was shredded and immersed in DMSO and then diluted with nutrient broth to achieve a concentration of 10% DMSO. In that percentage DMSO did not inhibit the growth of microorganism. We added 25 mg of the silken web per milliliter of described dilution. After mixing the dilution, the remains of the silken web were thrown away. For determining the antimicrobial effects we used microdilution method with resazurin (Sarker *et al*., 2007). The results were recorded in form of the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC).

The influence was tested on 20 species of microorganism, of which half were bacterial strains and half were fungal strains. The clinical isolate *Escherichia coli* could develope above the initial tested concentration of 25 mg/ml. The best results were obtained against bacteria of the genus *Bacillus*, then against *Staphylococcus aureus* ATCC 25923 and clinical isolate *Staphylococcus aureus* (MICs at 6.25 mg/ml).

Considering the results on the tested fungi, the dilution did not influenced 5 of the tested strains. The best results were obtained against *Rhodotorula sp*. and *Penicillium chrysogenum* (MICs at 6.25 mg/ml) and the dilution also affected against *Penicillium italicum, Trichoderma viride* and *Botrytis cinerea* (MICs at 12.5 mg/ml).

Keywords: Antimicrobial; *Plodia interpunctella*

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Antimicrobial Effects of Blueberry, Raspberry and Strawberry Aqueous Extracts on Pathogenic Bacteria and Their Effects on Virulence Genes Expression in *Vibrio cholerae*.

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Antibiotic-resistant bacteria represent a major concern worldwide. Therefore, intense-research efforts have focused on the search for new alternatives to prevent infectious diseases. Special attention has been paid to the antimicrobial activity of diverse plant polyphenols, which have been reported to show great inhibitory effects against pathogenic bacteria, yeast, fungi and viruses [1]. Among the potential candidates, berry phenolics are generating great interest because their components have been reported to show not only antimicrobial effects, but also other beneficial biological activities – for example anti-inflammatory, antioxidant, and anti-diabetic properties [2]. The aim of this reseach was to compare the antimicrobial properties of blueberry (BL), raspberry (RS) and strawberry (ST) aqueous extracts against 14 isolates of pathogenic bacteria. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the three berries were determined by a micro titer plate method. Pathogenic bacterial strains, both Gram-positive and Gram-negative, were selectively inhibited by the three berries. BL was the best inhibitor, and *Vibrio* and *Listeria* were the most sensitive bacteria. To understand the relationship between the phytochemical analysis of the three berries and their antimicrobial activities, the total contents of phenolics (TPC), flavonoids (TFC) and anthocyanidins (TAC) were measured by colorimetric methods. ST had the highest content followed by RS, finally, BL. Moreover, we studied the effect of sub-bactericidal concentration of the three berries on virulence genes expression in *V. cholerae*. Real-time quantitative reverse transcription-PCR revealed that the three berries effectively repressed the transcription of *tcpA* gene. RS repressed the transcription of *ctxA* gene, while BL and ST did not have any effect. In sharp contrast, the three berries did not affect the transcription of *toxT*. These results suggest that the three berries show potent antimicrobial effect and can inhibit the virulence factors production by *V. cholerae*. Further research should focus on exploring preventive and/or therapeutic formulations that show maximum antibacterial effects. However, comprehensive clinical studies are needed to determine the safety of these plant-derived extracts.

Keywords: Plant polyphenols; pathogenic bacteria, virulence genes.

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Antimicrobial efficacy of *Acacia saligna* (Labill.) H.L.Wendl. and *Cordia sinensis* Lam. leaves extracts against some pathogenic microorganisms

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Acacia saligna and *Cordia sinensis* used traditionally as medicine and food additives in Saudi Arabia. The antimicrobial activities of leaves ethanol extracts of both species were investigated against 7 medically important bacterial strains, namely *Bacillus subtilius*, MRSA, *Micrococcus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aurues* and *Klebsella pneumoniae* and four fungi *Aspergillus flavus, A.niger, A.fumigatus* and *Candida albicans* .The antibacterial activity was determined by agar well diffusion method. The most pronouns effect was shown by that of *Acacia saligna*. The most susceptible bacteria was *Klebsella pneumoniae* , flowed by *Micrococcus*, while the most resistant bacteria was MRSA followed by *Bacillus subtilius*. The most Pronouns effect on fungi was shown by that of *Acacia cyanophylla*. The most susceptible fungi was *Aspergillus fumigatus* while the most resistant fungi was *A.niger*. The HPLC analysis indicated the presence of 8 phenolic compounds as major active constituents (Gallic, protocatioic, chlorogenic, syringic, p.Hydroxy Benzoie, p.coumaric , vanillic and salicylic acid). Result obtained indicated variable differences in compounds concentration of *Acacia saligna* leaves extract. Results of HPLC showed (Gallaic), p.coumaric and syringic in high concentrations 54.31, 8.27 and 3.71 µg/g respectively. The concentrations of other phenolic compounds ranged from 0.44 - 2.01 µg/g. The least concentration was of chlorogenic with 0.44µg/g.

Key words: Acacia cyanophylla, *Cordia sinensis*, Antibacterial, antifungal , phytochemical constituents, crude extract.

Antimicrobial efficacy of natural agents against *Listeria monocytogenes* and spoilage microorganisms in meat products

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The objective of this study was to evaluate the antimicrobial efficacy of natural agents against the food pathogen *Listeria monocygenes* and lactic acid bacteria (LAB), which are one of the main spoilage microorganisms in meat products, and to investigate the effect of food meat ingredients on the efficacy of these agents. The natural compounds assessed were essential oils (EO) of oregano, sage and thyme, and its main components, carvacrol, thymol and eugenol. These compounds were initially screened at different concentration levels (0.1, 0.5 or 1%) against several strains of *Listeria monocytogenes*, *Lactobacillus sakei, Lactobacillus curvatus, Lactobacillus plantarum, Lactobacillus brevis, Leuconostoc citreum, Leuconostoc mesenteroides.* The assay used to evaluate the efficacy of these compounds was the drop plate method (fig.1), which allowed assessing the inhibition of microbial growth at different levels (10^7-10^2 cfu/ml). Different concentrations of compounds were included in the culture media (PCA or MRS agar). Carvacrol, thymol and oregano EO showed the best results. All strains at level of 10^6 - 10^7cfu/ml were inhibited when these compounds were present at 0.1% of concentration. In addition, combinations of carvacrol, thymol and oregano EO at lower concentrations were assessed to evaluate the possible synergic effects. The most efficient combination was 0.05% thymol and 0.05% oregano EO.

On the other hand, the effect of food meat ingredients on the antimicrobial efficacy of thymol (0.1%), oregano EO (0.1%) and their mixture (0.05% thymol and 0.05% oregano EO) was assessed using drop plate method. The selected ingredients for the model media were beef extract (10 or 20%) as protein, lard (10 or 20%) as oils and sodium tripolyphosphate (0.5%, maximum legal limit) as phosphate. These components were selected to simulate the conditions of the meat product matrix. The antimicrobial efficacy of the natural agents was found to be a function of ingredient combination. Oils concentration of 10% and 20% had a negative impact on the antimicrobial efficacy of natural agents. On the contrary, the phosphate enhanced the antimicrobial effect of the natural compounds, and in general, the high concentration of protein improved the antimicrobial effect of these natural agents. Moreover, in this study, different sensitivity to natural agents was observed between the species used, determining that *Lactobacillus plantarum* was the most resistant of all and *Listeria monocytogenes* was the most sensitive. These results are in agreement with the work of other authors ([1]; [2]; [3]).

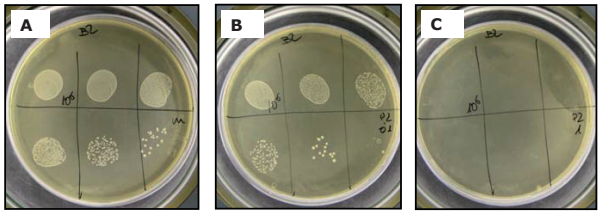

Figure 1. Antimicrobial efficacy by Drop plate method. A. *L. mesenteroides* growth in MRS without additives. B. *L. mesenteroides* growth in MRS with 0.1% eugenol. C. *L. mesenteroides* inhibition of growth in MRS with 1% eugenol.

Keywords: Antimicrobial; lactic acid bacteria; *Listeria monocytogenes*, essential oil; food ingredients, meat products

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Antimicrobial evaluation and fatty acid compositions of essential oils of *Helianthus annuus* Seed in multiresistant *Staphylococcus aureus* derived from milk

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Mastitis constitutes an inflammatory process of the mammary gland, causing great economic loss in dairy cattle due to reduced milk production and quality, increased use of drugs and the risk of death for the animals. Although many microorganisms can affect the region causing intramammary infection, *Staphylococcus aureus* is the main causative agent, responsible for chronic bovine mastitis. The concern about the presence of antibiotic residues in milk and the emergence of resistant bacteria has stimulated the search for alternatives, especially natural products [1] for the treatment of mastitis. The aim of this study is to evaluate the antimicrobial activity of essential oils from the *Helianthus annuus* seed on isolates from mastitic milk, as well as examining their fatty acid composition. To test the antimicrobial, multiresistant *Staphylococcus aureus* (n = 10) isolates from milk of cows with subclinical mastitis were selected. The constituents of the oils were determined by gas chromatography and the antimicrobial activity was determined by the microdilution broth [2]. The constituents are as follows: Myristic acid (C 14:0), palmitic acid (C 16:0), palmitoleic acid (C 16:1), margaric acid (C 17:0), stearic acid (C: These fatty acids in the oils studied were found 18:0), oleic acid (C18: 1n9c), linoleic acid (C18: 2n6c), linolenic acid (C18: 3N3), arachidic acid (C20: 0), gadoleic acid (C20: 1n9), behenic acid (C22: 0) and lignoceric acid (C24: 0) were found in the oils studied. The mean MIC to S. *aureus* was 23.5 µg.mL-1. It was observed that the CBM coincide with an MIC of 70.0%, indicating that the oils showed bacteriostatic. The results show the essential oils have antimicrobial activity against these microorganisms, and considering the difficulty in the control and treatment of bovine mastitis, as well as the issue of the presence of antibiotic residues in milk and dairy products, the data obtained demonstrate the potential use of the *Helianthus annuus* seed oil as an alternative therapy to control this disease.

Keywords: oil essencial; suscetibility; bacteria

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Antimicrobial screeening of *Plectranthus madagascariensis* and *P. neochilus* extracts

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Natural products are widely used as traditional medicines and are a common source of bioactive molecules for the treatment of bacterial infections. In particular, some plants of the genus *Plectranthus* (Lamiaceae) have demonstrated several applications, including the treatment of various infections. The use of plant extracts as therapeutic agents is an interesting area to be explored, as it may allow the isolation of antimicrobial metabolites [1]. *Plectranthus madagascariensis* is often used in the treatment of wounds, in influenza, cough and chest pain [2], while extracts of *Plectranthus neochilus* are used for the treatment of dyspnea and liver failure [3]. In this study, we have screened the antibacterial activity of different *P. madagascariensis* and *P. neochilus* extracts.

Aqueous, acetonic and methanolic extracts of *P. magascariensis* and *P. neochilus* were prepared using several extraction methods (infusion, decoction, microwave, ultrasound, supercritical fluid and maceration). All extracts were screened for their antimicrobial activity against Gram positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*, *S. epidermidis*, *Bacillus subtilis* and *Mycobacterium smegmatis*) and Gram negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*) and two yeast (*Candida albicans* and *Saccharomyces cerevisiae*) strains. The *P. madagascariensis* acetonic extracts obtained using ultrasound and maceration methods and *P. neochilus* acetonic extract obtained by ultrasound technique were active against the five Gram positive bacteria tested (5–24 mm of inhibition zone, well diffusion test). These antimicrobial activities were further evaluated by the microdilution method (MIC values between 250–0.49 µg/mL) and bioautography assay against *S. aureus*.

The *P. madagascariensis* ultrasound acetonic extract was the most active extract against all the Gram positive bacteria tested (MIC values between 31.25 - 0.49 µg/mL). It was also active against resistant MRSA and VRE strains (MIC values between 31.25-0.98 µg/mL). Previously, the *S. aureus* bioautography showed that the polar compounds were the responsible for the antimicrobial activity. Following, the chemical profile of the acetonic extract was also analysed by HPLC-DAD and the isolation of the antimicrobial diterpenoids is under study.

The antimicrobial *P. madagascariensis* extract seems to be a potential key source for new bioactive chemical entities. A study to know its contituents is being carried out using several chromatographic techniques. *P. madagascariensis* is a promising candidate in key therapeutic areas such as infectious diseases.

Keywords: Antimicrobial activity; natural products; *Plectranthus* spp; extraction methods

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Antimicrobial study of capacity of *Lactobacillus plantarum* strains isolated from mare's milk

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In the present study the antimicrobial activity of three strains of *Lactobacillus plantarum* isolated three milks obtained from three mare of free life was determined. From three cultures overnight in Man Rogosa Sharpe Broth (MRS) were proceeded to separate the liquid phase by centrifugation and the pellet.

Then, we proceeded to evaluate the antimicrobial activity of the two components (liquid phase and pellet) against pathogens bacteria or bacteria contaminant in products intended for food or feed. The technique used is the agar diffusion method. The three strains of *Lactobacillus plantarum* showed activity against *Salmonella* sp, *Proteus* sp, *Pseudomonas* sp, *Bacillus cereus*, *Klebsiella* sp, *Staphylococcus aureus, Enterococcus faecalis, Kocuria lutea, scherichia coli, Enterobacter amnigenus, Pantoea* sp and *Listeria monocytogenes.*

Antimicrobial activity was detected both in the liquid phase of the culture and in the pellet. These results establish a value to mare's milk in which isolated or their extracts lactic microorganisms are staying active.

Keywords: mare milk, *Lactobacillus plantarum* ; antimicrobial activity

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Antimicrobial hop extracts and their application on fresh produce

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Fresh-cut fruits and vegetables combine the aspect of convenience food with healthy nutrition. Therefore they get a growing share on the worldwide food market. Nevertheless, these non-treated food products bear a risk of microbial contamination with pathogenic microorganisms and limited shelf-life due to their fresh character and big surface.

Treating food with antimicrobial substances can minimize the inherent microbiological risk and is even able to prolong shelf-life and increase the quality of these products. On the other hand chemically synthesized preservatives get more and more rejected by the consumer. Natural substances are preferred instead. For example lupulone, a beta-acid of the hop plant, has a high antimicrobial potential.

Beta-acids containing hop extracts showed high bactericidal effect on different food-related (pathogenic) bacteria such as *Listeria monocytogenes*. As a second step these extracts have been applied on fresh cut produce such as endive salad or strawberries. The application (by spraying or dipping) can be either directly or via an edible coating.

The efficiency of the extracts on the quality of the products was evaluated in storage test. They showed that natural hop extracts were able to contribute significantly to the safety and quality of fresh produce. Thus they could be a serious alternative to conventional additives for food preservation.

Keywords: hop, beta-acids, natural antimicrobials, Listeria, food preservation

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Antioxidant activities of nine medicinal plants used in treating inflammatory ailments in Zulu traditional medicine of South Africa

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Inflammation is a complex interaction between pro-inflammatory and anti-inflammatory mediators in response to induced negative stimuli in which the former predominates. Many allopathic medications currently used in alleviating inflammation are associated with some major side effects such as intestinal and cardiac toxicity. However, medicinal plants are also used in many traditional practices against inflammatory complications. If the active components present in medicinal plant extract affect the same molecular targets as mainstream interventions with no side effects, such extracts could provide attractive and cost-effective alternatives to the conventional anti-inflammatory agents. Considering the importance of oxidation in inflammatory mechanisms, the free radical scavenging activities of *Pentanisia prunelloides, Pomaria sandersonii, Alepidea amatymbica, Gunnera perpensa, Carissa bispinosa, Artemisia afra, Eucomis autumnalis, Ledebouria revoluta* and *Berkheya setifera* used to remedy inflammation in Mabandla Village, KwaZulu Natal, South Africa was carried out against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals. Results from both assays indicated that some of the plants had good antiradical properties. For $DPPH^{\bullet}$ radical assay, EC_{50} values ranged between 1.9 mg/L from *L. revoluta* crude to 467 mg/L from hexane fraction of *C. bispinosa*. The trend of activity in $ABTS^{\bullet+}$ radical scavenging was similar to the DPPH trend. However, *P. sandersonii* extracts was the most active in this case inhibitory with EC_{50} values of 1.27 mg/L for crude extract, 1.66 mg/L for DCM and 1.27 mg/L for acetone fraction. *Carissa bispinosa* crude extract had the lowest activity against the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals (190.6 mg/L and 25.45 mg/L respectively). The good antiradical results obtained for some of the plants indicate that antioxidant activities may contribute to their anti-inflammatory potential, therefore validating their traditional use as therapeutic in inflammatory disorders.

Key word: Medicinal Plants; Anti-oxidant; DPPH

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Assessment of antibacterial activity of essential oils of two thymus species from organic growth in meat homogenates

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Organic food is one of the fastest growing sectors of the food and agriculture industry worldwide. In these products no chemical additives can be used to prevent microbial spoilage, partly as a consequence, aromatic herbs or spices, especially their derivates such as essential oils (EOs), are gaining interest for their potential as preservatives and as decontamination agents. Thus, the antimicrobial properties of aromatic plant EOs have been widely assessed in a wide variety of foods.

The aim of this study was investigated the effectiveness *in vitro* of the EOs from two species of *Thymus* such as *Thymus zygis* and *Thymus capitatus* obtained from organic growth on development of pathogenic microorganism indicators or as spoilage microorganism such as *Serratia marcescens*, *Listeria innocua* and *Alcaligenes faecalis* in homogenate meat products (minced meat, dry-cured meat and cooked meat). Ten grams of different meat products were added to 90 mL of one-quarter-strength buffered peptone water (pH 7.2) and homogenized in a Stomacher until smooth. All meat homogenates were autoclaved separately, in order to obtain a final solid media solution with 1.5% agar. The agar disc diffusion method (ADDM) was used to determine the antibacterial activities of the essential oils. A suspension (0.1 mL of 10^6 CFU/mL) of each microorganism was spread on the meat homogenates medium plates. Filter paper discs (9 mm) were impregnated with 30 µL of the each EO and placed on the inoculated plates; these plates were incubated at 37 or 26ºC for 24 h. The concentration effect (CE) was studied for to ascertain which EO concentration (30, 15, 7.5 and 3.75 µL) had an inhibitory effect on bacterial growth. All tests were performed in triplicate.

The *in vitro* antibacterial activities of *T. capitatus* and *T. zygis* EOs on several bacteria strains were qualitatively and quantitatively assessed by the presence or absence of inhibition zones using the agar disc diffusion method. All bacteria tested were sensible to *T. capitatus* and *T. zygis* EOs in all meat homogenates medium. In minced meat, the ADDM indicated that *T. capitatus* EO showed the highest ($p<0.05$) antibacterial activity against two bacteria tested, with inhibition zones ranging from 29.06 mm on *S. marcescens* and 37.12 mm on *A. faecalis*. On the other hand, *T. zygis* EO showed highest inhibition halos against *L. innocua* (45.37 mm). As regards cooked meat, no statistical differences were found ($p>0.05$) between the *T. capitatus* and *T. zygis* EOs against *L. innocua* and *S. marcescens*. For *A. faecalis*, again, *T. capitatus* EO had the highest ($p<0.05$) inhibition halos. As regards dry-cured meat, *T. capitatus* and *T. zygis* EOs were not actives against *A. faecalis*. For *L. innocua*, *T. zygis* EO produced an average inhibition zone ($p<0.05$) of 50.97 mm, while *T. capitatus* EO provoke an inhibition halo of 38.59 mm. For *S. marcescens*, *T. capitatus* and *T. zygis* EOs showed ($p>0.05$) inhibition zones of 16.60 and 16.64 mm, respectively.

Regarding to CE, in minced meat, *T. capitatus* and *T. zygis* EOs showed ($p<0.05$) inhibitory effect, in all added concentrations, for all bacteria tested, being *T. zygis* EO more effective ($p<0.05$) than *T. capitatus* EO against both *A. faecalis* and *L. innocua*. In cooked meat the *T. capitatus* EO showed inhibitory effects ($p<0.05$) in all added concentrations, against all bacteria strains tested. In the same way, *T. zygis* EO showed inhibitory effects ($p<0.05$) on all tested bacteria at all concentrations. *T. capitatus* EO showed higher inhibition halos than *T. zygis* EO, at all concentrations, against all bacteria strains tested. In dry cured meat any of the two EOs studied did not show inhibitory effects in any added concentrations, for *A. faecalis*. In the case of *L. innocua* and *S. marcescens,* both EOs showed inhibitory effect in all concentrations added, with no statistical differences ($p>0.05$) between them.

The essential oils of *Thymus capitatus* and *Thymus zygis* obtained from organic growth might be used to control the development of bacteria related with the food spoilage in a variety of meat and meat products. However, the use of plant-derived antimicrobials in food may be hampered by effective concentrations, interference by food constituents or other food-grade compounds, unsuitable water activity, incompatible pH or processing regiment.

Keywords: Essential oils; Thymus,organic growth, antibacterial, food homogenates

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Benzoxaborol-containing derivatives of amphotherycin B

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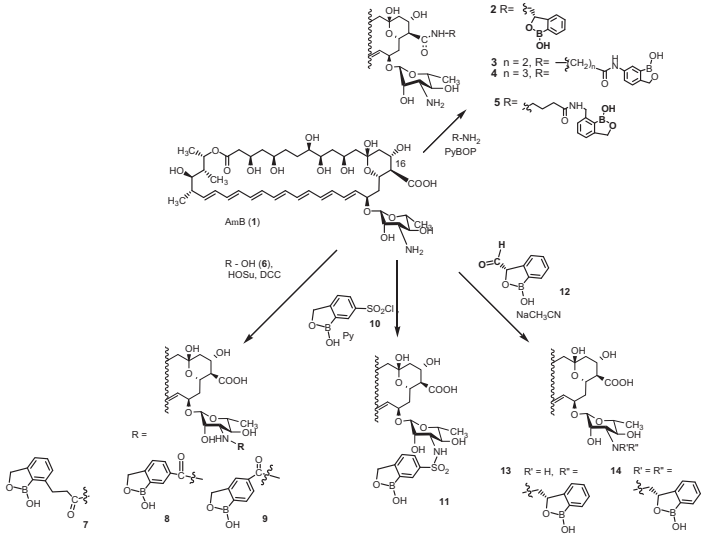
The aim of this study was the introduction of a fragment of benzoxaborole in a molecule of antibiotic amphotherycin B (AmB) in order to change the properties of the latter – broaden spectrum of activity, overcome resistance to the antibiotic as boronic acids and boroles readily interact with different nucleophile structures including dioles, aminoalcohols, hydroxyl- and amino acids, and in living cells with carbohydrates, peptides and RNA [1]. We We developed methods of synthesis of different types of benzoxaborole containing derivatives of AmB (Scheme 1) and evaluated their biological activity. Four types of conjugates of benzoxaborol with AmB 1 were synthesized: C-16 amides, 3'-N-acyl и 3'-N-alkyl derivatives, and 3'-N-sulfo-derivatives. Four types of conjugates of benzoxaborol with AmB 1 were synthesized: C-16 amides, 3'-N-acyl и 3'-N-alkyl derivatives, and 3'-N-sulfo-derivatives. Amides were obtained by the interaction of AmB with amines [(*S*)-3-(aminomethyl)benzo[c][1,2]oxaborol-1(3H)-ol or its derivatives acylated with ω-aminoacids] at the presence of PyBOP. For the synthesis of 3'-N-acyl-derivatives we used activated (N-OSu) eithers of bezoxaborol-containing carboxylic acids. The reaction of AmB with 1-hydroxy-1,3-dihydrobenzo[c][1,2]-oxaborol-6-sulfonyl chloride **10** in pyridine-DMF at the presence of Et_3N led to the sulfamide **11**. Reductive alkylation of AmB with (*R*)-2-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-3-yl)acetaldehyde **12** at the presence $NaBH_3CN$ led to the mixture of mono- and di-alkyl derivatives **13** and **14**, which were separated by column chromatography. Antifungal activity of the derivatives obtained was investigated in vitro against *Candida albicans* (ATCC 14053)*, Cryptococcus humicolus* (ATCC 9949), *Aspergillus niger* (ATCC 16404), *Fusarium oxysporum* (VKM F-140). The derivatives **5** and **13** had activity superior to the activity of AmB.



Scheme 1.

The work was partly supported by grant of Russian Foundation for Basic Research 13-03-00643 a.

Keywords: amphotherycin B; benzoxaboroles

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Biological and Phytochemical Evaluation of *Ajuga chamaepitys* ssp *palestina* and *Ajuga chamaepitys* ssp *cypria* from Turkey

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Ajuga L. is one of the largest genera of the Lamiaceae family with 301 species all over the world. *Ajuga* species are used in traditional medicine to treat diabetes, malaria, dysentery, and are known to have antiinflammatory, antifungal, antimicrobial activities [1].

The purpose of this study was to investigate the potential antimicrobial and antioxidant activity the air dried aerial parts of the 70 % methanol extract of *A. chamaepitys* (L.) SCHREBER ssp *palestina* (BOISS.) BORNM. (collected from Tavas-Denizli) and *Ajuga chamaepitys* (L.) SCHREBER ssp *cypria* P. H. DAVIS (collected from Babadağ-Denizli). Antimicrobial activity was performed against *Escherichia coli* NRRL B-3008, *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 13311, *Bacillus cereus* NRRL B-3711, *Candida albicans* ATCC 90028, *Candida tropicalis* ATCC 1369 and *Candida parapsilosis* ATCC 22019 strains, according to the *in vitro* CSLI microdilution methods [2,3]. Ampicilline, tetracycline, ketoconazole and oxiconazole were used for comparision as standart antimicrobial agents. Antioxidant activity was determined and calculated as IC_{50} values by a photometric absorbance based *in vitro* DPPH radical scavenging method compared to the standard agent butylated hydroxytoluene (BHT).

Minimum inhibitory activity was determined both extracts as 312.5 µg /ml against the tested bacterial strains, whereas the antifungal activity was found more effective for *A. chamaepitys* ssp *palestina* extract against *Candida tropicalis ATCC 1369* with the MIC= 78.12 µg /ml value. The antioxidant activity of *A. chamaepitys* ssp *palestina* and *A. chamaepitys ssp cypria* extracts were IC_{50} = 1.30 and 1.80 mg/ ml, respectively, when compared to BHT [IC_{50}= 0.06 mg/ml].

Phytochemical constituents were determined by LC-MS/MS analysis; where luteolin and derivatives, apigenin and derivatives, caffeoyl and coumaroyl glucosides, citric acid and forsythoside A were identified as the major constituents within the plant extracts.

The i*n vitro* experimental results suggest that the traditional use of *Ajuga sp.* plant extracts may have some impact on the antimicrobial utilization in the connection with rather weak radical scavenging activity.

Keywords: Antioxidant; antimicrobial activities, LC-MS/MS analysis;

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Bottlenecks to pseudomonic acid C production by *Pseudomonas fluorescens*

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Mupirocin is a polyketide antibiotic produced by the soil bacterium *Pseudomonas fluorescens* NCIMB 10586. It is a mixture of pseudomonic acids (PA) (Fig 1A) with PA-A comprising 90%. Mupirocin targets bacterial isoleucyl-tRNA synthase and is used topically to combat methicillin resistant *Staphylococcus aureus* (MRSA). A related compound thiomarinol (Fig 1B) produced by isolates of the marine bacterium *Pseudoalteromonas* is an amide formed from the condensation of a pyrrothine to marinolic acid. Marinolic acid is most similar to PA-C, which has a 10,11-alkene, except that it possesses an 8-hydroxyoctanoic acid side chain instead of 9-hydroxynonanic acid. Mupirocin and marinolic acid are produced by similar type I polyketide synthase pathways[1,2].

The 10,11-epoxide in PA-A makes it susceptible to intramolecular attack by the 7-OH outside a narrow pH range, which limits its clinical utility. PA-C, lacking the epoxide, has enhanced chemical and *in vivo* stability and may have the potential for improved clinical use. Our aim therefore was to create a strain of *P. fluorescens* in which the epoxidase activity had been knocked out and all production was directed to PA-C.

Through in-frame mutagenesis we identified the gene responsible for epoxidation of PA-A[3]. However PA-C titres were still low. This suggests that later biosynthetic steps have strong substrate specificity for epoxidised PA. Since *Pseudoalteromonas* produces non-epoxidised marinolic acid the *tml* genes that work after epoxidation may allow efficient processing of a non-epoxidised molecule. We therefore propose to identify the rate limiting steps to PA-C production by cross complementing the mupirocin pathway with genes from the thiomarinol pathway.

Metabolite profiling of wild type and various mutants indicates that epoxidation occurs at the end of monic acid assembly but prior to tetrahydropyran (THP) ring formation. The enzymes responsible for THP formation in thiomarinol are the Rieske oxidoreductases TmlW and TmlT. These were expressed *in trans* to a triple mutant of the mupirocin cluster which has the epoxidase and Rieske oxidoreductases homologues MupW and MupT knocked out. Results on the levels of PA metabolite production will be presented.

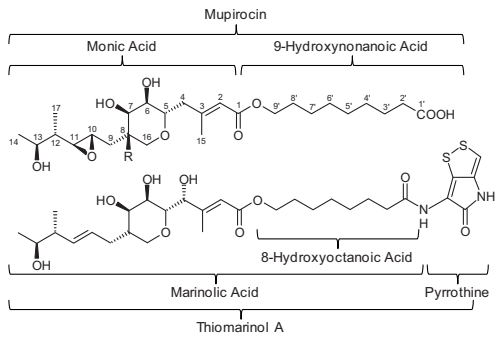

Figure 1A. Structure of Mupirocin

PA-A: R=H
PA-B: R=OH
PA-C: R=H, C10/C11 *E*-alkene
PA-D: R=H, C4′/C5′ *E*-alkene

Figure 1B. Structure of Thiomarinol A

Keywords: mupirocin; polyketide, antibiotic, MRSA

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Candicid's activity of exopolysaccharides and polysaccharides fungi extracts from Amazonian

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The diversity of microbiota in Amazon Rainforest has an amazin underexplored and unknoleged biological and chemist potential. Basidiomycets and *Pestalotiopsis* fungis are usually find in this area. The both appears great potential polysaccharides, basidiomycetes are fungi with high potential for production of enzymes used in bioremediation of environments contaminated by toxic molecules, as food, natural insecticides and other biotechnological applications. *Pestalotiopsis* are found as endophytc fungi, phytopathogens or saprophytes. *Candida* is a fungal pathogen responsible for opportunistic infections in humans. Grows on the surface of medical devices causing infections in debilitated patients. We started these way with two cultures: one basidiomycete and another *Pestalotiopsis* sp. Material and Methods: Samples of the fungi collection of work GEMMA Group (Study on Mass Spectrometry and Microorganisms Amazon Group) were reactived in PDA culture medium for 8 days at 26 ^{o}C. After this period two fungi (basidiomycetes and *Pestalotiopsis* sp.) was inoculated into 100 ml liquid medium and cultivated in BDL static mode at room temperature (+ / - 26 ^{o}C) for 18 days. Then the mycelium was separated from the medium by vacuum filtration to proceed to extraction of the mycelium and culture medium. Was added 5% methanol to the culture medium and stored at -20 °C. The mycelium was macerated with ethyl acetate for 24 h repeated process was 3 times and then the mycelium was covered with 100% methanol at 24 h which was filtered and the mycelium discarded. The experiment was performed in triplicate and the means of culture of each fungus were combined, totaling 300 ml for each fungus, which was added 1 L of methanol for 48 left at 4 °C. The precipitate formed was centrifuged off and the remaining organic phase the solvent was evaporated in rotary evaporator (RE) The organic phase was left was subjected to liquid-liquid extraction and extracts all the solvent had been removed and concentrated by rotary concentration. Four samples were selected for testing candicids: (1) inorganic partitioned basidiomycete RE (2) *Pestalotiopsis* mycelium (3) Basidiomycete culture medium, (4) basidiomycete of the organic medium. After testing the biological activity was observed in all extracts candicid subsequently tested concentrations were 1000, 750, 500 and 250 ug / ml to determine the value of the minimum inhibitory concentration, and it was found that sample 1 showed no inhibition less satisfactory than 1000 pg / ml, while 2 showed inhibition up to 500 ug / mL, 3 and 4 have a potential of less than 250 mg / ml satisfactory inhibition. These good results show the potential of bioactive molecules Amazonian fungi.

Keywords: polysacharide; endofitics fungi, candida inibitor

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Caraway essential oil combinations with antifungal drugs against *Candida* sp. and cytotoxicity assessment

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Caraway (*Carum carvi* L.) is a well-known aromatic plant of the Apiaceae family and is used in the formulation of liqueurs, mouthwashes, toothpastes, soaps, and perfumes, *etc*. Also, caraway herbal preparations are popularly used as antispasmodic, carminative, appetite stimulants, and for its antimicrobial effects [1, 2].

In the present research work, commercially available Pharmacopoeia Grade (PhEur) Caraway essential oil (Carvi aetheroleum) was used for the anticandidal combination experiments. Prior the combinations with the current antifungal agents, the essential oil was fractioned using a silica column by *n*-pentane, diethyl ether and methanol, respectively. The chemical composition of the oil as well as its fractions were determined both by GC/FID and GC/MS techniques. All fractions of the essential oil were also tested in combinations for their inhibitory activities against a panel of pathogenic *Candida* sp.

The caraway essential oil combination with antifungal drugs oxiconazole and terbinafine against clinical *Candida* sp. and standard *Candida albicans* ATCC 90028, *C. glabrata* ATCC 66032, *C. tropicalis* ATCC 1369 strains were screened in comparison, using the *in vitro* CLSI microdilution method with the aid of the automated liquid handling system (Biomek 4000, Beckman & Coulter). *C. carvi* essential oil and antifungal drug combinations were evaluated using the chequerboard assay method. Resulting fractional inhibitory concentrations were calculated and interpreted as synergic, additive or antagonistic effects. Cytotoxicity of compounds were evaluated using Cytotox-XTT-1 Parameter Kit (Xenometrix AG, Switzerland) as well as WS1 cells (ATCC® CRL-1502™, human normal skin fibroblast cell line). IC_{50} values were calculated by non-linear regression analysis.

According the chromatographic and spectroscopic analyses limonene (43.4 %) and carvone (54.5) were determined as the main compounds of *C. carvi* essential oil, which was in agreement with the EurPharm. monograph. Indifferent interactions were noted for *C. carvi* essential oil and antifungal drug combinations of oxiconazole and terbinafine in the anticandidal assay. Essential oil fractions resulted in synergic and additive effects against some *Candida* species, but no antagonistic effects were observed at the tested concentrations. Additionally, the fractional inhibitory concentration index (FICI =0.53; 0.14; 0.26; 0.51 microgram/mL, respectively) for the essential oil combinations were determined. *In vitro* cytotoxicity for C. carvi essential oil, oxiconazole and terbinafine were measured in a WS1 cell line assay, where the IC_{50} values were calculated as 204.58, 27.08 and 56.68, respectively. Overall, the initial results suggested that the cytotoxic effects of essential oil-drug combinations decreased.

Keywords: *Carum carvi* L. essential oil; anticandidal and cytotoxic activity; antifungal combination

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Characterization of the Chamomile (*Matricaria recutita* L.) essential oil, its fractions and antimicrobial effects in combination with antimicrobial agents

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As a popular member of the Asteraceae family, German Chamomile (*Matricaria recutita* L.) and its preparations are used among many other uses against many skin diseases as well as antiinflammatory.

In this present study, commercial Pharmacopeia (PhEur) grade chamomile essential oil (Matricariae aetheroleum) was combined with different antimicrobial agents such as ampicilline sodium, cefuroxime acetyl, tetracycline hydrocloride, fluconazole and nystatine, respectively. All combinations were evaluated *in vitro* against pathogenic standart and clinical resistant Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus)* bacterial isolates as well as against *Candida albicans* for their broad antimicrobial effectiveness. Additionally, the essential oil was fractioned by column chromatography using *n*-hexane, diethly ether, dichloro methane and methanol, respectively. Additionally all fractions of essential oil were tested in combinations for their Minimum inhibitory concentrations (MIC) as well as for their Fractional inhibitory concentrations (FIC) against the resistant microbial pathogens.

The essential oil, fractions and antimicrobials were evaluated for their efficacy by the standard CLSI microdilution method [1-2] using an automated liquid handling system (Biomek 4000, Beckman&Coulter). Initially, the antimicrobial interactions were assayed using the chequerboard assay. According the results, FICs were calculated and interpreted as synergic, additive or antagonistic effects. The chemical compositions of the investigated oil as well its fractions was determined both by GC/FID and GC/MS techniques.

The analyses proved that α-bisabolol oxide A (47.7 %), *(Z)-β*-farnesene (21.5 %), chamazulene (4.1 %), α-bisabolol oxide B (% 6.2), α-bisabolone oxide A (5,8 %), and α-bisabolol (2.2 %), respectivetly, were the major compounds and in compliance with PhEur. Antimicrobial evaluations using the chequerboard method of *M. recutita* essential oil in combination with ampicilline, cefuroxime, tetracycline resulted as “indefferent” against the tested resistant clinical bacterial isolates. However, the essential oil combination of fluconazole and nystatine showed “synergic and additive inhibitory effects” againts the clinical *Candida* strain. Observed FICs were calculated as 0.3125 and 0.56 µg/mL respectively**.** As a conclusion, essential oils and antimicrobial agent combinations may be potential alternatives againts resistant clinical isolates and may reduce the adverse impact of synthetic antimicrobial drugs.

Keywords: *Matricaria recutita* L.; essential oil; chequerboard; antimicrobial combination.

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Chemical Characterization and Biological Evaluation of the Volatiles of *Alnus glutinosa* subsp. *barbata* Gaertn. and *A. orientalis* var. *pubescens* Decne.

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The genus *Alnus* Mill. of Betulaceae, is represented by 5 species in Europe and by 23 sp. all over Asia. It is also represented by two species, namely *Alnus glutinosa* Gaertn. and *Alnus orientalis* Decne. as well as six subsp. in Turkey [1,2]. Natively *Alnus* species are used traditionally because of anticancer, antibacterial, antiviral and antifungal potential [3].

In this present study, air dried leaves of *A. glutinosa* subsp. *barbata* and *A. orientalis* var. *pubescens* were collected from Artvin and Antalya, respectively, which were hydrodistilled for chemical characterization. The volatiles obtained were investigated by GC-FID as well as GC-MS. As analytical results, the major compounds were identified as rimuene (33.3 %); kaur-16-ene (32.7 %) for *A. glutinosa*; and geraniol (13.7 %); hexadecanoic acid (7.9 %) for *A. orientalis*, respectively. The antimicrobial activity of the volatiles of *Alnus* sp. was evaluated against a pannel of 8 different Gram-positive and -negative as well as *Candida* sp. using CLSI microdilution method.

Keywords: *Alnus sp.*, volatile, rimuene, geraniol, antimicrobial activity, CLSI.

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Chemical Characterization of the Volatiles and Fixed Oil of the Seeds of *Nigella damascena* L. and Biological Evaluation

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Nigella damascena L.*, a* member of the Ranunculaceae, is a as relative of black cumin and its seeds are also used similarly in Turkey. To evaluate the chemical composition and for comparison the air dried seeds of *N. damasacena* were hydrodistilled using a Clevenger apparatus and extracted in Soxhlet apparatus, respectively. Column chromatography of the essential oil resulted in two fractions. Both the volatiles and fixed oil were analysed by both GC-FID and GC-MS on different polarity columns. β-Elemene (64.2 and 50.5%), α-selinene (10.6 and 10.9%) and 7-*epi*-α-selinene (8.6 and 6.8%) were the major volatile component while linoleic acid (48.5 and 50.3%), oleic (28.3 and 29.6%) and palmitic acids (10.7 and 11.0%) were the major fatty acid components. In addition biological activity of *N. damascena* seed volatiles and fatty acids were evaluated using *in vitro* photometric antioxidant, antimicrobial and soy bean lipooxygenase bioassays. As a result of the antimicrobial evaluation against a pannel of 8 different Gram-positive and -negative as well as *Candida* sp. MIC values of 0.8-1.25 mg/mL were observed using a microdilution assay according the CLSI microdilution strandards.

Acknowledgements: This project was supported by the Anadolu University BAP 1404S106

Keywords: *Nigella damascena*, essential and fixed oil, β-elemene, damascenine, linoleic acid, biological activity.

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Cinnamic acid in the control of planktonic and sessile cells of *Escherichia coli* and *Staphylococcus aureus*

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Surface disinfection is one of the frontline strategies to prevent infectious diseases. However, the emergence of resistance against the commonly used biocides has lead to an increasing demand for new antimicrobial compounds. Furthermore, there is strong evidence of a possible relationship between resistance to biocides and cross-resistance to antibiotics. Consequently, the effective control of bacterial growth is of prime importance. Therefore, it is critical to explore new resources to produce effective biocides and plant secondary metabolites (phytochemicals) are an interesting and sustainable source of new bioactive molecules.

In this study, two phytochemicals, cinnamic acid and ferulic acid, were tested for their antimicrobial properties in comparison with two commonly used disinfectants, sodium hypochlorite and hydrogen peroxide. Minimum inhibitory and bactericidal concentrations (MIC and MBC), for *Escherichia coli* and *Staphylococcus aureus*, were determined for the selected phytochemicals. The effects of the selected antimicrobials were also assessed on monolayer adhered bacteria and on biofilm prevention by colony forming units (CFU) determination.

One of the benchmark disinfectants, hydrogen peroxide, was not successful in controlling adhered and biofilm cells of *S. aureus*. Contrarily, sodium hypochlorite was the most efficient chemical with almost 100% CFU reduction achieved for adhered and biofilm cells of *S. aureus* and *E. coli*. Regarding the chosen phytochemicals, ferulic acid was only capable of reducing aproximatly 3 log CFU/cm^2 of monolayer adhered *E. coli,* being inneffective against *E. coli* biofilms and adhered and biofilms of *S. aureus*. Curiosly, the effciency of cinnamic acid was comparable to sodium hypochlorite in controlling adhered bacteria, despite its higher MIC and MBC (Table 1). In fact, total CFU reduction was achieved for the monolayer adhered cells of both bacteria. A mild CFU redution of biofim cells was obtained.

The overal results demonstrate that cinnamic acid has antimicrobial activity against a Gram-positive and a Gram-negative bacterium. This phytochemical is able to effectively control monolayer adhered bacteria and reduce significantly the viability of biofilms, to values comparative to those obtained with sodium hypochlorite application. Moreover, this study reinforces that phytochemicals are an auspicious alternative to commonly used disinfectants for general disinfection practices.

Table 1 - Minimal Inhibitory and Bactericidal Concentration (MIC and MBC, respectively) of each chemical for *Staphylococcus aureus* and *Escherichia coli*

	Staphylococcus aureus		*Escherichia coli*	
	MIC (mM)	MBC (mM)	MIC (mM)	MBC (mM)
Cinnamic Acid	25	25	15	> 25
Ferulic Acid	25	> 25	> 25	> 25
Hydrogen Peroxide	400	450	16	20
Sodium Hypochlorite	9	10	5	7

Keywords: Antimicrobial resistance, Disinfection, Phytochemicals

Common human parasites and pathogens and their natural remedies in the USA

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This presentation is based on our diagnostic work and research at the Parasitology Center, Inc. (PCI), in Scottsdale, Arizona, USA. A brief introduction addresses exposure to parasitic infections, testing, impact on public health, and the diagnosis of common parasitic infections. The most common protozoan and helminth (worm) parasites as well as pathogenic bacteria in the USA are cosmopolitan. Our work and products are, thus, applicable world-wide where they are also distributed. The primary focus here is to introduce our natural herbal anti-parasitic remedy comprising three formulas, FREEDOM/CLEANSE/RESTORE (F/C/R). F/C/R is the most popular and effective anti-parasitic remedy in the USA today. FREEDOM frees and protects the body from parasitic infections. CLEANSE cleanses the intestine from parasite toxins, promotes regularity, and integrates organ system functions. RESTORE supports the integrity and regeneration of tissues damaged by parasite feeding, migration, and toxic byproducts. F/C/R also has a wider application for use in infected pets. At PCI, we also diagnose pet parasites. Published sources used to formulate F/C/R as well as reports from clients who have used the product are included. All topics are presented with illustrated labeled pictures of the various kinds of parasites and bacterial pathogens tested for in our facility and their gross pathology in human tissues, when applicable. GI symptoms caused by pathogenic bacteria are similar to those caused by intestinal parasites.

Key words: Natural herbal remedies; Freedom/Cleanse/Restore (FCR); USA; anti-parasitic; anti-bacterial; anti-fungal; Freedom/Cleanse/Restore (FCR);

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Comparative study of phytochemical compounds, antioxidant and antimicrobial capacities of six ecotypes of Chilean quinoa (*Chenopodium quinoa* Willd.)

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Quinoa (*Chenopodium quinoa Willd.)* is a native plant of the Andean region [1]. The Incas appreciated its high nutritional value due to the exceptional balance between oil, protein and fat contents [2]. Quinoa has gained an increasing interest in recent years due to its nutritional value as well as its antioxidant capacity. The aims of this work were to determine isoflavone, flavonoid, polyphenol as well as the antioxidant and to explore the antimicrobial capacity of six ecotypes of quinoa cultivated in three different zones of Chile. The samples are called Ancovinto and Cancosa (North), Cáhuil and Faro (Center), and Regalona and Villarica (South). Total phenolic content (TPC) were determined colorimetrically using Folin-Ciocalteau reagent and Isoflavones by means of HPLC analysis. Total flavonoid content of the quinoa extracts was performed following the protocol described by Kim et al. (2003) [3]. Antioxidant capacity was determined by means of DPPH and ORAC analysis. Regarding microbiological analysis, *Listeria innocua* and *Saccharomyces cerevisiae* were analyzed. Results have shown that all ecotypes of Chilean quinoa could be considered good sources of polyphenolics (161.32±14.40 mg/ 100 g DM). The North and Central ecotypes exhibited the highest isoflavones concentration. The Cancosa from the North was the ecotype that showed the highest flavonoid content (211.06 mg CET 100/ g DM). Based on the ORAC assay, Ancovinto, Cancosa and Faro presented the highest antioxidant capacity (67.6 mmol/ 100 g DM). Regarding antimicrobial activity, Regalona ecotype showed the best performance against *S. cerevisiae and L. innocua* (Fig 1).

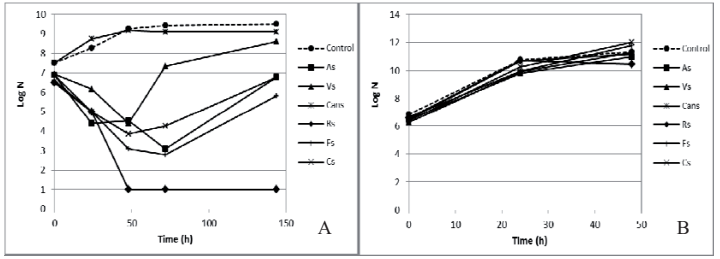

Fig. 1. Antimicrobial activity against *Saccharomyces cerevisiae* (A) and *Listeria innocua* (B). Control: broth without quinoa, A_f: Full suspension of Ancovinto, V_f: Full suspension of Villarrica, A_s: Supernatant of Ancovinto, V_s: Supernatant of Villarrica, Can_s: Supernatant of Cancosa, R_s: Supernatant of Regalona, F_s: Supernatant of Faro, C_s: Supernatant of Cahuil.

Keywords: Quinoa ecotypes; isoflavones; total polyphenolics; antimicrobial activity.

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Comparative study of the antimicrobial activity of garlic against allicin

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The use of natural products or components thereof in order to control the activity of pathogenic microorganisms or cause changes in foods has increased dramatically during recent years.

In this study we evaluate the antimicrobial activity of crude garlic and allicin derived from the same against bacteria and fungi is provided.

The method used is that of disk diffusion. Microorganisms under study were: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella sp*. *Bacillus cereus*, and *Pseudomonas aeruginosa*, among bacteria and *Candida albicans*, *Penicillium rugulosum*, *Aspergillus flavus* and *Fusarium moniliforme* among yeasts and mold.

After studies we can state that raw garlic posses a remarkable and more marked inhibitory activity than allicin, against all microorganisms tested The most sensitive microorganisms among all tested in front of raw garlic, were *Escherichia coli* and *Pseudomonas aeruginosa* between bacteria *and Penicillium rugulpsum, Fusarium moniliforme* and *Candida albicans* between moulds and yeast assayed. Allicin only showed activity in front of *Escherichia coli.*

Keywords: allicin; garlic; bacteria; inhibitory activity; moulds; yeast

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Compare the antimicrobial activities of various commercial essential oils of cinnamon and rosemary

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The aim of this study was to investigate and compare the antimicrobial effects of commercially cinnamon and rosemary oils over the standard strains of some clinically important microorganisms. Standard strains of eleven bacteria and two *Candida albicans* strains were included in this study. Also 3 different trademarks of cinnamon oil and 5 different trademarks of rosemary oil were used in the study. Essential oil compositions were illuminated by GC-MS. The antimicrobial activities of oils were determined by the disc diffusion method. The study was conducted double repetitive. The cinnamon oil of all trademarks showed strong antimicrobial activities against to the all isolates. The zone diameters of oil are listed in table. The results indicate that especially cinnamon oil can be an alternative in pharmaceutical industry due to its antimicrobial activity.

Table: The zone diameter of cinnamon oil and rosemary oil (mm).

	Cinnamon oil			Rosemary oil				
Strains	1	2	3	1	2	3	4	5
***Enterococcus faecalis* ATCC 19433**	20	17	33	-	12	-	-	-
***Enterococcus faecalis* ATCC 29212**	30	15	34	-	14	-	-	-
***E.coli* ATCC 25922**	38	20	39	-	18	-	-	16
***E.coli* ATCC 35218**	33	14	36	-	16	-	-	24
***S.aureus* ATCC 29213**	37	13	40	-	12	-	-	-
***S.aureus* ATCC 25923**	50	15	45	-	12	-	-	-
***MRSA* 43300**	50	24	>50	-	13	-	-	-
***Pseudomonas aeruginosa* ATCC 9027**	20	15	22	-	12	-	-	-
***Pseudomonas aeruginosa* ATCC 27853**	18	18	21	-	14	-	-	-
***Bacillus subtilis* ATCC 6633**	40	19	40	-	-	-	-	-
***Klebsiella pneumonia RSKK* 574**	35	19	39	-	14	-	-	-
***Candida albicans* ATCC 10231**	>50	>50	>50	-	14	-	-	16
***Candida albicans* ATCC 033**	>50	>50	>50	-	14	-	-	16

Key words: antimicrobial activity; cinnamon; rosemary; essential oil

Comparison of *In vitro* anticandidal activity of *Thymus vulgaris* and *Myrtus communis L* against *Candida albicans* strains isolated from the patients with oral candidiasis with Nystatin

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Candida albicans is the most frequent etiological agent of oral candidiasis. Nystatain is a choice drug in the treatment of this infection, but it has some problems through its side effects. In recent years, the use of plant products has been increased for treatment of different fungal infections. The aim of this study was to determine the Minimum Inhibitory Concentration (MIC) of the essence of *Thymus vulgaris*, *Myrtus communis L* and the mix of these essences on the growth of *Candida albicans* in solid media, and also their MIC was compared with the MIC of Nystatain in the same conditions.

Thirty-two strains of *Candida albicans* isolated from patients with oral candidiasis were studied in this research. We provided a yeast suspension of candida yeast cells, and also a serial dilution from these essences and Nystatin in Sabouraud Dextrose Agar(SDA) medium. A loop of candida suspension was cultured on all of the solid media and was incubated at 25°C. The obtained results were recorded during 7 days.

Our finding showed that the MIC of *Thymus vulgaris*, *Myrtus communis L*, mix of these essences and Nystatin was 0.390µl/ml, 12.5 µl/ml, 0.78 µl/ml and 160 IU/ml respectively.

It seems that *Thymus vulgaris* has antifungal activity against *Candida albicans*. These plant products are inexpensive and their side effects probably are very lower than Nystatin.

Key words: *Thymus vulgaris*, *Myrtus communis L*, Nystatin, *Candida albicans*, oral candidiasis

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Comparison of treatment effect of propolis with griseofulvin on improvement of dermtophytic lesions resulting *of Trichophyton violaseum* and *Microsporum gypseum*

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Bee propolis ethanolic extract was investigated for its antidermatophytic properties and then was compared with griseofulvin. The lesions resulted from *Trichophyton violaseum* and *Microsporum gypseum* were treated by propolis extract and griseofulvin in separate groups. All lesions were improved after treatment with propolis extract and eliminated 35 days after starting of treatment. Also, the treatment of lesions with griseofulvin was done successfully but, the response period to drug was different for *Trichophyton violaseum* and *Microsporum gypseum*. The response to treatment in case of *Trichophyton violaseum* was better than *Microsporum gypseum*. Finally, we could conduct that the propolis has a potential value for treatment of dermatophytic infections but further researches is necessary.

Keywords: *Trichophyton violaseum*, *Microsporum gypseum*, Propolis, Griseofulvin, Dermthopytic lesions

Composition of fatty acids and antimicrobial activity of essential oils of the *Bertholletia excelsa*

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The etiology of bovine mastitis can be infectious, toxic, traumatic, allergic, or metabolic, with infectious causes being the majority of cases. Despite bacteria being the most frequently isolated agents, there are records of sporadic cases of environmental microorganisms in the literature, among which stand out the yeasts, especially the *Candida* spp. and *Cryptococcus* spp. [1]. The objective of this study is to evaluate the antimicrobial activity of the essential oil of *Bertholletia excels* oil, against yeasts, as well as to analyze its fatty acid composition. To test the antifungal, yeasts were selected from seven species: *Candida* spp. and *Cryptococcus laurenti*. Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the oil was performed using the technique of broth microdilution, as provided in CLSI 2008 (M27-A3 protocol for yeasts), adapted to a phytochemical compound. The constituents of the Brazil nut oil were determined by chromatographic, analysis performed using a Shimadzu GC-2010 (Shimadzu, Japan) capillary column Elite Wax (0.25 µmx30mx0, 25mm). The identified fatty acids were myristic, palmitic, palmitoleic, margaric, stearic, oleic, linoleic, linolenic, arachidonic, gadoleic, behenic and lignoceric. The essential oil analyzed had activity against the yeast at low concentrations, which ranged from 10.41 to 20.83 µg.mL^{-1}. It was observed that cyclophosphamide coincided with an MIC of 70.0%, indicating that this oil has fungistatic and fungicidal action against these strains. The importance of this essential oils antifungal activity against these micro-organisms is enhanced due to the rarity of antifungal drugs available suitable for the treatment of fungal mastitis, especially when compared to antimicrobial drugs commonly used to fight fungal disease. Noteworthy is the high sensitivity verified by *Cryptococcus laurentii*, when using *Bertholletia excels* oil, an average MIC and MFC 1.2 µg.mL-1. With respect to the isolates of *Candida* spp. oil analysis also showed inhibitory activity at low concentrations, which becomes relevant since among the yeasts,

Keywords: yeast; essencial oil, suscetibility

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Cymbopogon nardus against *Candida glabrata*: antifungal activity and time-kill assay

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Candida glabrata is an important fungal pathogen in the clinical practice due the level of inherent resistance to azole and the *emergence* of *antifungal resistance* in human medicine. Although it is a normal component of microbial flora, it can induce severe infections in immunocompromised patients [1]. The search for new molecules presenting antifungal activity is necessary, and so plant extracts may be candidates due to their high activity of secondary metabolites. The aim of this study was to test the antifungal activity of essential oil from *Cymbopogon nardus* (EOCn) against *C. glabrata* ATCC 2001 and clinical isolates. The minimal inhibitory concentration (MIC) of EOCn was determined according to the protocol described by Araújo et al. [2], with modifications. The initial concentration of EOCn was 2000 µg/mL. 0.1 mL was placed in a 96-well microtiter plate containing RPMI 1640 medium. Each well was inoculated with 0.1 mL of a suspension containing 2.5x10^3 cfu/mL of yeast. Amphotericin-B and fluconazole were used as controls of the antifungal activity with incubation for 48 h at 37°C. The MIC of sample was detected following the addition of 0.02 mL 2.0% triphenyltetrazolium chloride. The time-kill assay was performed according to Zore et al. [3], with modifications. In brief, Sabouraud broth medium (10 mL) containing 2.5 x 10^3 cfu/mL of *C. glabrata* and 2 x MIC of EOCn were incubated and aliquots of 0.4 mL were removed at different time intervals (30 min, and 1, 2, 4, 8, 12, 24 and 36 h), and re-suspended in Sabouraud broth medium and 100 µL were inoculated on Sabouraud agar plates. All plates were incubated at 37°C for 48 h. Amphotericin-B (32 µg/mL) was used as control. The number of colonies was counted and compared with controls. The results showed EOCn has effective antifungal activity with MIC of 1000 µg/mL for ATCC and clinical isolates, while fluconazole and amphotericin-B had MICs of 128.0 µg/mL (resistant) and 1.0 µg/mL, respectively. The time-kill assay curve showed that EOCn killed 100% of the ATCC strain within 24 h of exposure (Fig.1) and clinical isolates within 12 h (Fig.2). As *C. glabrata* has an intrinsic resistance to fluconazole, EOCn is a promising antifungal agent in the treatment of candidiasis caused by *C. glabrata.*

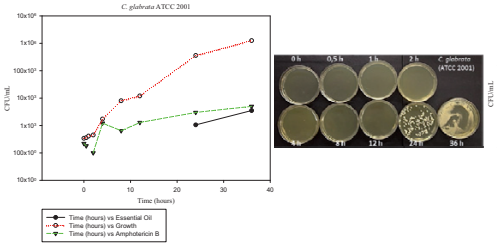

Figure 1: Effect of essential oil of *Cymbopogon nardus* on time-kill assay curve of *Candida glabrata* ATCC 2001

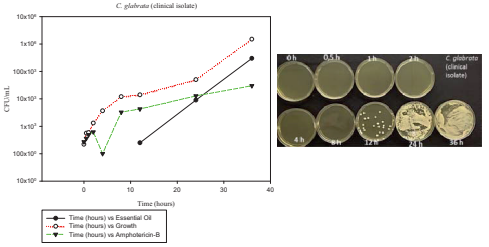

Figure 1: Effect of essential oil of *Cymbopogon nardus* on time-kill assay curve of *Candida glabrata* clinical isolates

Keywords: *Cymbopogon nardus*; essential oil; *Candida glabrata;* antifungal activity; time kill;

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Cymbopogon nardus: evaluation of the inhibitory effect on *Candida albicans* hyphae growth

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Candida albicans is a prevalent human fungal pathogen that exists as benign commensal in immunocompetent individuals but can become invasive and cause infections when the host immunity is impaired [1].The ability of *C. albicans* to colonize and proliferate in humans is closely related to its pathogenicity. *C. albicans* may exhibit yeast and filamentous forms (pseudohyphae, true hyphae). The hyphal form may be important for penetrating tissue surfaces [2]. Several chemical compounds have been analyzed for their ability to inhibit fungal cell, especially the azole and polyene. However, overuse of antifungal agents often leads to emergence of resistant strains. Therefore the search for new drugs with antifungal properties is a wide challenge. Plants produce a variety of medicinal components that can inhibit the growth of pathogens, and a considerable number of studies have been conducted to evaluate the antimicrobial activity of extracts and essential oils of medicinal plants. In this sense, the aim of this study was to evaluate the antifungal potential, *in vitro*, of essential oil (EO) and ethanol extract (EtE) from leaves of *Cymbopogon nardus* (L.) Rendle (citronella). A microassay was developed for evaluating the inhibition effect on the growth of fungal strains. Growth of *C. albicans* (ATCC 10231) from a 48 h culture were transferred to microplate with RPMI 1640 medium supplemented with fetal bovine serum to obtain a final concentration of $2.5x10^3$ yeast/ml. EO and EtE were added to the growth medium to concentrations ranging from 1000 µg/mL to 7,5 ug/mL, and the cultures were incubated for 12 and 24 h at 37 °C. The hyphal formation of *C. albicans* was observed through an inverted light microscope. Amphotericin B (16 ug/mL) was used as a positive control. The results exhibited the effect of EO and EtE on *C. albicans* hyphae growth. Microscopic observation of EO-treated fungal cells revealed an absence of filamentous cells to concentrations ranging from 1000 ug/mL to 15 ug/mL (after 12 h) and from 1000 ug/mL to 31ug/mL (after 24 h) (Fig. 1). EtE-treated cells showed the inhibition on the *C. albicans* hyphae growth to concentrations ranging from 1000 ug/mL to 250 ug/mL (after 12h and 24 h). Inhibition of hyphal development represent an effective measure to decrease *C. albicans* pathogenesis. In addition, the results presented this study are very encouraging for the development of new antifungal therapy from plants.

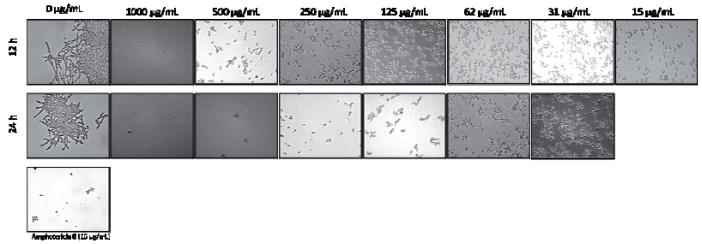

Figure 1: Effect of essential oil of *Cymbopogon nardus* on *C. albicans* hyphae growth to concentrations ranging from 1000 ug/mL to 15 ug/mL (after 12 h) and from 1000 ug/mL to 31 ug/mL (after 24 h). Amphotericin B (16 ug/mL) was used as a positive control.

Keywords: Cymbopogon nardus; Candida albicans; hyphae growth;

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Cytotoxicity and antifungal activity of cyanobacterial lipopeptides puwainaphycins and muscotoxins

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Among the less known cyanobacterial secondary metabolites with interesting bioactivities the but also potential toxic effects for human, cyclic lipopeptides may play an important role. They represent an abundant group of cyanobacterial peptides characteristic by the presence of lipophilic β-amino fatty acid and up to 14 residues in the cycle. Some 16 structural classes have been identified up to date. They display a broad range of bioactivities including cytotoxicity or antifungal and antibacterial activity and share some similarities to iturin family antibiotics. Recently, we have isolated two types of cyanobacterial lipopeptides (puwainaphycin [1] and muscotoxin) and characterized their structures and effect on human in vitro cell lines. We have demonstrated that their bioactivity lays in permeabilization of the cell membrane for calcium ions analogously to bacterial lipopeptide surfactin. Furthermore, we have found that this effect is caused solely by the interaction of the compound with phospholipid bilayer. Aside from the cytotoxic activity puwainaphycin variants were found to have anti-fungal activity against several parasitic fungi (e.g. *Sclerotinia sclerotiorum*) and only mild phytotoxicity when tested in field experiments. In present contribution we are discussing the possible use of puwainphycin as antifungal agent if field together with possible toxicological aspect caused by these compounds and the structure activity relationship for different puwainphycin and muscotoxin variants.

Keywords: lipopeptides, puwainaphycin, muscotoxin, cytotoxicity, anti-fungal activity

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Degradation of bacterial DNA by methyglyoxal, a highly bactericidal natural product from Manuka flowers

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Despite widespread availability of antibiotics, it is currently advised that clinical administration of antibiotics against bacterial infections requires a thorough monitoring due to emergence of multidrug resistant pathogenic strains. Therefore newer and more effective antimicrobials are in demand to treat such cases. On the basis of a report that aldehyde form of pyruvic acid called methylglyoxal (MG) could induce lanthanum sensitive Ca^{2+} transients for growth inhibition in *Escherichia coli* [1], a detailed study was carried out to determine the antibacterial potentiality of MG. It was observed that MG alone was distinctly active against *Staphylococcus aureus*, *Escherichia coli*, salmonellae, shigellae and *Vibrio cholerae*, while *Klebsiella pneumoniae*, *Pseudomonas* and *Acenetobacter* were less sensitive to this agent. MG was found to be highly bactericidal in nature since there was a sharp fall in the number of viable bacteria after addition of MG at the logarithmic growth phase and all the cells were killed within one hour. The rod shaped cells of *Escherichia coli* became round and adhered to each other after treatment with MG in one hour. Agarose gel electrophoresis study with the genomic DNA extracted from *Escherichia coli* ATCC 25922 revealed that addition of MG could completely degrade the DNA within one hour, pointing out to the distinctly high degree of sensitivity towards MG. Since it was known that MG is an ingredient of New Zealand's Manuka flower honey supplementation of commercially available honey distinctly augmented the antibacterial action of MG to a great extent.

In an intensive study to determine the mechanism of action of MG, molecular interaction between lipid and MG within the liposomal membrane was investigated. Multilamellar and unilammelar vesicles were prepared from 1, 2-dipalmitoyl-en-glycero-3 phosphocholine (DPPC) [2]. The effect of MG on DPPC liposomal membrane was studied by fluorescence spectroscopy and differential scanning colorimetry. Results indicated that MG could interact mainly with the DPPC head group that produced a significant increase in the fluidity of liposomal vesicles, which was possibly the cause of a fusion /aggregation effect in microbial cells.

Keywords : Methylglyoxal, Manuka honey, Bactericidal, Antimicrobial, Liposomal Membrane

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Detection of Minimum Inhibitory Concentration of Methanoloic Extract of Propolis against *Epidermophyton fluccosum* and its comparison with some species of genus *Microsporum* and *Trichophyton*

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The dermatophytes are filamentous fungi belonging to three genera *Trichophyton, Microsporum* and *Epidermophyton* that are able to cause infection of the skin, hair and nails.

The spread of resistance of fungi to available drugs, makes it necessary to find new antifungals for treatment of fungal infections.

The aim of this study was to detect the Minimum Inhibitory Concentration (MIC) against Epidermophyton fluccosum and its comparison with Trichophyton schhenlienii, Trichophyton tonsorans, Trichophyton violaseum, and Microsporum canis.

The antifungal activity of the methanolic extracts of the Iranian propolis was measured against Epidermophyton fluccosum, Trichophyton schhenlienii, Trichophyton tonsorans, Trichophyton violaseum, and Microsporum canis samples.

The minimal inhibitory concentration was determined following the agar dilution method. The susceptibility rate of them to extract was determined as follow: *Epidermophyton fluccosum> Trichophyton violaseum> Trichophyton tonsorans> Microsporum canis> Trichophyton schhenlienii*

The antifungal potential of the methanolic extracts of green and red propolis demonstrated suggest an applicable potential as an alternative treatment for dermatophytosis caused by these species.

Key words: propolis, dermatophytes, *Epidermophyton fluccosum*, *Trichophyton schhenlienii*, *Trichophyton tonsorans*, *Trichophyton violaseum*, and *Microsporum canis*

Effects of *Matricaria recutita* essential oil on non-albicans *Candida* biofilms

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Chamomile has many pharmacological activities such as anti-inflammatory, immunomodulatory and antibacterial [1]. However, few studies studied the effect of chamomile against *Candida* species. *Candida* spp. are related to superficial and invasive infections. Nowadays, there is a considerable increase in the *Candida* non-*albicans* isolation rate with pontential to develop antifungal resistence [2, 3]. The aim of this study was verify the antifungal activity of *Matricaria recutita* essential oil against *Candida* species in planctonic cultures and bioflms.

Matricaria recutita essential oil was obtained comercially (Ferquima Ind. Com. LTDA-Brazil). The oil was diluted in propylene glycol (1:9 v/v). The following reference strains were used in this study: *C. glabrata* ATCC 90030, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, *C. tropicalis* ATCC 13803 and *C. dubliniensis* NCPF 3108. Standardized inocula were obtained from 24-hours cultures suspended in fisiologic solution (10^6 cells/ml). The Minimum Inhibitory Concentration (MIC) were determined by microdilution assay using RPMI buffered with MOPS. After 24 hours of incubation (37 °C), MIC was determined by visual analysis and Minimum Fungicidal Concentration (MFC) was determined by subculture. Biofilms were formed on the bottom of 96-well plates containing RPMI buffered with MOPS supplemented with 2% glucose (n=6). After incubation for 24 hours (37 °C) under agitation (80 rpm), biofilms were exposed to concentrations of 2 and 4 times MIC for 5 minutes. Cell viability were determined by CFU/ml counting and compared with non-treated group (One-way anova, Tukey‘s test, 5%).

The essential oil showed inhibitory and fungicidal activity for all strains studied. The lowest MIC and MFC values were observed for *C. glabrata* (0.19 %; 0.39 % v/v) and the higher values were observed for *C. krusei* (12.5 %; 25 % v/v). Two and 4 times MIC significantly reduced CFU/ml counting for *C. tropicalis*, *C. parapsilosis* and *C. krusei* biofilms ($p < 0.01$). However, no significant reduction in biofilm viability was observed for *C. glabrata* and *C. dubliniensis* ($p > 0.05$).

Keywords: Candida; antifungal; Matricaria recutita

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Essential oils use as an alternative to antimicrobials in *Staphylococcus xylosus* and *Staphylococcus epidermidis* infections in horse

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Coagulase negative staphylococci (CoNS) are frequently isolated from nasal carrier and diseased horses, being *S. xylosus* and *S. epidermidis* the most prevalent species of CoNS detected in horses assisted at the Veterinary Clinical Hospital in Cordoba, Spain. An important percentage of them showed resistance to one or more antimicrobial agents (76.6%), highlighting the resistance to β-lactamics (66.6%), to methicillin (16.6%) and to clindamycin (13.3%). An *in vitro* study has been designed to determine the inhibitory and bactericidal effect of nine essential oils whose effectiveness has been showed against different pathogens, including Gram negative and Gram positive infectious agents (Huerta *et al*., 2004; Prabuseenivasan *et al*., 2006; Dussault *et al*., 2014). For this, the Kirby-Bauer disk-diffusion method, using impregnate disk with each essential oil and 30 isolates (27 *S. xylosus* and 3 *S. epidermidis*) was used. Furthermore, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the five essential oils with the major inhibitory effect in the Kirby-Bauer assay were determined by broth microdilution method (CLSI, 2009). Each test was conducted in triplicate, calculating the mean ± typical deviation for each isolate and bacterial specie. Reference quality control strain of *S. aureus* (ATCC 25923) was used.

According to the inhibition diameters obtained with the disk-diffusion method (Table), three categories were established: *oils without antimicrobial activity* (rosemary), *oils with moderated antimicrobial activity* (cinnamon, palmarosa, Indonesian clove, Madagascan clove and Niaouli) and *oils with notable antimicrobial activity* against *S. xylosus* and *S. epidermidis* (mint, oregano and *Thymus zygis*).

Essential oil	Disk-diffusion method		Microdilution method			
	S. xylosus	*S. epidermidis*	*S. xylosus*		*S. epidermidis*	
	∅ (mm) ± sd	∅ (mm) ± sd	MIC ± sd	MBC ± sd	MIC ± sd	MBC ± sd
Rosmary	6 ± 0.0	6 ± 0.0	-	-	-	-
Palmarosa	14 ± 2.5	15 ± 0.0	-	-	-	-
Cinnamon	16.25 ± 3.1	15 ± 0.0	-	-	-	-
Madag. clove	16.3 ± 3.7	15 ± 0.0	-	-	-	-
Indon. clove	16.8 ± 3.1	15 ± 0.0	0.45 ± 0.2	1.76 ± 1.1	0.25 ± 0.0	0.67 ± 0.3
Niaouli	21.9 ± 9.6	15 ± 0.0	0.84 ± 0.4	7.52 ± 4.6	1.11 ± 0.2	5.33 ± 2.3
Peppermint	41.5 ± 11.9	20 ± 0.0	1.36 ± 0.8	8.88 ± 5.1	1.67 ± 0.3	9.33 ± 6.1
Oregano	45.3 ± 7.3	35 ± 0.0	0.12 ± 0.04	0.52 ± 0.42	0.06 ± 0.0	0.21 ± 0.1
Thymus zygis	52 ± 0.0	52 ± 0.0	0.07 ± 0.05	0.31 ± 0.38	0.06 ± 0.0	0.12 ± 0.0

∅ (mm) ± sd: zone of inhibition in diameter ± typical deviation
MIC ± sd and MBC ± sd: Minimal inhibitory concentration and Minimal bactericidal concentration (% v/v) ± typical deviation

By microdilution assay, *Thymus zygis* (original plant of Iberian Peninsula), oregano and Indonesian clove oils showed the best results against both staphylococcal species. No significant differences were obtained between antimicrobial-sensible and multi-resistant isolates ($P>0.05$).

Keywords: *Staphylococcus xylosus*; *Staphylococcus epidermidis*; horse; essential oils; antimicrobial activity.

Eucalyptus bark derived extracts applied to *Helicobacter pylori* infection management

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Background: The spiral shaped Gram-negative bacterium *Helicobacter pylori* (*H. pylori*) is the etiologic agent of several gastric disorders, such as chronic gastritis and peptic ulcer disease [1]. Although asymptomatic in most individuals, persistent infection with this pathogen may lead to gastric carcinoma development [2]. The current therapeutic scheme relies on a combination of several pharmacological substances, but the cure rates obtained have been declining over the years, mostly due to bacterial resistance to antibiotics [3,4]. In this scenario, discovery of novel non-antibiotic substances that may be employed in managing *H. pylori* infection is of the utmost importance. In the last years, the bio-and pharmacological properties of Triterpenic Acids (TAs) have been highlighted, such as their potent antibacterial activity [5]. *Eucalyptus* spp bark residues have demonstrated to be a rich source of TAs, namely of betulinic, ursolic and oleanolic acids [6].

Methods: Four *H. pylori* strains (*H. pylori* J99; *H. pylori* 094UK; *H. pylori* 101UK and *H. pylori* 131UK) were used to access the anti-*H.pylori* potential of *Eucalyptus nitens* and *Eucalyptus globulus* lipophilic extracts, as well as that of pure TAs (betulinic, ursolic and oleanolic acids). For that, determination of Minimal Inhibitory Concentrations (MIC) were performed by the microbroth dilution method, with a range of concentrations from 8 µg/ml to 2 mg/ml. Due to the intrinsic coloration and turbidity that the compounds confer to the solutions, which makes the standard naked eye visualization of the results impracticable, the MIC protocol was adapted by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: Our results demonstrated that *E. nitens* bark lipophilic extract inhibited 3 of the 4 tested *H. pylori* strains with a MIC of 64 µg/ml, except the 101UK strain, which was inhibited by this extract in a concentration of 1mg/ml. Regarding the *E. globulus* extract, all the 4 *H. pylori* strains were inhibited in a concentration of 256 µg/ml. Concerning the pure tested TAs (oleanolic, betulinic and ursolic acid) all demonstrated to inhibit the tested *H. pylori* strains. However, when compared to the *Eucalyptus* extracts effect, the pure TAs required higher concentrations to achieve anti-*H. pylori* action. Of the three pure acids, ursolic acid was the one with the lower MIC (512 µg/ml), while oleanolic and betulinic acid MIC for the tested strains was established at 1mg/ml.

Conclusions: *Eucalyptus* nitens and *Eucalyptus globulus* lipophilic extracts have demonstrated anti-*H.pylori* activity in low concentrations. Moreover, a synergetic effect of the TAs present in both extracts is thought to contribute to the final observed anti-*H.pylori* effect, since the concentration required to inhibit the pathogen is lower than when using the TAs separately. These results bring new perspectives to employ these bioactive compounds in the quest for novel non-antibiotic therapies against *H. pylori*.

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Keywords: *Helicobacter pylori*; Gastric Cancer; New Therapies; Phytotherapy, Triterpenic Acids

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Evaluation of antimicrobial activity of processed spices used in sausage manufacturing

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The 'Nduja is a traditional Calabrian sausage manufactured with high amounts of different spices (ca. 5%), mostly represented by the red hot chili pepper that confers its typical flavor and piquancy. In addition, spices are well known for their antimicrobial activity against several microorganisms; in fact, no yeast and mould growth was found on this sausage throughout ripening and storage. In this study, we assayed the antimicrobial activities of processed sweet, hot chili pepper, and three spice mixtures used in industrial manufacturing of 'Nduja against ten food-borne bacteria (*Bacillus cereus* DSM4312 and DSM4313, *Yersinia enterocolitica* DSM4780, *Listeria monocytogenes* DSM20600, *Enterococcus faecalis* V583, *Aeromonas hydrophila* DSM30187, *Escherichia coli* ATCC 35401, *Helicobacter pylori* ATCC 43504, *Salmonella enterica* ATCC 13311, and *Staphylococcus aureus* NCTC 832). Each spice was extracted with eight parts of water, ethanol or methanol for 24 h. Extracts were five-times concentrated, and tested using agar disc and well diffusion assays. The antimicrobial activity was found only for hot chili pepper methanol extract and only against *Bacillus cereus* strains whereas the remaining extracts did not show any relevant activity. This work reports that the control played by industrial spice mixtures against foodborne bacteria is lower than that expected; this result suggests that it could be lost when spices are processed in order to be applied in meat curing.

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Keywords: Foodborne pathogens; spices extracts; Southern Italian sausage; meat curing

Evaluation of the activity of potential antimicrobials against lactic acid bacteria isolated from spoiled food sauces in media with or without added sucrose

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Although lactic acid bacteria (LAB) do not negatively affect human health and may have potential desirable effects on the organoleptic characteristics of food, they can also cause food spoilage by producing off-flavours and carbon dioxide. On this circumstance the intrinsic high resistance of LAB to acidic conditions and antimicrobial agents poses a problem to the food industry. Furthermore, most of the *in vitro* antimicrobial testing does not take into account the effect of certain abiotic conditions of the food matrices that may reduce or eliminate the antimicrobial effect.

The aim of this work was to evaluate the potential of three antimicrobials (chitosan, rosmarinic acid and sodium diacetate) against four aerobic LAB isolated from spoiled sauces under standard culture conditions and in media supplemented with sucrose at 10 g/L thus approximating as much as possible sauce conditions. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) values were determined and inhibition curves were plotted.

The results obtained showed that under standard conditions all compounds exhibited significant antimicrobial activity, with MIC values varying between 0.25 and 0.5 mg/ml for chitosan, 0.5 and 1 mg/ml for sodium diacetate and of 4 mg/ml for rosmarinic acid, and complete bacterial growth inhibition within 24 h of exposure. On the other hand, it was found that the activities of sodium diacetate and rosmarinic acid in the sucrose supplemented media were strongly affected. Growth of the selected LAB strains was not inhibited by rosmarinic acid upon 48h incubation and it was only 30% inhibited by sodium diacetate. Only chitosan was not affected by the presence of sugar as evidenced by the MIC/MBC values and the inhibition curves obtained that were the same in the presence of sugar..

The results show that despite the demonstrated potential *in vitro,* the use of rosmarinic acid and sodium diacetate as antimicrobial compounds can be impaired due to interactions with the food matrix and/or to the protection offered by food components to the microorganisms. Remarkably, chitosan was capable of mantaining an effective antimicrobial activity under the sauce simulated conditions.

Keywords: Antimicrobial; LAB; abiotic factor; sucrose supplementation

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Formulated natural plant extracts from nutmeg and cardamom show antifungal activity against clinical isolates of *Candida albicans* and affects cellular morphology and ergosterol

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Candida is an opportunistic fungal pathogen accounting for high rates of mortality and morbidity in immunocompromised individuals. Of all *Candida* spp, *Candida albicans* is the fourth most common cause of nosocomial infections globally. Emergence of multidrug resistance and limitations in availability of broad spectrum drugs with minimum host side effects have been hindering the control and treatment of fungal infections.

In the recent times, natural products have gained impetus in their use as antimicrobials owing to their broad spectrum, multiple targets in the microbial cells and negligible host toxicity. For this study we prepared formulations of two plant products, nutmeg and cardamom. The formulations were prepared by extracting the active components using isopropanol followed by subsequent distillation and emulsification by ethylacetate. Anti-*Candidal* activity of the formulations of these two extracts were confirmed by both microdilution assay and spot assays. The growth inhibition was found to be more in nutmeg than cardamom. Formulations of nutmeg and cardamom revealed more than 80% growth inhibition at respective concentrations of 50µl/ml and 100µl/ml (v/v). Treated cells showed reduced ergosterol content, altered cell morphology, cell wall thickening, membrane aberrations and cytoplasm displacement. The antimicrobial effects observed may be due to the phenol, polyphenols, terpenoids, flavanoids, alkaloids and quinones present in the plant extracts. Further investigations are underway. This study has the potential to contribute to the development of new therapeutic strategies for clinical applications in the treatment of Candidiasis.

Keywords: *Candida albicans*; antifungal agents; natural plant products; ergosterol; cardamom; nutmeg

Fungi treated with small mass chemical effectors exhibit increased antimicrobial activity against facultative bacterial and yeast pathogens.

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For decades fungi have been one of the main sources for the discovery of natural antimicrobial compounds. The screening of virtually every easily available and cultivable fungus lead to the discovery of presumably all antimicrobial compounds produced under laboratory conditions. However, recent sequencing efforts in fungi revealed a still high number of so far unknown "cryptic" compounds which may contain yet unknown antimicrobial compounds. The expression of these compounds is mainly related to the secondary metabolism (SM). Compounds produced in the SM are not essential for the survival of the fungus but lead to a competitive advantage. The SM is not strictly linked to the stationary growth phase or the sexual reproduction state in fungi. During the primary metabolism most genes linked to the SM are transcriptional silenced due to the formation of „facultative heterochromatin". Furthermore genes related to the SM are physically linked on the genome forming clusters. Recent work in the field of heterochromatin linked the formation of heterochromatin to epigenetic regulation. Epigenetic regulation is based on several markers like chemical modifications of histones. Influencing of the histone modifications thus may lead to expression of otherwise silent "cryptic" compounds. One approach to influence epigenetic regulation is the use of small mass chemical effectors (SCE).

The aim of this work was to determine if the use of five SCEs leads to increased "cryptic" antimicrobial activity in spore forming fungi. We used sodium butyrate (Sob), Valproic acid (Valp) and TichostatinA (Tricho) which directly interferes with histone modification. Furthermore we used Azacytidine (Aza), which interferes with DNA methylation and N-acetyl-D-glucosamine (Nacet), which may function as a proxy for bacterial co-cultivation conditions. Thus we investigated the effect of these SCEs on the production of "cryptic" antimicrobial compounds in 54 spore forming fungi. The antimicrobial effect of fungal samples was tested against clinically facultative pathogens and multi-resistant indicator organisms.

In total, 30 samples of treated fungi belonging to six different genera reduced significantly growth of different indicator organisms compared to the untreated fungal sample (growth log reduction 0.3 – 4.3). For instance, in the mycelium of *Penicillium restrictum* treated with butyrate revealed significant higher antimicrobial activity against the indicator organism *Stapphylococcus* (*S.*) *aureus* and multi-resistant *S. aureus* strains and displayed no cytotoxicity against human cells; thus making it an ideal candidate for antimicrobial compound discovery. Furthermore we observed that antimicrobial activity already present in untreated samples could also be significantly increased after SCE treatment. This could be shown in *Hypocrea koningii* with antimicrobial activity against the gram negative *Pseudomonas aeruginosa* and *Aspergillus clavatus* with activity against gram positive and gram negative bacteria.

Our study shows that presumable every fungus, even well described fungi, has the potential to produce "cryptic" antimicrobial compounds and that this approach is capable of rapidly filling the pipeline for yet undiscovered antimicrobial substances with initial hit candidates.

Keywords: epigenome; *fungus*; antimicrobial; secondary metabolism

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Impact of plant-derived isothiocyanates on growth and synthesis of biomolecules of various bacteria species

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Isothiocyanates (ITC) derive from glucosinolates abundant in *Brassicaceae* plants and exhibit a wide range of biological activities. Especially their chemopreventive and anticancer potential has been explored in details. It is documented that ITC modulate activity of carcinogen activating and detoxifying enzymes in healthy cells as well as inhibit cell cycle progression and induce apoptosis of cancer cells, which is accompanied by modulation of numerous signaling pathways. Moreover, ITC reveal anti-inflammatory, immunomodulatory and anti-oxidant properties, thus showing cardioprotective and neuroprotective activities in animal models of stroke, ischemia/reperfusion, neurodegeneration and spinal cord injury [1]. Their antimicrobial potential is however underexplored – only few reports show that some ITC inhibit bacteria growth but the mechanisms underlying this activity are largely not known [2-4].

We studied the effect of SFN (1- isothiocyanato-4- (methylsulfinyl)-butane) and PEITC (phenethyl isothiocyanate) on different bacteria species, including potential pathogens, such as: *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis* and *Enterococcus faecalis*. Bacteria were treated with different concentrations of SFN or PEITC (0, 25, 50 or 100 μM) and growth rate, as well as DNA and RNA synthesis were assessed. ITC were more potent toward Gram-positive than Gram-negative bacteria however response of individual bacteria species varied. Moreover, ITC induced synthesis of the stringent response alarmon, (p)ppGpp, in *E. coli* cells.

Keywords: isothiocyanates; sulforaphane, PEITC, stringent response

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In vitro anti-cariogenic *Streptococcus mutans* activity of 30 herbal formulas used for dental caries in Southern Thailand

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Dental caries is considered as one of major public health problem globally. *Streptococcus mutans* is a major bacterial pathogen responsible for the development of dental caries, especially for young people. The uses of medicinal plants for primary health care have steadily increased worldwide in recent years. Thai herbal formulas have long been used and prescribed for dental caries by Thai traditional healers for centuries. Nevertheless, only single medicinal plants have been receiving attention in various areas of oral health research. Therefore, the scientific evaluation to ensure the rational use of herbal formulas containing two or more different single medicinal plants in traditional medicine is scarce. This present study we evaluated the anti-*S. mutans* activity of ethanol and water extracts of the 30 herbal formulas traditionally used for dental caries in Southern Thailand. Agar disc diffusion method was used to determine the antibacterial activity of both extracts. From the results, only 12 formulas produced antibacterial property revealed by the zones of inhibition ranging from 8-15 mm. The ethanol extract from THF-DC12 containing *Capparis micracantha*, *Clerodendrum petasites*, *Harrisonia perforata*, *Tiliacora triandra* produced the largest zone of inhibition, 15 mm. The ethanol extract from 10 formulas exhibited antibacterial activity while only three water extracts displayed such activity. Both ethanol and water extracts from THF-DC29 (*Acmella oleracea*, *Avicennia marina*, *Piper betle*, *Streblus asper*, *Syzygium aromaticum*) produced zone of inhibition 11 and 8 mm, respectively. The results from this study revealed that the herbal formulas did not show strong antibacterial activity. However, these formulas have had a long history of use in traditional Thai medicine as treatments for dental caries. Due to their weak antibacterial activity, other mechanisms such as anti-biofilm and anti-quorum sensing activities may be responsible for their therapeutic efficacies in traditional use as medicines. As a part of our continued interest in the anti-cariogenic property of Thai herbal formulas used in Southern Thailand, the upcoming work is carried out in order to determine their anti-biofilm and anti-quorum sensing activities against *S. mutans*.

Keywords: *Streptococcus mutans*; Thai herbal formula; antibacterial activity

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In vivo protection against *Salmonella enterica* infection by natural compound from Alliaceae

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Currently salmonellosis remains one of the most prevalent zoonoses associated with food consumption. The social impact of this problem is significant by the large number of reported outbreaks of foodborne salmonellosis, considered a major problem in the field of animal production and public health. The indiscriminate use of therapeutic antibiotics for years, by the productive sectors, for the control of this pathogen has led to an increase in cases of resistance to them.

The aim of this study was to evaluate the anti-salmonella effect of a natural compound from Alliaceae. For this, a commercial extract rich in allium thiosulfinates and thiosulfonates (from DOMCA, Garlicon 40®) was used.

To evaluate the in vitro antimicrobial activity, well diffusion assay were used to provide semi-quantitative measures of anti-salmonella activity, and Minimum Bactericidal Concentration (MBC), were determined by a micro dilution assay against different strains of *Salmonella enterica.* Results indicated that the natural product was active against all the target bacteria. Differences were evident in the diameters of clearance zones, which were 43.5 mm to 46.0 mm for all dilutions between different target strains. MBC values obtained were 1 – 4 µg/mL. Finally, lethal dose curves for each strain was performed to study the kinetics versus antimicrobial bacterial observing the elimination of all viable Salmonella cells within 6 hours of exposure to the extract of allium.

To investigate the potential of natural extract to prevent the invasion of mice by Salmonella, seven week-old female Balb/C mice were used for *in vivo* studies. At least ten mice were used per group. Mice were fed with 150 ppm of Garlicon 40 in drinking water for 3 days. On the day 20, mice were challenged with *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* DT014 (DSM – 26529) (each mouse was administered by oral pipette with 10^8 CFU of bacteria). Control mice received a water placebo for 3 days before Salmonella infection. Three or six days after infection, mice were killed by cervical dislocation, livers and spleens were excised, and CFU Salmonella per organ was determined. Results are showed in Figure 1 in which can be observed that spleens and livers of extract-fed mice (black bars) are significantly less infected ($P \leq 0.05$) than organs of placebo-fed mice (white bars).

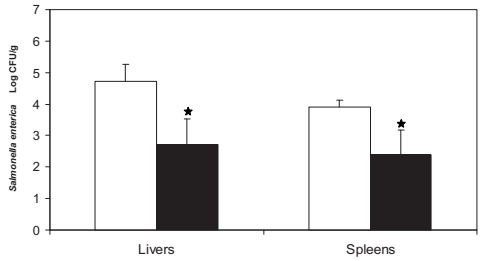

Figure 1. *In vivo* protection against *Salmonella enterica subsp. enterica* serovar *Typhimurium* infection in spleens and livers after oral dosing of mice with natural compound from Alliaceae **(**150 ppm of Garlicon 40) for 3 days before Salmonella infection. (*) P 0.05, indicating the statistically significant difference between the numbers of bacteria infecting organs compared with placebo-fed control mice.

These results demonstrate the great potential of the use of extracts of Alliaceae in controlling *Salmonella enterica*, appearing as a safe and effective therapeutic alternative to antibiotics in livestock farms.

Keywords: Alliaceae; protective effect; *Salmonella enterica*; mice.

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Inhibitory effect of resveratrol encapsulated in hydroxypropyl-γ-cyclodextrin against *Arcobacter butzleri*

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Arcobacter is a genus belonging to the *Campylobacteraceae* family, currently, with 18 recognized species of which *A. butzleri* is the most relevant since it has been reported as the fourth most common *Campylobacter*-like organism isolated from human faeces. Its detection in food products, associated with the antimicrobial resistance and its ability to survive to numerous physical and chemical treatments, highlights the need for the design and implementation of new strategies to control this bacterium.

Resveratrol (3,4',5 trihydroxystilbene) is a naturally occurring phytoalexin synthesized by plants in response to stress that has drawn attention over the past years due to its health-promoting effects, namely its antimicrobial activity, with a recent demonstrated effective inhibitory activity against *Arcobacter* [1]. This compound's reduced stability and solubility have been amended through the complexation with numerous polymers, including cyclodextrins like hydropropyl-gamma-cyclodextrin (HP-γ-CD), which showed a considerable increase in resveratrol aqueous solubility and photostability [2].

Taking this into account, this study focused on the antimicrobial activity of resveratrol inclusion complexes (IC) with HP-γ-CD against two *A. butzleri* strains isolated from poultry (AB36/11) and human (INSA776).

The antibacterial activity of resveratrol IC was assessed by the microdilution method, showing a minimum inhibitory concentration (MIC) of 64 and 256 μg/mL against *A. butzleri* AB36/11 and INSA776, respectively. Time-kill curves revealed a bactericidal activity dependent on incubation time and antimicrobial concentration. The viability reduction was similar to both poultry and human origin strains with 2× and 4× MIC; however when exposed to MIC of IC, the human strain showed to be more susceptible. Furthermore, using flow cytometry, it was possible to determine that this antibacterial action was a result of a decrease in metabolic activity as well as alterations in the membrane potential of cells, with this effect being dose-dependent. The effect of resveratrol IC on biofilm formation and on established 48 h-old biofilms was tested using a crystal violet assay as indicator of total biofilm biomass. Results revealed that the inhibition effect of resveratrol IC on biofilm formation was observed even for sub-inhibitory concentrations. A biomass reduction on 48 h-old biofilms of about 50% was observed after an exposure for 48 h to MIC values of resveratrol IC, with a greater effect being detected for higher IC concentrations.

Overall, the results suggest that resveratrol-HP-γ-CD IC have inhibitory activity against *A. butzleri* planktonic cells and biofilms, either on biofilm formation or in the biomass reduction of established biofilms.

Keywords: Resveratrol; inclusion complex; *Arcobacter butzleri*; antibacterial activity; biofilm

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Interaction between major compounds from essential oils and antimicrobial drugs against *Staphylococcus aureus* strains

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An increasing number of methicillin-resistant *Staphylococcus aureus* (MRSA) strains present resistance to diverse antimicrobial drugs and are implicated as nosocomial infections causes. The combinatory effect between conventional antimicrobial drugs and phytochemical products (essential oils, extracts and majority compounds from essential oils) can be an interesting strategy to increase the effectiveness of antimicrobial drugs currently in use. Recent studies have demonstrated their antimicrobial activities as well as their mechanisms of action. This study aimed to verify the possibility of synergism between phenolic compounds present in essential oils and conventional antimicrobial drugs against *Staphylococcus aureus.* Five phenolic compounds (citronellol, geraniol, eugenol, terpineol and cinnamadheyde (Sigma Aldrich) were tested using methodology adapted from Kirby&Bauer [1] on about 20 strains of *Staphylococcus aureus* (10 methicillin-resistant (MRSA) and 10 methicillin-sensitive (MSSA). Culture medium (Mueller-Hinton agar plus 0.5% Tween 80 in Petri plates of 10x150mm) were mixed individually with the compounds in proportion of 1/4 of Minimal Inhibitory Concentration ($MIC_{90\%}$), previously obtained. Discs of 9 drugs (oxacillin (1 μg), gentamicin (10 μg), erythromycin (15 μg), sulfazotrim (25 μg), vancomycin (30 μg), penicillin G (10 U) levofloxacin (5 μg), tetracycline (30 μg) and linezolid (30 μg)) were laid on culture medium inoculated with a standardized *S. aureus* culture. Assays were performed in duplicate and values of inhibitory zones (millimeters) for controls antibiograms (without compounds) and treatments (with compounds) were recorded after incubation (37°C/24 hours). Mann-Whitney test was used as statistical analysis. Synergistic effects were observed against MRSA: citronellol/gentamicin, citronellol/vancomycin, citronellol/levofloxacin, citronellol/linezolid, eugenol/gentamicin, eugenol/vancomycin, eugenol/linezolid and terpineol/linezolid. Against MSSA were: citronellol/gentamicin, citronellol/vancomycin, citronellol/levofloxacin, citronellol/linezolid, citronellol/sulfazotrim, citronellol/tetracycline, eugenol/gentamicin, terpineol/gentamicin, cinnamaldehyde/linezolid, cinnamaldehyde/gentamicin, cinnamaldehyde/erythromycin, cinnamaldehyde/sulfazotrim , cinnamaldehyde/vancomycin, cinnamaldehyde/levofloxacin and cinnamaldehyde/ tetracycline. There was no synergism in any of associations with geraniol (MSSA and MRSA). In recent years MRSA has appeared more and more in communities outside of the hospital settings and has emerged as a major public health concern worldwide. Results showed that the strategy of combining antimicrobials of different origins can be a promising way of treatment for diseases caused by MRSA and MSSA, as well as probably against other bacterial species.

Keywords: antibiotics, essential oils, *Staphylococcus aureus*, synergism

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Introduction to glycobiology of enzymes: Enzyme glycome, enzyme-lectins complexes/ assemblies/ somes/ particles/ architectures, enzymes as true lectins

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Enzymes can be served as effective and selective antimicrobials. The data on relationships between 115 carbohydrate-sensitive reagents (mainly carbohydrate recognizing proteins) and approximately 500 enzymes and their protein modulators and stabilizators from 446 taxonomic sources are presented [1]. Their using carbohydrate-sensitive proteins for enzymes isolation, separation, carbohydrate moiety studying, assembling and modulation, in microanalysis and biocatalysis is shown and evaluated. The following advantages and features are marked: effective combinations of lectins, glycosyl hydrolases and amidases; HPLC and FPLC steps; best purification and multiple glycoforms separation steps (calculations); glycan moieties (calculations and prognostics). Evaluation of biomarker and diagnostic enzyme forms in normal and pathological states of organism (human, etc.) is given. The book is useful for any of glycobiology aspects, glycome studies, glyco(nano)biotechnology and glycomedicine. Additional glycobiological aspects include bifunctional enzymes (esterases as proteases/proteinases, proteinases as amidases, glycosyl hydrolases/transferases, peptidoglycan hydrolases as separated ones or cascade block), intramolecular cofunctioning enzyme catalytic site and non-catalytic lectin site (examples are from all known enzyme 6 classes), and bacteriophages -induced targets lysis involving hydrolases alone or cascades, as donor or/and acceptor systems are discussed [2, 3]. In addition to antimicrobial enzymes described, prognoses of antimicrobial features of other enzymes are possible.

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Key words Enzymatic antimicrobials, glycoconjugates, lectins, antimicrobial assemblies, (glyco)recognition

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Investigation of the Antituberculous Effect in Vitro of the New Remedies

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Objects of the research are two new remedies. The first remedy is a syrup based on extracts of wartwort, aspen bark and licorice root, aimed for oral use in the treatment of inflammatory and infectious diseases of the respiratory organs and complex therapy of pulmonary tuberculosis.

The second remedy is a two-component emulsion of terpenoid fraction (essential oil) balsam poplar buds and pfokalin in water intended for use in aerosol form for the treatment of respiratory diseases and pulmonary tuberculosis and to prevent bacterioexcretion. For the second medication there has also been studied independent action of substances in its composition.

The aims of the work: the research of the new medication activity in experiments in vitro on Mycobacterium tuberculosis (strain N_{37}Rv, wild sensitive and multidrug resistant wild strains isolated of the ill people) as well as Staphylococcus aureus and Streptococcus pneumoniae.

The research was conducted at the National Reference Laboratory of the National Center of Tuberculosis problems (Almaty, Kazakhstan) in accordance with the normative document [Guideline on experimental (preclinical) study of new pharmacological agents. Under the general editorship of Corresponding Member of RAMS, professor R.U.Habriyev, M.: 2005. - 832 p.].

According to the research, the syrup has got bactericidal activity to the museum strain N_{37}Rv, wild strain sensitive and multidrug resistant strains in concentrations ranging from 100% to 25%.

Terpenoid fraction (essential oil) of poplar buds has bactericidal activity to the museum strain N_{37}Rv, wild sensitive and multidrug resistant strains in concentrations ranging from 100% to 50%. Pfokalin has no bactericidal activity. Two-component emulsion has bactericidal activity against all strains in concentrations ranging from 100% to 50%.

In studying the action of experimental medications on Staphylococcus aureus and Streptococcus pneumoniae bactericidal effect of the syrup was set up to both strains only in concentration of 100%. Bactericidal effect of terpenoid fraction of balsam poplar buds was observed in concentrations of 100% and 50% to the strains Streptococcus pneumoniae and 100% concentration of strains Staphylococcus aureus. For pfokalin the bactericidal effect was observed in all triplicates in 100% concentration to the strains of Staphylococcus aureus and Streptococcus pneumoniae. Two-component emulsion has bactericidal activity against both strains in concentrations ranging from 100% to 50%.

Based on the studies the experienced agents are recommended for preclinical tests on the experimental animals.

Keywords: aspen, wartwort, licorice, balsam poplar, tuberculosis, multidrug resistance.

Investigation of the Antituberculous Effect in Vivo of the New Remedies

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Object of the research is a new drug based on extracts of wartwort (Chelidonium majus), aspen bark and licorice root, aimed for oral use in the treatment of inflammatory and infectious diseases of the respiratory organs and complex therapy of pulmonary tuberculosis.

The aims of the work: the research of the antituberculous activity of the new remedy by the experiments on guinea pigs.

The experiment was conducted on 16 males and 16 females (weighing 250-300 g). Animals were challenged with a museum strain of Mycobacterium tuberculosis $N_{37}Rv$.

The research was conducted at the National Reference Laboratory of the National Center of Tuberculosis problems (Almaty, Kazakhstan) in accordance with the guidance [Practical policies for the Study of antituberculous activity of pharmacological substances. Moscow: 2000. – 7p.].

Treatment of animals was started after the development of tuberculous process, which was tested by the general state (decreased activity, difficulty in breathing, weight loss) and by the results of histological, pathological and microbiological studies. The same parameters were monitored in experimental animals. Treatment was started on the 20th day after infection.

Animals of the first group (uninfected) during the whole experiment retained their normal motorial and behavioral activity. Feed intake corresponded to normal. During the experiment the animals weight increased by 90 ± 6 g.

Animals of the second group which received no treatment died within 36 days after infection. In the experiment, they had the full range of behavioral, morphological and microbiological pathological changes attributable to tuberculosis. Mass loss during the experiment was about 100 ± 3,1 g.

In the third group (was treated with rifampicin – 10,0 mg/kg, isoniazid – 15,0 mg/kg, per orally) during the experiment only one female died on the 30th day after treatment. Mass reduction of the survived animals was 70 ± 3,5 g. At thanatopsy 3 animals had no pathological changes in the internal organs. Homogenate culture of internal organs of animals of this group gave a single growth of Mycobacterium tuberculosis (single colony from 1 to 5). Two animals had isolated small hillocks of grayish-yellow color, with no tendency to merge on the surface of the lungs and liver. Organs culture of these animals showed a slight increase (1+) of all samples.

The best results were achieved in the fourth group with a combination of rifampicin (10,0 mg/kg) and isoniazid (15,0 mg/kg) with the test remedy (0,5 ml/kg) per orally. One animal in this group died on the 23rd day of treatment. Mass recovery of the remaining animals was faster, and compared with the third group, the weight of animals of this group was about 20 g more by the end of the experiment. Also, compared with the third group, as a result of treatment cough attacks were completely absent. 2 animals were completely healthy by the end of treatment, and the remaining 3 had minor lesions with minimal growth (+1) only in the inguinal lymph node closest to the site of infection.

In the fifth group by self-use of the researched remedy in a dosage of 0,5 ml/kg of body weight as well as in the third and fourth groups, only one animal died on the 23rd day of treatment, and the other animals survived till the end of the experiment (60 days). Mass reduction of animals significantly slowed down and was 90 ± 5,2 g by the end of the experiment. Although physical activity was reduced, and there was a depression at the same time the amount of begma and cough attacks decreased significantly in comparison with the animals of the 2nd group (non-treated) and the culture growth of internal organs was 2+.

Thus, we can conclude that the self-use (without antibiotics) in the studied dosage the researched medication has bacteriostatic action against Mycobacterium tuberculosis in the experiment in vivo, thus effectively reducing the amount of begma and cough attacks, which helps to reduce bacterioexcretion.

Keywords: aspen, wartwort, licorice, tuberculosis.

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Liquid and vapour phase antibacterial activity of *Eucalyptus globulus* essential oil = susceptibility of selected respiratory tract pathogens

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Essential oils (EO) produced by medicinal plants have been traditionally used for respiratory tract infections, and are used nowadays as ethical medicines for colds. Although several studies of *Eucalyptus globulus* essential oil (EGEO) have been reported, there are no reports describing vapour activity of EGEO against bacterial respiratory tract pathogens. The aim of this study was to test the efficacy of the Algerian EGEO against some respiratory tract pathogens by disc diffusion and vapour diffusion methods at different concentrations.

Chemical composition of the EGEO was analysed by Gas Chromatography-Mass Spectrometry. Fresh leaves of *E. globulus* on steam distillation yielded 0.96 % (v/w) of essential oil whereas the analysis resulted in the identification of a total of 11 constituents, 1.8 cineole (85.8%), α-pinene (7.2%), and β-myrcene (1.5%) being the main components.

By disc diffusion method, EGEO showed potent antimicrobial activity against Gram-positive more than Gram-negative bacteria. The Diameter of Inhibition zone (DIZ) varied from 69 mm to 75 mm for *Staphylococcus aureus* and *Bacillus subtilis* (Gram +) and from 13 to 42 mm for *Enterobacter* sp. and *E.coli* (Gram -), respectively. However, the results obtained by both agar diffusion and vapour diffusion methods were different. Significantly higher antibacterial activity was observed in the vapour phase at lower concentrations. *A. baumanii and Klebsiella pneumoniae* were the most susceptible strains to the oil vapour with DIZ varied from 38 to 42 mm. Therefore, smaller doses of EO in the vapour phase can be inhibitory to pathogenic bacteria. Else, the DIZ increased with increase in concentration of the oil.

There is growing evidence that EGEO in vapour phase are effective antibacterial systems and appears worthy to be considered for practical uses in the treatment or prevention of patients with respiratory tract infections or as air decontaminants in hospital. The present study indicates that EGEO has considerable antimicrobial activity, deserving further investigation for clinical applications.

Keywords: *Eucalyptus globulus*; Essential oils; Respiratory tract pathogens; Antimicrobial activity; Vapour phase.

Lithium as broad spectrum biocide enhancer in mineral dispersions

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Calcium Carbonate ($CaCO_3$) in solution or as dry powder is widely used for the coating of paper, production of paints and varnishes, as filler in polymer products and as filler in the pharmaceutical and cosmetic industry. Antimicrobials and biocides play an immense role in the preservation of the mineral in order to maintain product quality. Governmental regulations force industries to reduce the amount of biocides added to industrial products. Therefore, biocide enhancers, which can reduce the amount of biocides needed to show the same antimicrobial effect, may offer a solution. These components do not exhibit antimicrobial activity themselves, but increase the antimicrobial activity of biocides.

Lithium ions have been discovered to enhance the activity of biocides against bacteria in aqueous solutions and to show antibacterial properties against gram-negative and gram-positive bacteria [(1)]. This effect might be due to an increase in cell membrane permeability triggered through lithium ions [(2)].

We tested the biocide enhancement of a diverse set of lithium salts, which provide free lithium ions, in $CaCO_3$ aqueous solutions in combination with defined classes of biocide. We could show that the lithium not only enhances the biocide activity, but also renders biocide resistant bacterial strains susceptible to the biocide again. The effect was apparent mainly in the biocide classes consisting of Ampicillin, Bronopol, Formaldehyde, Glutaraldehyde or 2-Phenylphenol. The lithium enhances the antimicrobial activity of these biocides more than 10-fold.

Our results indicate a large potential of lithium added to mineral suspensions, providing a better preservation in general of aqueous products. The addition of lithium could therefore reduce the use of biocides in mineral slurries significantly.

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Lupinifolin from *Albizia myriophylla*: Antibacterial activity against cariogenic *Streptococcus mutans*

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Dental caries is one of the most common chronic oral diseases affecting a large portion of population throughout the world. The mutans streptococci, especially *Streptococcus mutans*, have been strongly implicated as the principal etiological agent of dental cavities and a normal inhabitant of dental plaque, a biofilm on tooth surfaces. Recently, we reported the antibacterial activity of 35 Thai herbal formulas used by Southern Thai traditional healers for dental caries against cariogenic *S. mutans* [1]. The results revealed that the ethanol extract of *Albizia myriophylla* wood show strong antibacterial activity. Lupinifolin, an active compound, was isolated from this plant and demonstrated good antibacterial activity [2]. This present study we investigated the antibacterial properties of lupinifolin against 11 clinical isolates of *S. mutans*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using broth microdilution method. Lupinifolin showed a strong activity against all isolates of *S. mutans* with MIC and MBC ranging from 2-4 and 2-8 µg/ml, respectively. Time kill assay was performed to determine how quickly the compound act on *S. mutans*. At concentrations equivalence to 16MIC, lupinifolin displayed 99% killing activity after 16 h. It is suggested that lupinifolin produced a good promising anti-*S. mutans* activity.

Keywords: Lupinifolin; *Albizia myriophylla*; *Streptococcus mutans*; Thai herbal formulas

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Microbial cell viability of clinical strains of *Candida* spp treated by CROTALICIDINS - Cathelicidin-Related Antimicrobial Peptides (CRAMPS) from South American rattlesnake venom gland

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Crotalicidin, together batroxicidin, lutzicidin, and lachesicidin constitute the group of the vipericidins, the novel cathelicidin-related antimicrobial peptides (CRAMPs) from the venom gland of the South American pit vipers. As members of the large cathelicidin family, vipericidins are prepropetides that are post-translational processed by proteases to release the pharmacophore (34 amino acid residues). In a broad sense, cathelicidin-related antimicrobial peptides are a family of AMPs acting as multifunctional effector molecules of innate immunity and effectively inhibit the growth of gram-negative bacteria. In previous studies conducted by our research group, the full crotalicidin sequence (Crotalicidin-AB) was prepared by solid peptide synthesis and biochemically characterized. Here, we demonstrate the viability related of clinical strains of *Candida* spp after treatment with Crotalicidin-AB(C-AB), and two crotalicidin fragments named Crotalicidin-A1(C-A1) and Crotalicidin-B1(C-B1). The minimum inhibitory concentration (MIC) was established by the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute-CLSI (formerly NCCLS; M27-A2). As assay control, Amphotericin B (Sigma Chemical Co.) was used. Two-fold serial dilutions of peptide solution, ranging from 0.039 to 40 µM, were prepared in RPMI-1640 in a final volume of 100 µL. Subsequently, 100 µL/well of washed fungal, containing 2×10^7 cfu/mL (0,5 of McFarland's scale), were added to the wells in 96-plates. To evaluate of microbial cell viability related we performed growth assays with *Candida* spp treated by crotalicidins in RPMI broth at 30°C for 48 hs, using a 96-well plate format microbial cell viability assay with luminescence readout (BacTiter-Glo™, Promega). C-AB was active against all strains so far tested, with MIC value of 40µM. The C-A1 was also active against strains of *Candida* spp, with MIC values of 20 – 40µM. The C-B1 showed more active against all strains of *Candida* spp, with MIC values of 2,5 – 10µM. In the all MIC´s we observed less than 1% of microbial cell viability related, after 48hs incubation. CRAMPs isolated from the pit vipers' venom glands are active *in vitro* against *Candida* spp, demonstrating good fungicide properties. Moreover, this results shows differences between C-AB and fragments forms, and data here reported indicate that C-B1 is a promising peptide candidate to be used as a biodrug for the control of human infectious diseases, include drug-resistant strains like *Candida* spp. For biotechnological development of crotalicidins, further research will include the cytotoxic tests and the *in vivo* evaluation of their antifungal efficacy.

Keywords: Vipericidins, Antimicrobial peptides, *Candida* spp.

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Microbiological testing of flavonoids and tannins contained in the aqueous-ethanolic extract from the endocarp of coconut (*Cocus nucifera L.*)

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In recent years, the incessant searching for natural drugs has led to use a great diversity of natural resources. In various regions of the planet, the researchers are trying to provide new evidence of the effectiveness of secondary metabolites against different sufferings. Among many others, the antimicrobial activity of coconut peel (endocarp) has tested against three common microorganisms that cause infectious diseases in humans: *Staphylococcus aureus*, *Candida albicans* and *Shigella dysenteriae*. The aqueous-ethanolic extract of coconut shell was obtained from the dried fruit peels by ultrasound with a water-ethanol (7:3) mixture. The extract was tested for biological activity and was subsequently made a partition using the solvents: hexane, ethanol, acetone and water. The fractions obtained from the original dry matter were monitored by thin layer chromatography (TLC), these were revealed by UV, vanillin/H_2SO_4 and $CeSO_4$ in order to identify the generated fractions. Results allowed us to discard the hexane extract formed basically by waxes. The ethanol and acetone fractions, containing mainly flavonoids, were put together and leaving the aqueous fraction with tannins and sugars. These three fractions obtained were concentrated to dryness and weighed. Flavonoids and tannins were used to microbiological tests using susceptibility disk agar Mueller Hinton method. It was found that both fractions have antimicrobial activity and it is consistent with the evidence of previous analysis to the crude extract. The results lead us to believe that both flavonoids and tannins contribute to the biological activity of the extract, it is possible that the compounds responsible for the biological activity are stable and their activity does not change during the separation from the crude extract.

Keywords: flavonoids; tannins; coconut; antimicrobial activity.

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Monitoring of Aflatoxin M_1 in Some Dairy Products in Local Market of Alexandria, Egypt: Attempts for Detoxification

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Aflatoxins are carcinogenic, toxic metabolites produced by a variety of molds. Aflatoxin M_1, AFM_1, accumulates in animal livers and is excreted in milk, which can be consumed by human. The underlying study was undertaken aiming at examining the dairy milk products for any contamination of AFM_1 in Alexandria local market, Egypt and conducting trials for elimination of AFM_1 contamination by using ozone gas and gamma radiation. Levels of AFM_1 contamination of 0.001 to 0.06 µg/l were detected in milk and dairy products. The maximum contamination was in raw and pasteurized milk and minimum levels were in infant milk. The results indicated that yoghurt manufacturing decreases or even eliminate the presence of AFM_1, which may be attributed to factors such as low pH, formation of organic acids or other fermentation by-products. Detoxification by treatment of contaminated milk with ozone gas (200 mg per hour on ambient air) resulted in a decrease or even loss in the contents of AFM_1. The effective interval for complete elimination of contamination was10 minutes of ozone treatment which resulted in curd milk protein and change in its odor. Therefore, it can be stated that ozone gas is a non-convenient method for complete detoxification of milk and dairy products. Treatment by gamma radiation (with a dose of 10 kGy) seems to be more suitable as a detoxification procedure than ozone treatment because there was no change in milk properties upon treatment.

Keywords: Mycotoxins, Aflatoxin M_1, Aflatoxin B, AF M_1, Milk, Dairy products

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New antibiotics from nature: SLU-Medivir collaboration

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Antibiotic resistance is increasing – new antibiotics are urgently needed. The challenges to antibacterial discovery have kept the output of novel antibacterial drug classes at extraordinarily low levels over the past 50 years and rational drug design of new compounds has failed.

International expert organizations, such as Infectious Diseases Society of America (IDSA) and British Society for Antimicrobial Chemotherapy (BSAC) are now proposing a return to nature as a place to find new antibiotics As an example the IDSA 10 X ’20 initiative[1] has an aim to create an inventive research environment that allows the development of ten new antibacterial drugs by 2020. The most effective strategies to find antibiotics are, however, not clearly stated.

Collaboration between SLU and Medivir started in August 2012. It has a primary objective to discover and develop new antibiotics that are active against bacterial pathogens resistant to present therapies. Microorganisms from the existing SLU-collection as well as microorganisms that are isolated from new environmental samples are used as a source for recovery of secondary metabolites with antibacterial activity.

Since then, over fifty novel natural products with varying antimicrobial profiles and capacity to inhibit the growth of bacterial pathogens, some of them resistant to existing drug therapies, were discovered and at least partially characterized.

Keywords: antibiotic resistance; microbial metabolites; purification, characterization

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Novel *Enterococcus* strain isolated from midgut of mosquito induce expression of antimicrobial peptide in human intestinal epithelial cell

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Objective The insects including mosquito are known to possess microbiota. Previous studies have shown that lactic acid bacteria (LAB) constitute a part of mosquito microbiota. Recently, some novel LAB, including *Enterococcus* strain were isolated from insects and were identified[1,2]. However, the identification of LAB from mosquito has never been reported. The mosquito midgut microbiota activate vector innate immunity and block *Plasmodium* development[3]. Then, we predicted that the LAB from midgut of mosquito contribute largely to activate innate immunity. Previous study indicated that LAB induce expression of antimicrobial peptides (AMPs) in human intestinal epithelial cells[4]. Here, we attempt to identify LAB isolated from mosquito and whether induce expression of AMPs in human epithelial cells.

Method In September 2012, 20 female *Aedes albopictus* were collected at Ohori Park (Fukuoka, Japan). Each mosquito midguts was collected and was homogenized with 40% glycerin. The homogenate were plated onto the GYP-$CaCO_3$ agar and were incubated at 30°C for 96 h. After incubation, One of the colonies with halo was submitted for following analysis. The isolated bacteria named Ohori was characterized by genetical (phylogenetic analysis of 16S rRNA and *pheS* gene seqencing, DNA-DNA hybridization), morphological (colony observation, Gram staining, electron microscopy) and physiological analysis (biochemical test, growth test). Subsequently, we studied whether Ohori induce expression of antimicrobial peptide. The Caco-2 cells were incubated with Ohori. After incubation, total RNA of Caco-2 cells were extracted and purified, after then total RNA were reverse-transcribed into cDNA. The AMPs expression was measured using real-time PCR.

Results & Discussion The genetical analysis indicated that Ohori was *Enterococcus* strain and different from previously-identified *Enterococcus* species. The morphological analysis showed that Ohori had the characteristics of *Enterococcus* and had unique morphological properties. The physiological analysis indicated that Ohori had different biochemical and growth characteristics with other closest relative. Furthermore, the Caco-2 cells incubated with Ohori increased the expression of human beta-defensin-2 (HBD-2). This study elucidate that Ohori isolated from midgut of mosquito was classified in a novel *Enterococcus* strain. Additionally, Ohori have the potential to activate human innate immunity by secretion of AMPs and can contribute to protect against infection.

Keywords: mosquito; lactic acid bacteria (LAB); *Enterococcus*; identification; antimicrobial peptides(AMPs)

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Olive oils from Algeria: phenolic compounds composition and antibacterial activity

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Phenolic compounds present in olive oil have received much attention in recent years due to their beneficial functional and nutritional effects. In addition to extending the shelf life of foods by inhibition of lipid peroxydation, the phenolic act in the scavenging of free radicals and can protect the human body against damage caused by them [1].

Phenolic composition, antioxidant activity of phenolic extracts of olive oil varieties from Algeria were investigated. The analysis of polyphenols was performed by Folin-ciocalteu colorimetric method HPLC. The antioxidant activity was assessed by the scavenging effect on the DPPH and $ABTS^{\cdot+}$ radicals. The results showed many phenolic compounds were identified and quantified by using HPLC. Derivatives of oleuropein and ligstroside, hydroxytyrosol, tyrosol, flavonoids, and lignans reporting unique and characteristic phenolic profile. These phenolic fractions also differentiate the total antibacterial activity. The bacterial strains used as test organisms were *Escherichia coli* Nalidixic Acid Resistant NAR**,** *Klebsiella pneumoniae* E47, *Listeria innocua* CLIP 74915, *Pseudomonas aeruginosa*; ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633. Among the bacteria tested, *S. aureus* and to a lesser extent *B. subtilis* showed the highest sensitivity; the MIC varied from 0.6 to 1.6 mg·mL-1 and 1.2 to 1.8 mg·mL-*1*, respectively. The results reveal that Algerian olive oils may constitute a good source of antimicrobial agents. The results obtained denote that Algerian olive oils may constitute a good source of healthy compounds, phenolics compounds, in the diet, suggesting that their consumption could be useful in the prevention of diseases in which free radicals are implicated.

Keywords: Antibacterial activity; Olive oil; Phenols, HPLC.

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Piper betel and *Phylanthus niruri* extract as natural antimicrobial solution

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There has been growing interest in the investigation of natural products for the discovery of a new antimicrobial and antioxidant agents as an alternative route for the substitution of synthetic chemicals, of which side effects are always in question.

Chitosan has received extensive interest for its commercial applications especially in food technology due to its antimicrobial activity. Chitosan mixed with *P. betel* and *P. niruri* extract solution was developed for enhancement of antimicrobial properties in chitosan solution. *P. betel* and *P. niruri* both are ancient medicinal plants that contain high antimicrobial properties. Combination of *P. betel* and *P.niruri* creates synergism resulting in better inhibition towards Gram-positive and Gram-negative bacteria. Addition of *P.betel* and *P.niruri* enhances chitosan antibacterial activity towards both Gram-positive and Gram-negative bacteria.

Synergistical modified antibacterial blend can be done using bio-switch technique. Using soxlet extractor, both *P.betel* and *P.niruri* were extracted and then concentrated using rotary vacuum evaporator. Control solution of chitosan and *P.betel* and *P.niruri* extract was prepared separately. The ratio of *P.betel* to *P.niruri* in the hybrid chitosan solution was varied into 7 different formulations in order to find the optimum inhibition.

From agar diffusion test, chitosan mixed with *P.betel* and *P.niruri* (ratio 6:4) was found to have the highest antibacterial activity against gram-positive (*Bacillus subtilis*) and ratio 1:9 against gram-negative bacteria (*Escherichia coli*). Ratio 6:4 of Pb:Pn solution suggests that its contain high amount of phenolic chemical constituents such as flavonoids and tannins which are proven to posses antioxidant activity. The results show that hybrid solution increased the antimicrobial and antioxidant activity up to 20-50% compared to solely chitosan solution and mixed of *P.betel* and *P.niruri* solution.

Keywords: Antimicrobial, Antioxidant, Chitosan, *Piper betel, Phyllanthus niruri*, Bio-switch

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Plant extracts rich in gallotannins show greater inhibition of spoilage bacteria and antioxidant activity than extracts high in flavonoids and phenolic acids

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Non-volatile natural extracts derived from tree and fruit components could be used in Active Packaging of chilled products to extend their shelf-life and may also find use as antioxidant dietary supplements. The aim of the present study was to compare the antibacterial and antioxidant properties of extracts rich in gallotannins or flavonoids and phenolic acids, respectively. The extracts were either commercial (tannic acid, mimosa tannin, grape seed extract), extracted with 80% acetone from finely ground avocado and mango seed and/or skin powders, or extracted with water from pine bark (pine tannin).

Table 1 shows the total phenol (Folin-Ciocalteau assay) and flavonoid contents (colorimetric assay) of the extracts. Based on these results and LC-MS analysis, the main phenolic components of tannic acid and mango seed extract are gallotannins (polygalloyl glucoses). The LC-MS also showed that the phenols in avocado skin and seed extracts and condensed (pine and mimosa) tannins comprised mainly flavonoids and phenolic acids.

Table 1. Results of extract analysis, antioxidant assays and antibacterial activity against spoilage bacteria

	TPC, mg GAE/g	TFC, mg CE/g	EC50, mg/ml	Antibacterial properties as minimum inhibitory concentration (MIC), mg/ml (±SE)				
				Shewanella putrefaciens	*Photobacterium phosphoreum*	*Pseudomonas fluorescens*	*Brochothrix thermosphacta*	*Clostridium estertheticum*
Extract								
Tannic acid	885 ± 55	74 ± 2	1.3 ± 0.2	0.16 ± 2.60	0.94 ± 0.71	3.75 ± 3.33	0.31 ± 1.57	0.18 ± 0.19
Mango seed extract	406 ± 35	53 ± 8	3.6 ± 0.1	0.47 ± 1.56	1.56 ± 3.09	1.56 ± 2.34	0.47 ± 1.20	0.12 ± 0.04
Grape seed extract	676 ± 19	506 ± 14	3.6 ± 0.1	2.50 ± 2.50	20.00 ± 2.78	20.00 ± 3.15	7.50 ± 3.09	0.31 ± 3.23
Avocado skin extract	403 ± 38	370 ± 18	7.6 ± 0.4	2.50 ± 2.32	20.00 ± 0.00	10.00 ± 2.10	0.63 ± 1.21	0.94 ± 2.39
Avocado seed extract	303 ± 23	297 ± 27	7.2 ± 0.7	0.94 ± 0.14	20.00 ± 2.11	5.00 ± 1.25	0.63 ± 0.74	0.47 ± 0.34
Pine tannin	567 ± 13	405 ± 8	4.1 ± 0.5					
Mimosa tannin	498 ± 13		4.4 ± 0.2	12.50 ± 3.39	12.50 ± 3.16	11.25 ± 3.37	3.75 ± 3.75	0.47 ± 0.75
Reference compound								
Ascorbic acid			3.0 ± 0.1					
(+)-Catechin	977 ± 40		4.3 ± 0.1					
Gallic acid			0.9 ± 0.1					
Ferulic acid			5.9 ± 1.5					
Trolox			4.8 ± 0.1					

TPC = total phenol content; TFC = total flavonoid content; GAE = gallic acid equivalent; CE = catechin equivalent; EC50 = amount of antioxidant necessary to decrease DPPH (2,2-diphenyl-1-picrylhydrazyl) radical absorbance by 50%; SE = standard error

As shown in Table 1, the extracts rich in gallotannins (tannic acid and mango seed extract) exhibited greater antibacterial activity (lower MICs) against spoilage bacteria than the other extracts whose phenolic components were predominantly flavonoids and phenolic acids. The Gram-positive bacteria (*B. thermosphacta* and *C. esterthearum*) were mostly more susceptible than the Gram-negative ones. In terms of antioxidant activity, tannic acid outperformed (lower EC50 = higher activity) all the other extracts while mango seed and grape seed extracts showed equal activity. These three extracts also compared favorably against antioxidant reference compounds. Neither antibacterial nor antioxidant properties could be directly predicted from the total phenol content.

Based on this study, extracts with a high gallotannin content have more potential than those containing mainly other types of phenols for Active Packaging of chilled products and antioxidant dietary supplement applications.

Keywords: Active Packaging; antibacterial; antioxidant; dietary supplements; extract; gallotannin; flavonoid

R191A mutant displays defective GTPase activity and impairs cytokinesis in *Bacillus subtilis cells*

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FtsZ is highly conserved prokaryotic cytoskeleton protein, playing a crucial role in bacterial cell division. During division, FtsZ polymerizes to form a dynamic Z-ring at the mid-cell position and orchestrates the division process. The perturbation of Z-ring has been found to be lethal to bacteria. Recently, plumbagin [1] and SB-RA-2001[2] were suggested to inhibit bacterial cell proliferation by disturbing the formation and functioning of the Z-ring at the mid cell. Plumbagin interacted directly with purified FtsZ and inhibited the assembly and GTPase activity of *Bs*FtsZ *in vitro*. Determination of the putative binding site by molecular docking analysis showed that both plumbagin and SB-RA-2001 bind to the cleft region between H7 helix and C-terminal domain of the *Bs*FtsZ. Arg191 (R191) was identified as the common residue involved in H-bonded interaction with these two compounds. We constructed a strain R191A-*Bs*FtsZ. The secondary structure of the mutated FtsZ (R191A-*Bs*FtsZ) was similar to that of WT-*Bs*FtsZ. The mutation (R191A) strongly diminished the GTPase activity of FtsZ. R191A-*Bs*FtsZ exhibited less polymerization ability as compared to WT-*Bs*FtsZ. It could also poison the assembly of WT-*Bs*FtsZ in concentration dependent manner. Moreover, the polymer morphology of R191A-*Bs*FtsZ was distinctly different from that of WT-*Bs*FtsZ. Further, we observed that when R191A- *Bs*FtsZ was transformed into *B. subtilis* PL2084 strain, the *B. subtilis* cells became filamentous indicating that the mutation had a strong adverse effect on the division of *B. subtilis* cells. The results suggested that the GTPase activity of FtsZ plays an important role in Z-ring formation. The study also suggested that designing antimicrobials targeting the Arg191 residue might help to curb the division process.

Keywords: FtsZ assembly dynamics; Z-ring; R191; *B. subtilis*; cell division

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Screening for wide spectrum polyphenolic antimicrobials from plants using a fast AlamarBlue® based method

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Despite the numerous advances made in medicine and pharmacology, there is still a need for new compounds with antimicrobial activity, especially due to outbreak of antibiotics resistance cases. One of the main strategies to solve this problem is the search of new antimicrobial phytochemicals in plant sources. In this work, four species of *Cistus* genus (*C. ladanifer*, *C. clusii*, *C. salviifolius* and *C. albidus*) and four other herbal species (*Hypooxis rooperi*, *Hibiscus sabdariffa*, *Hibiscus arnottianus* and *Lippia citriodora*) have been selected based on previous results [1-4] and also due to their traditional use, with the aim to find for broad spectrum antimicrobials.

The inhibition of microbial growth by these aqueous extracts was determined by an optimized AlamarBlue® cell viability assay. *Escherichia Coli* WA321, *Staphyloccocus aureus* 533R4 Serovar 3 and *Candida albicans* BSMY 212 were the strains tested for the screening. Furthermore, main compounds of each extract were identified by HPLC-DAD-ESI-IT-MS/MS.

The screening showed that the most active extracts bearing a wider antimicrobial spectrum were *H. sabdariffa* and *C. salviifolius***.** The analysis of the polyphenolic composition of the most active extracts by HPLC-MS/MS, indicated that ellagitannins and flavonols are strongly associated with the antibacterial activity, whereas the presence of chlorogenic acid is related with antifungal activity.

The results indicate that extracts enriched in these compounds would increase the potential use of formulations and/or extracts with wide spectrum of antimicrobial activity for hygiene or cosmetic purposes.

Keywords*:* Antibacterial, *flavonoids*, polyphenols, alamarBlue® assay, HPLC-DAD-ESI-IT-MS/MS.

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Screening of Inhibitors of the β-Sliding Clamp of *Staphylococcus aureus* from Caatinga plants

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Among human and animal pathogens, *Staphylococcus aureus* has been highlighted owing to its ability to express a variety of virulence factors and increased resistance to antimicrobial agents. The multi enzyme machinery responsible for DNA replication represents an attractive and underexploited target for identification of new antimicrobial substances, since these proteins are distinct from those in eukaryotes and archaea. For instance, the β-sliding clamp (DnaN) has been indicated as a potential target for new antibiotics, due its essential role regulating DNA polymerase traffic at the replication fork. The β-clamp interacts with many different proteins (DnaE, PolC, δ, PolIV, PolV, PolI, MutS, MutL, DNA ligase and Hda) through the conserved β-binding motif ($QL^S/_PLPL$ or $QL^D/_SLF$) which binds a hydrophobic pocket located in each DnaN protomer. Caatinga (or Semi-arid region) is an exclusive biome from Brazil marked by an accentuated dryness (rainfall is usually less than 900 mm/year). As a result of the environmental conditions to which they are exposed, the Caatinga plants have developed interesting chemical features and they have been described as excellent weapons against microorganisms. The aim of this study was to investigate the ability of 30 extracts from 22 Caatinga plant species to prevent in vivo the dimerization of β-sliding clamp from *S. aureus.* The selection of extracts able to inhibit DnaN-DnaN interaction was performed using a Bacterial two-hybrid system (BTH), based on the interaction-mediated reconstitution of the adenylate cyclase (*cya)* activity from *Bordetella pertussis* in *Escherichia coli.* Briefly, *E. coli* BTH101 was co-transformed with two recombinant plasmids both containing DnaN sequence from *S. aureus*, but one expressing the T25/DnaN fusion of *cya* (p25N-DnaN) and the other one expressing the T18/DnaN fusion (pUT18-DnaN). The extracts were applied on the surface of LB plates containing X-gal (5-bromo-4-chloro-indolyl-β-D-galactoside) and relevant antibiotics where transformed BTH101 was plated; the inhibition of DnaN-DnaN interactions resulted in development of white colonies. The specificity of active extract was confirmed using the control plasmids of BTH (pKT25-zip and pUT18C-zip) and assessing the effects of extracts in the expression of lacZ operon of 5 different *E. coli* strains. Finally, the anti-*S. aureus* activity of extracts was evaluated by determination of minimum inhibitory and bactericidal concentrations (MIC and MBC). The initial screening revealed that despite that 8 extracts were active against *S. aureus*, only 3 of them inhibited the interaction of DnaN-DnaN: *methanolic and ethyl acetate extracts* from *Buchenavia tetraphylla* leaves and aqueous extract from *Libidibia ferrea* fruits. The antimicrobial activity of these plants has been reported by our group. The complementation of T25-zip and T18-zip fusion proteins was not interrupted by these extracts. Similarly, any effect was observed in the expression of lacZ operon of tested strains. Regarding the anti-*S. aureus* activity, the most activity sample was the aqueous extracts of *L. ferrea* (MIC of 97.65 µg/mL and MBC of 390 µg/mL), followed by methanolic and ethyl acetate extracts from *B. tetraphylla* (MICs values of 195,3 µg/mL and 390 µg/mL; and MBC values of 1562 µg/mL and 1562 µg/mL, respectively). The evaluation presented in this study confirms the antimicrobial potential of *L. ferrea* and *B. tetraphylla*, and shows by the first time that inhibition of DNA replication is a possible target of their action. The purification and structural characterization of active compounds from these plants are the next step of our research.

Keywords: antimicrobial activity; synergistic effects; caatinga biome.

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Screening seaweeds from Mauritius Islands for antimicrobial activity

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Emergent antibiotic resistance of conventional drugs against microorganisms has stimulated studies in finding natural products which can be used as alternative. For the past five decades biologists and chemists have explored alternative sources of medicine from nature, including the great biodiversity of marine life in the search for compounds with pharmacological and antifouling activities. Seaweeds also known as marine algae, are sources of functional foods used nowadays for curing and controlling diseases such as diabetes, cancers, obesity and is also used as immune boosters. The algal flora of Mauritius is the best known of all Indian Ocean tropical Islands and the coast of Mauritius includes a total of 122 species of algae out of which 34 are green algae, 20 are brown algae and the remaining 67 are red algae. This work reports the antimicrobial activity of common seaweeds species from the coastline of Mauritius Islands namely *Ulva*, *Sargassum*, *Gracilaria*, *Padina, Enteromorpha and Amphiroa.* Crude and fractionated extracts of the selected seaweeds were screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella oxytoca* by using the broth microdilution assay. Remarkable antimicrobial activity with minimum inhibitory concentrations (MIC) ranging from 0.585±0.28 mg/ml to 4.688±2.21 mg/ml was obtained against the tested bacteria. The lowest MIC (0.585±0.28 mg/ml) was recorded from dichloromethane extracts of *Amphiroa fragilissima* against *S. aureus.* Total phenolic profiling of the seaweed extracts showed positive correlation with the antimicrobial profile of these algae. The above findings confirm the potential of Mauritian algae as a source of pharmaceutical leads against infectious and degenerative diseases.

Keywords: algae; Mauritius; *Ulva*; *Sargassum*; *Gracilaria*, *Padina ; Enteromorpha; Amphiroa*; antimicrobial activity.

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Silver nanoparticles synthesis based on essential oil and its antimicrobial properties

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Effective synthesis of metallic nanoparticles particularly silver nanoparticles (AgNPs), is an important issue, because they are widely used in various applications, especially as antimicrobial agents [1]. In this work we report a novel strategy with biological approach for AgNPs synthesis. It involves the use of essential oil which is commonly utilized in cosmetics and food production. This natural compound was found to play dual role of both reducing as well as capping agent. The structure and composition of AgNPs synthesized by this method were characterized by X-ray diffraction, scanning electron microscopy, transmission electron microscopy and UV-vis spectroscopy. DLS analysis was performed as well and zeta potential were determined. The various pH and temperature conditions were tested to find their stabilizing effects on AgNPs synthesis peak at 415 nm. Synthesized AgNPs particles were almost spherical in shape with an average diameter of about ~ 2,5 nm. Their activity against several bacterial pathogens such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* were determined by growth inhibition assay.

Keywords: silver nanoparticles, nanomaterials

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Staphylococcal enterotoxins production influenced by phenolic compounds from plants essential oils

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Staphylococcus aureus is a pathogen of major concern for clinical infection besides possibility of resistant to antimicrobials [1]. Staphylococcal enterotoxins A and B (SEA and SEB) are a major cause of foodborne diseases and milk is involved in food poisoning due to bovine mastitis. Studies have shown that major phenolic compounds from plant essential oils may influence bacterial toxins production, contributing to reduction of antimicrobial drugs consumption [2]. 76 *S. aureus* isolated from milk of animals with subclinical mastitis originated from six Brazilian states and storage at Embrapa Dairy Cattle Bank were studied. The presence of *sea* and *seb* genes encoding enterotoxins was determined by Polymerase Chain Reaction (PCR) and genes expression was assessed by Reverse Passive Latex Agglutination (RPLA). The antimicrobial tests were performed with two phenolic compounds (eugenol and geraniol) against *S. aureus* enterotoxins producers using sub-inhibitory doses (60% of $MIC_{90\%}$) of each compound previously obtained. The procedures and interpretation of results were performed according to manufacturer's instructions kit. Each treatment was inoculated with an overnight culture of each *S. aureus* strain standardized by 0.5 McFarland scale and incubated (37ºC/24h). TSB inoculated with *S. aureus* was used as control. Colony forming units were counted to ensure that minimum number for toxin production was reached. Assays were performed in duplicate. *Sea* gene was found in 12 (15%) out of 76 strains studied, while 7 (9%) had *seb* gene, and 6 (7%) had both. Of these, 4 (33%) strains expressed *sea* and 5 (71%), *seb*. The susceptibility tests to compounds showed that SEB production of all strains tested were inhibited by both compounds (p <0.001), while the production of SEA was not affected when compared to controls. The presence of staphylococcal genes encoding toxins and the ability of expression of these genes in samples demonstrates the possibility of poisoning, which is a public health problem. Therefore, phenolic compounds from essential oils could be a possible alternative to controlling enterotoxin B production and thus contribute to reduction of antimicrobial drugs consumption.

Keywords: enterotoxins, natural products, *Staphylococcus aureus*

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Studies on Finishing of Silk using Aloe Vera

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Natural Silk in its native form contains fibroin, which has a gummy coating of sericin possessing antimicrobial properties. However, sericin is removed during the pretreatment to improve the absorbency of silk for further value addition through coloration. Silk being a natural and hygroscopic fibre gets attacked by microbes easily. Hence antimicrobial treatment becomes essential to add value to wear and care of silk textiles. Since synthetic antimicrobial agents may be toxic or even carcinogenic, natural and eco-friendly antimicrobial agents are essential substitutes. Aloe Vera is a natural plant product which finds common uses in skin protection, Antimicrobial and UV protection. Aloe Vera is used in cosmetics and soap industries abundantly due to its above mentioned properties.

In the present paper, ready-for-dyeing soft silk fabric was treated with Aloe Vera using 1, 2, 3, 4- butane-tetra-carboxylic acid (BTCA) as a cross linking agent and sodium hypophosphite (SHP) as a catalyst. The treated fabric with a concentration of 15% of Aloe Vera showed excellent antimicrobial properties. Since BTCA is used as cross-linking agent, durable press properties namely crease recovery angle, improved due to the treatment. Strength losses in breaking strength and in tearing strength were minimal. The mechanism of finishing of Aloe Vera was found to be its chemical binding with silk instead of simple coating or impregnation. FTIR studies showed that the carboxyl side groups and short chain amino acids' side groups act as sites for BTCA cross linking *inter-alia* chemically binding of Aloe Vera with silk fibroin. SEM studies revealed that no coating or tangible impregnation on the surface of the fibre was visible supporting the postulation that Aloe Vera has been chemically bound to silk. This is further substantiated by the durability of the finish to dry cleaning. Since Aloe Vera is a natural product and BTCA is an eco-friendly cross linking agent, the finishing recipe optimized in these studies can serve as eco-friendly antimicrobial finish for silk fabrics. The finished silk fabric was found to be effective antimicrobial agent for both gram positive bacteria (*S. aureus*) and gram negative bacteria (*K. Pneumonia*). Antimicrobial property on the finished fabrics exhibited was 97 to 99%.

Keywords: Aloe Vera, Silk Fabric

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Study of the antifungal activity of essential oil extracted from peels of *Citrus aurantium*

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The objective of this study was to evaluate *in vitro* antifungal activity of peels essential oil extracted from *Citrus aurantium*. The extraction of fennel seed essential oil (EO) was made by a hydrodistillation using the Clevenger type apparatus. The fungus strains are *Alternaria sp*, *Aureobasidium sp, Aspergillus fumigatus* CIP 1082.74, *Fusarium sp , Penicillium sp , Rhizopus sp* and *Trichophyton rubrum* CIP 2043.92. The screening of antifungal activity was carried out by the diffusion and microatmospheric method. Results obtained indicate that peels' essential oil has an inhibiting effect on the tested strains. The method of dilution enabled us to evaluate the values of the minimum fungistatic concentration (MFSC) and the minimum fungicidal concentration (MFCC). These concentrations lie between 625 and 1250 $\mu g.ml^{-1}$. The antifungal index (AI50) was also estimated, *Alternaria sp.* strain seems to be most sensitive with an AI50 close to 23.20 ± 0.66 µg.ml-1. To obtain concentrations more precise than the MFSC and MFCC, another parameter is given: the AI50, which is the concentration which inhibits 50% the mycelia growth, According to the results obtained, it is clear that the strains tested do not have the same AI50, the strains belonging to the *Alternaria sp.* and *Aureobasidium sp* kinds is most sensitive. Our results indicate that the essential oil of peels has an interesting antifungal activity.

Key words: Essential oil, antifungal activity, peels , *Citrus aurantium*

Synergic behaviour of main polyphenolic compounds of *Cistus salviifolius* against *Staphylococcus aureus*

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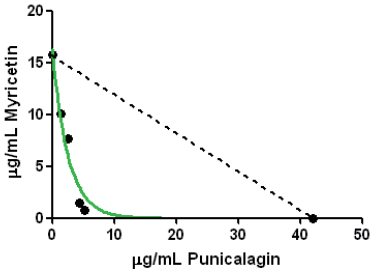
Plant polyphenols are a potential source of new antimicrobial molecules against bacteria since most newly developed antimicrobial agents do not improve the clinical management of infectious diseases. The potential synergism between the major polyphenolic compounds present in the most active extract screening in previous studies of our group [1], *Cistus salviifolius*, which was subsequently quantified for this assay by HPLC-ESI-MS/MS [2].

Pairwise combinations of main polyphenolic compounds in *C. salviifolius* extract were selected, among them were found as the major family of compounds, the ellagitannins and the flavonoids. These combinations were tested against the *in vitro* growth of *Staphylococcus aureus* by the isobole method and the fractional inhibitory concentration index determination. Some combinations revealed synergic effects, resulting in a reduction of minimum inhibitory concentration required to inhibit 50% growth (MIC_{50}) up to twenty times lower as compared with the individual compounds. Some of the combinations exhibited MIC_{50} values close to drug potency level (0.05-1 mg/mL). Punicalagin and myricetin were the major contributors in combinations.

The proportion between the compounds in the synergic mixtures is crucial and may explain the superior antimicrobial activity displayed by this extract when compared with other botanical extracts. The optimization of these combinations could lead to the design of potent antimicrobial phytopharmaceuticals, which may improve the performance of current antibiotics, with the rational use of multi-targeted and synergic molecular interactions of selected polyphenols.

Keywords: Antibacterial, *Cistus*, polyphenols, synergy, HPLC-ESI-MS/MS.



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Synergistic effects of soy sauce and essential oils on *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*

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Background Soy sauce is the third most popular condiment, frequently used for salad, meat products, seafood, and so on [1]. Although soy sauce has been regarded to control food deterioration because of its antimicrobial factors such as low pH, high cencentration of salts (NaCl), ethanol, and preservatives, several researches showed soy sauce has little bactericidal effects [2, 3]; however, those factors are expected to be enhanced by combining with other factors [4]. Plant essential oils (EOs) are known to control growth of microorganisms and have potential to be applied in foods as antimicrobial agents [5]. Unfortunately, there are a few barriers to apply EOs in foods; it requires high concentration to exhibit bactericidal effects in foods, it is more expensive than synthetic additives, and it has a strong smell. Thus, there have been various researches to develop a combining method using EOs to enhance the bactericidal activity of them [5].

Objectives In this study, synergistic bactericidal effects between soy sauce and EOs were investigated against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* at 4°C as well as 22°C.

Materials and methods Three strains for each bacteria were used in cocktail suspensions (*E. coli* O157:H7 ATCC 35150, 43890, and 43895, *L. monocytogenes* ATCC 19113, 19114, and 19115, and *S.* Typhimurium ATCC 19585, 43174, and DT104 Killercow). Bactericidal activity of soy sauce containing 1 mM of each EO (carvacrol, thymol, eugenol, *trans*-cinnamaldehyde, β-resorcyclic acid, and vanillin) was tested for 10 min to screen a variety of synergism. Carvacrol and thymol, which showed the greatest activity, were then evaluated for bactericidal efficacy in 0.25, 0.5, and 1 mM for 1, 5, and 10 min. For microbiological quantitative analysis, MacConkey Sorbitol Agar, Xylose-Lysine-Desoxycholate Agar, and Oxford Agar supplemented with *Listeria* selective supplement were used for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively.

Results and discussion The single treatments of soy sauce and each EO did not show any reduction for all tested bacteria; however, when either carvacrol or thymol was added in soy sauce, the reductions significantly increased ($p < 0.05$). One mM of carvacrol and thymol in soy sauce reduced all tested bacteria below detection limit within 1 or 5 min at 22°C (initial population = 7.10 ± 0.02 log CFU/ml). At 4°C, populations of *E. coli* O157:H7 and *S.* Typhimurium were less inactivated by soy sauce containing 1 mM of carvacrol or thymol, so they were reduced below detection limit within 10 min. On the other hand, *L. monocytogenes* was controlled better at 4°C; it was inactivated within 1 min at 4°C. These results represented that antibacterial activity of soy sauce could be enhanced significantly as combining with carvacrol or thymol in concentration of less than minimal inhibitory concentration (MIC = 1.5-3.0 mM).

Significance and impact Control of pathogen could be made in foods containing soy sauce by treating with only small amount of carvacrol or thymol (less than MIC) at 4°C as well as 22°C. The natural flavor of soy sauce can mask the strong smell of EOs which is a major concern in foods.

Keywords: Soy sauce; Carvacrol; Thymol; Antimicrobial efficacy; Foodborne pathogen

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The antibacterial effect of isothiocyanates on Shiga toxin-producing *Escherichia coli* strains

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Numerous compounds of natural origin e.g. plant derived secondary metabolites constitute research objects in recent years due to their health beneficial effects, including their antimicrobial properties. Isothiocyanates (ITC), widely distributed in plants of *Brassicaceae* family, such as sulforaphane (SFN), allyl isothiocyanate (AITC),benzyl isothiocynanate (BITC) and phenethyl isothiocyanate (PEITC) have been evaluated in this work for their effect on Shiga toxin producing *Escherichia coli* (STEC). Bacteria harboring enterotoxins comprise a grave threat for public health as proven by numerous outbreaks and epidemics also in highly developed countries, for example recent STEC outbreak in Germany in 2011, and may result in severe life threatening complications such as hemolytic uremic syndrome. Virulence of enterohemorragic bacteria requires production of enterotoxins which is strictly related to bacteriophage development. The induction of the lambdoid prophage integrated into host chromosome leads to efficient expression of *stx* genes and release of the toxin. The prophage induction factors include specific conditions, causing genome lesion or rearrangement and importantly, antibiotics used in standard therapy against infection. We showed in our previous studies that the control of development of prophages and subsequent expression of toxin genes is dependent on the global stress response system of the host cell, the stringent response [1]. We demonstrated as well that the mechanism of PEITC antimicrobial effect is related to stringent control of cellular metabolism [2].

In this work we evaluated the minimum inhibitory concentrations for each ITC, then to elucidate the mechanism of antimicrobial effect we assessed ITCs effect on DNA/RNA synthesis and found that both processed were inhibited by ITC with PEITC and BITC exhibiting the strongest effects. The evaluation of the stringent response induction as a result of isothiocyanate treatment showed that ITCs, especially PEITC, led to significant increase of the level of stringent control alarmons, guanosine tetraphosphate and pentaphospate, (p)ppGpp. To assess the ITC effect on phage development we employed several Shiga toxin converting lambdoid bacteriophages such as 933W and Phi24B. Moreover, we used *E. coli* O157:H7 strain to determine ITC effect on cytotoxicity in cell lines. Antimicrobial activities of isothiocyanates together with the effect on bacteriophage development indicate that these natural compounds could be promising factors in combating and prevention of infections by bacterial pathogens harboring enterotoxins.

Keywords: Shiga toxin; enterohemorragic *Escherichia coli*; isothiocyates; stringent response

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The comparison of antimicrobial activity of extracts obtained by subcritical water extraction process (SWE) from agro-food plant residues as raw material

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Every year millions tons of food are produced worldwide. It's a necessity to feed still growing billion human population all over the world. One of the main problems of food industry is to find ecological and relatively cheap way to increase the efficacy of usage of plant resources and also to preserve food. A Lot of substances obtained in synthetic way such as parabens and benzoate or sorbic salts are used for such purpose. Many scientists from all over the world are looking for more ecological substitutes to be used as food preservatives.

A high potential to be used as food preservatives have different plant raw materials (including the residues/waste), because of high polyphenolic and other active compounds that can act as antimicrobials and antioxidants. Subcritical water extraction process gives a chance to extract active substances from agro-food residues in a „green way". Additionally, the process can be performed at different conditions (the combination of pressure and temperature)that enables the increase of efficacy of active compounds and also has the impact on chemical composition of obtained extracts.

This study compares the antimicrobial activity of extracts obtained from four raw materials (agro-food residues) such as apple pomace, barley straw, corn straw and oat hulls. The process of extraction has been performed at two different temperature values.

The results showed that apple pomace extract had the highest antimicrobial activity among other extracts. The use of natural, cheap material as a preservatives seem to be very attractive.

Keywords: Subcritical water extraction; apple pomace; food preservatives

The considerable antibacterial effect of some natural mineral substances

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Mineral substances have long been acknowledged as therapeutic with an early detailed account of their properties as drugs given by Dioscorides in his *De Material Medica* in the 1st c. AD. Aluminium sulphates and boron minerals are widely known as antiseptics. Such minerals are described as hemostatics and as eye salves, respectively, in ancient texts dated to the 2nd millennium BC or earlier. Ancient texts do not specify which minerals exactly are associated with the prescribed therapeutic substances, nor do they point to their exact geographical localities from which they have been extracted. In the last ten years we have sought to look for some of these purported therapeutic inorganic substances in the field and within an archaeological context, and to establish whether there is evidence for their extraction and processing in antiquity. These geographical areas are associated with volcanic and geothermal activity, like the volcanic arc of the Aegean. We have identified two candidate groups, the colemanite of Samos and the alunogen of Melos, the two islands situated in the west and east Aegean respectively and their associated localities.

What is certain is that refined methods of separating these minerals in antiquity would have been impossible and that the minerals used and prescribed by ancient texts would have consisted of composite inorganic substances; in the case of alunogen, sulphur and other sulphate salts may have been present and in the case of borates, colemanite would have existed in association with smectite clays like montmorrillonite. We argue that the properties of composite or 'impure' minerals may have worked synergetically and in a beneficial manner.

We have tested some of these pure minerals and in association with other minerals (just as they would have existed in their natural state) against *staph aureus* and *pseudomonas aeruginosa* with positive results [2,3]. We would like to understand the mechanism behind their bactericidal effect and indeed the bactericidal effect of a whole range of 'recipes' suggested by the ancient texts, involving composite inorganic materials as they occur in their natural state. We are looking to work closer with microbiologists who might be willing to follow our 'from geo-archaeology to microbiology' approach into the elucidation of the therapeutic role of some of these natural mineral substances; many of these minerals are quarried today on an industrial scale, some of which have a documented track record of being therapeutic of over 3000 years. The ancient mineral-based *pharmacopoeia* is far too nuanced and it is only now that we are reaching a position to explore and test the contents of the ancient texts in the field and in the laboratory.

Keywords: alunogen, borates, antibacterial, *materia medica*

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The new weapon for nosocomial infections: *Cymbopogon citratus* essential oil

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Our ethnopharmacological studies, in several Angolan regions, showed that *Cymbopogon citratus* (DC) Stapf., it´s applied in folk medicine to treat feverish and anti-inflammatory conditions, as well as efficiency against some skin infections. In order to validate antibacterial activity several studies were performed using 12 bacterial strains.

The essential oil samples of *Cymbopogon citratus,* obtained by hydro-distillation*,* were analysed by GC and GC-MS. Constituents were identified from their retention indices on two different phases GC columns (polydimethylsiloxane and polyethyleneglycol) and from their mass spectra [1]. The antimicrobial activity of *C. citratus* and major natural volatile compound were tested by the disc agar diffusion technique and the dilution technique against *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228) and other bacterial strains*.*

Broad spectrum antibacterial activity was exhibited by the *Cymbopogon citratus* essential oil against both Gram-positive and Gram-negative bacteria. What is more interesting from this study is that MRSA isolates were more sensitive towards the test substance compared to the non-MRSA. When tested against a *S. Aureus* MRSA, resistant to amoxicillin-clavulamic acid combination, penicillin G and meticillin, *Cymbopogon citratus* essential oil show a significant increase in bactericidal activity when compared with the commercial antibiotics. The same results were obtained using vancomycin resistant *S. Epidermidis* strains, as well as the other strains used in these studies.

Our work was the first to show that *Cymbopogon citratus* essential oil has higher antibacterial activity against MRSA strains, than commercial antibiotics. These discover opens a new hope to fight against nosocomial infection.

This finding suggests that essential oils from *Cymbopogon citratus* showed a potential antimicrobial activity that can further be used for clinical treatment; thus, there is need for a study on the possible impact of PAE in the clinical situation

Keywords *Cymbopogon citratus*, MRSA, nosocomial infections, antibacterial activity

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Totarol Induced Proteome Alterations in *Bacillus subtilis* by Multipronged Quantitative Proteomics

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Background and objectives
The rapid emergence of microbial drug resistance indicates the urgent need for development of new antimicrobial agents. Bacterial cell division machinery is considered as a promising antimicrobial target. Totarol is a naturally existing diterpenoid, which has the ability to restrain bacterial growth by perturbing the cell division. The present study was conducted to investigate the proteomic alterations in *Bacillus subtilis* as a consequence of totarol treatment to decipher its mechanism of action and possible molecular targets by multiple proteomic technologies.

Methods
Fluorescence microscopy analysis of *B. subtilis* has showed totarol treatment (IC_{50}- 1.5 µM and MIC- 2.0 µM) leads tofilamentous morphology with multinucleoids per cell. The proteomic analysis of biological triplicate samples was performed using multiple quantitative proteomic techniques, 2D-DIGE and iTRAQ using three independent techniques such as LTQ-Orbitrap, Q-TOF and MALDI-TOF/TOF mass spectrometry. Bioinformatics analysis was performed using DAVID and KOBAS for pathways and network analysis. Additionally, multiple cellular assays including resazurin-based metabolic activity assay and fluorescence-activated cell sorting (FACS) analysis using 5-Cyano-2,3-ditolyl tetrazolium chloride (CTC) staining for respiratory activity assay were performed to validate the proteomics data.

Result and discussion
This study has identified 15 and 53 proteins in 2-DE and DIGE, whereas quantitative iTRAQ analysis identified 299, 679 and 1096 proteins using MALDI-TOF/TOF, QTOF and LTQ-orbitrap respectively. A total of 227 proteins (87 proteins up-regulated and 140 proteins down-regulated) showed differential expression (1.2 fold change with 1% FDR) with similar trends from at least two independent iTRAQ analysis. Further comparison between gel-based and gel-free analysis showed quite a few proteins were common with similar trend. Pathway analysis showed that the enzymes involved in heme synthesis and petose phosphate pathways were induced whereas ribosomes, glycolysis, TCA cycle and fatty acid synthesis were repressed. The cell division proteins and family of Clp protein involved in protein degradation were induced whereas the universal chaperone proteins involved in protein folding were repressed. In addition, few cell division proteins involved in septum formation were altered. Interestingly, the central metabolic dehydrogenases were all down-regulated, which indicated that aerobic respiration was significantly repressed. Interestingly, the anaerobic respiration enzymes such as lactate dehydrogenase and anaerobic marker protein YwfI were induced. Moreover, metabolic and respiratory activity of the totarol induced cells was significantly reduced. Overall, totarol perturbed the cell division by targeting the accessory cell division proteins and the essential cell physiological functions such as cell membrane permeability and respiration.

Conclusion
The findings obtained from our proteomic and cellular analyses corroborated with quite a few known cellular targets reported earlier; while many of the differentially expressed candidates and associated physiological pathways identified in our study were unique to this study. Our quantitative proteomic investigation and subsequent functional analysis suggests that cell division machinery is a prime target for totarol action by modulating the cell division accessory proteins and essential physiological pathways.

Keywords: Natural products, Quantitative proteomics, iTRAQ, *B. subtilis*, Totarol, Dehydrogenases, Cell elongation

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Use of antimicrobial films (active packaging) incorporating some essential oils and preservatives to control *Penicillium* in cheese

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Molds are the most important spoilage agents in ripened cheeses, with *Penicillium* being the most common genus. The presence of these fungi causes not only spoilage, but also a potential health problem due to their toxigenicity. Among the *Penicillium* responsible for fungal spoilage of cheeses, we have found previously *P. nordicum*, a poorly studied species and potential producer of ochratoxin A. Control of fungi is based on prevention, but also on the use of preservatives, and active packaging may be an alternative tool.

In this work, the inhibitory effect of several preservatives authorized under current European legislation (pimaricin, sodium propionate and potassium sorbate), some essential oils (rosemary and thyme) and several combinations (pimaricin and sodium propionate/sodium propionate and thyme) on several strains of *P. nordicum* (ochratoxin positive) was investigated. To perform this test, an impedimetric equipment (Bactometer, Biomerieux, S.A.), that allows quick results, was used. Moreover, we investigated the use of both active and non-active packaging. The active system essentially consisted of edible films based on gelatin containing these compounds placed on the surface of agar plates and of Castellano cheese slices, both inoculated with *P. nordicum* and *P. verrucosum* (also ochratoxin-positive).

The MICs (Minimum Inhibitory Concentration) corresponding to pimaricin, sodium propionate and potassium sorbate for *P. nordicum* were 5, 2000 and 5000 ppm, respectively; rosemary was less effective than thyme, this one being effective at high levels (10% or higher). Most combinations investigated (using concentrations bellow the MICs found) were effective in inhibiting the growth of *P. nordicum* and *P. verrucosum,* showing a synergic effect; also, an inhibitory effect using films containing these preservatives against these two *Penicillium in vitro* was found; however, when testing the effectiveness of the films on cheese, an inhibitory effect on the growth of *Penicillium* occurred with the use of both active and no-active films. Our findings open the possibility to the use of both active and non-active protein-based films in the protection of some varieties of cheese against surface fungal growth.

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Keywords: preservatives, antimicrobial films, protein-based edible fims, *Penicillium*, cheese.

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Biocontrol. Biosynthesis of antimicrobials

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Activities of Lactic acid bacteria populations and fungi flora in fermented wheat

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In Algeria, wheat is fermented in underground silo called *MATMOUR.* This traditional way of processing would disappear because of continuous farming population migration to the urban area. Today, the fermentation is carried out in metal or plastic barrels. During this process, the wheat is subjected to the micro-organisms and/or the enzymes action with desirable biochemical changes. In this context, we study the modifications of biochemical composition of fermented wheat, the evaluation of microbial biodiversity and the production of extracellular hydrolase (α-amylases, proteases, and lipases). Two wheat samples are used, with vinegar (BFV) or without vinegar (BFSV), and fermented during 15 months.

The significant modification of the lipids had confirmed and the starch is the more degraded substrate during the process of fermentation in the two wheat samples.

Forty lactic bacteria strains (6 *Pediococcus*, 8 *Streptococcus*, 13 *Lactococcus,* 13 *Lactobacillus*) and 10 fungi strains (6 *Penicillium*, 1 *Mucor*, 1 *Saccharomyces*, 1 *Candida*, 1*Pichia*) were isolated on the basis of the morphological, biochemical and physiological properties.

The description of the hydrolytic activities showed that the majority of the fungus strains are amylolytic (5 moulds, 1 yeasts), proteolytic (4 moulds, 1yeasts) and lipolytic (5 moulds, 1 yeasts). The 40 isolates of the lactic bacteria are amylolytic for 12 isolates and proteolytic for 27 isolates.

Keywords: Fermented wheat, barrels, isolation, fungus, bacteria, extracellular hydrolyses

Biography
Farida BEKHOUCHE, Assistant Professor and researcher at the institute of Nutrition, food and food technology of the University Constantine 1, Algeria. My academic qualification is a PhD in Microbiology and Enzymology (food industry). My Scientific activity is PhD supervisor of two research works. On the other hand, I direct a scientific team concerning the wheat's fermentation for producing the couscous "*Lemzeïet*" (traditional food).

Analysis of the complete genome sequence of batumin producing strain *Pseudomonas batumici* UCMB-321 revealed that the whole batumin synthesis encoding operon was acquired by horizontal gene transfer

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Staphylococcus aureus is a major human pathogen that is capable of causing a wide range of human diseases that result in significant morbidity and mortality in both community and hospital acquired infections. Methicillin-resistant *S. aureus* that have developed antibiotic resistance through the process of natural selection became especially problematic in hospitals. A new antibiotic was isolated from *Pseudomonas batumici* that selectively suppress *Staphylococcus* including multi-drug resistant strains in concentration 0.05 μg/ml [1].

A similar antibiotic kalimantacin was isolated in 1996 from *Alcaligenes* sp. [2], but then works on this strain stopped and it is not available anymore for further investigation. Another producer of an antibiotic termed by the authors as kalimantacin/batumin was *Pseudomonas fluorescens* BCCM ID 9359 [3]. The authors isolated and sequenced the whole *kal/bat* operon encoding a polyketide synthase [3,4]. But eventually this strain was lost and it is not supported anymore by BCCM. Currently *P. batumici* UCMB-321 is the only batuming synthesizing strain that is available for further studies.

Genome UCMB-321 was sequenced in Macrogen (South Korea) using Illumina PE Hiseq2000 technology. DNA reads were assembled by CLC Genomics Workbench 7 into 127 contigs. Whole *kal/bat* operon was identified in the mid of the large contig 3. Surprisingly the identified 77 kbp long operon containing in total 28 protein coding genes showed 100% DNA similarity to *kal/bat* operon isolated from *P. fluorescens* BCCM ID 9359 except for 1 nucleotide deletion in a non-coding part. This operon sequence is significantly less GC-rich (50% against 64% in average) and the whole genome sequence and the program SeqWord Genomic Island Sniffer predicted horizontal acquisition of this region.

Availability of the whole genome sequence of UCMB-321 allowed precise identification of its phylogenetic position among other sequenced *Pseudomonas*. The closes relatives of UCMB-321 were *P. gingeri* NCPPB 3146 and *P. protegens* CHA0; both have no *kal/bat* operon in their genomes. The highest similarity to *kal/bat* operon in terms of gene synteny and amino acid sequence conservation was observed for gene operons encoding bacillaen biosynthesis in *Bacillus*. Failure in obtaining clones of UCMB-321 lacking batumin synthesis pointed out that this compound despite its horizontal acquisition most likely is indispensible for *P. batumici* and is involved in the regulation of cell wall fatty acid biosynthesis [3]. Antibiotic activity of the batumin most likely is not directly linked to its prime biological function. Genome comparative analysis and molecular docking modeling will aid in understanding the functionality of complex secondary metabolites and the impact of acquisition of these genes on bacterial speciation that in its own turn will improve our ability for predicting new antibiotics among them.

Keywords: batumin, antibiotic, *Pseudomonas*, *Staphylococcus*, NGS, comparative genomics

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Antibiotic resistance and molecular characterisation of seafood isolates of nontyphoidal *Salmonella* by PFGE

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Emergence of multidrug resistant nontyphoidal *Salmonella* isolates is a major health concern worldwide with the most predominant occurrence of *Salmonella* Typhimurium phage type 104 (DT104) resistant to ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracycline. Apart from antibiotic resistance the identification and genotypic characterization of pathogens are essential for epidemiological surveillance and outbreak investigations. Pulse-field gel electrophoresis is currently considered the gold standard method for subtyping foodborne pathogens. A total of 39 environmental strains of *Salmonella* were isolated from seafood samples and examined for their susceptibility to various antibiotics and used for PFGE analysis using *Xba*1 restriction enzyme. Of the 39 seafood isolates the highest rates of resistance were observed for erythromycin (100%), nalidixic acid (15.38%), co-trimoxazole (15.38%), chloramphenicol (12.82%), ampicillin (12.82%) and tetracycline (10.25%). Six (15.38%) of the 39 isolates were multidrug resistant. The *Xba*I digested chromosomal DNA of 39 strains of *Salmonella* produced 7 different clusters indicating the presence of diverse *Salmonella* strains in seafood. The Discriminatory Index (DI) value for PFGE obtained by using *Xba*I restriction enzyme was 0.91. The PFGE method has been proven to be highly discriminating for subtyping *S*. Weltevreden and *S*. Newport. The *Xba*I PFGE analysis used in this study could also be able to distinguish multidrug-resistant strains from the sensitive strains as they shared different pulsotypes. The study also showed that multiple clones of *S*. Weltevreden, *S*. Newport and *S*. Oslo can be isolated throughout the south west coast of India. Genetic diversity between different seafood sources would not be unexpected. Genetic diversity among the similar seafood sources suggests the presence of different clones of *Salmonella* which further, increases the risk of seafood being a potential source of highly pathogenic bacteria like *Salmonella*.

Antimicrobial activity and probiotic potential of piglets microbiota

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Gastrointestinal microbial homeostasis plays a major role in maintaining good animal health. Therefore, reach work have been undertaken to obtain lactic acid bacteria (LAB) strains able to inhibit most common pathogens of pigs. Most probiotic strains occur among lactic acid bacteria, particularly *Lactobacillus*, *Bifidobacterium* and *Enterococcus*. These bacteria are part of the normal intestinal microflora of animals and can be used as probiotics. Their biological activity is closely linked with the ability to produce bioactive metabolites.

The aim of this study was to isolate active probiotic bacteria strains characterized with targeted pro-health action against pathogenic strains of *Clostridium perfringers* and *Escherichia coli.*

The material consisted of faecal samples from piglets, a sample swab from the mouth, as well as samples of intestinal contents taken from canulated piglets.

Altogether 226 isolates were studied. It was found that most of the isolated lactic acid bacteria (LAB) showed a narrow range of activity against *C. perfringers* and *E. coli*. This activity was mostly a consequence of competition. Only a few isolates affected *C. perfringers* and *E. coli* with exogenous metabolites, mainly organic acids and bacteriocins. All the bacteriocin produced by the tested isolates were characterized with strain dependent activity.

The isolates showing the strongest activity against *C. perfringers* and *E. coli* were the representatives of the following species: Enterococcus faecium, Enterococcus hirae, Enterococcus avium, Leuconostoc mesenteroides and Carnobacterium divergens. It was found that most of the active strains with anti-clostridium and anti-escherichia activity showed tolerance to low pH and the action of bile salts and survived *in vitro* digestion. In addition, five strains were characterized by strong adhesion properties.

The resulting strain has the potential to develop new probiotic preparations in targeted elimination of the most common pathogens in piglets.

Keywords: LAB, probiotic, eubiotic, pathogens, health

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Antimicrobial activity of Se^0/Te^0 – based nanoparticles of bacterial origin

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In the last few decades, the emergence of bacterial resistance to antibiotics has become a common phenomenon in both community and hospital setting. As a consequence, the effectiveness of antibiotic treatment of bacteria-based infection has progressively decreased [1]. In particular, the treatment of biofilm-associated infection is problematic, since bacteria grown in biofilm mode are more tolerant to conventional antibiotics and biocides compared to free swimming cells [2]. Therefore, it's necessary to develop and test new antimicrobial compounds having both bactericidal potential and biofilm eradication activity against multidrug-resistant bacteria.

In recent years, the employment of metallic nanoparticles has emerged as an alternative to the use of organic compounds as antimicrobial agents [3]. Several studies has been focused particularly on the antimicrobial activity of silver nanoparticles: however other metal or metalloid nanoparticles have exhibited a promising bactericidal capability [4]. However, one of the major drawback for the employment of nanoparticles is the cost associated with the traditional physical-chemical methods of synthesis and the production of toxic substances as byproduct [5]. For these reasons, there is a considerable amount of interest in developing new and eco-friendly processes for the manufacturing of nanoparticles.

In the present work, Se^0 and Te^0-based nanoparticles were bio-synthesized employing the selenite and tellurite-reducing capability of two bacterial strains isolated from polluted environments: *Stenotrophomonas maltophilia* SeITE02 and *Ochrobactrum* sp. E. By regulating culture conditions and exposition time, we were able to produce nanoparticles of different dimensions, between 50 and 200nm.

The nanoparticles were tested against planktonic and biofilms cultures of three common pathogenic strains: *Escherichia coli* JM109, *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* ATCC 25923. We evaluated both the inhibition activity against biofilm and planktonic growth and the eradication activity against biofilms established for 24 hours. To measure these parameters we determined both the minimum biocidal concentration (MBC) and the minimum biofilm eradication concentration (MBEC). In addition, we observed the effect of increasing concentrations of nanoparticles on biofilm structure using Confocal Laser Scanning Microscopy (CLSM).

Our results indicate that both Se^0 and Te^0 nanoparticles possess antimicrobial and biofilm eradication activity. In particular Se^0 nanoparticles exhibited antimicrobial activity at lower concentration. Preliminary data suggests that the activity seemed to be dependent on the dimension of the nanoparticles: indeed, the highest activity was shown by the nanoparticles smaller in size. The key observation is that bacteria growth in biofilm mode didn't exhibit a higher level of resistance against the nanoparticles antimicrobial action.

Results described in this study suggest a possible application of both Se^0 and Te^0 nanoparticles as an effective antimicrobial agent with a high biofilm eradication capacity.

Keywords: antimicrobial; metalloids; nanoparticles; biofilms

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Biocontrol bacteria effects on postharvest performance of *Gladiolus grandiforus* L. 'Mammoth'

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A study was conducted at Department of Horticultural Science, North Carolina State University, Raleigh, USA, to elucidate the efficacy of different bacterial strains on controlling detrimental bacteria and vase life extension of *Gladiolus grandiflorus* L. 'Mammoth'. In a preliminary study, three bacteria strains (*Bacillus pumilus*, *Delftia acidovorans*, and *Herbasperillum* sp.) were isolated from the vase solutions of cut *Gladiolus* and cultured to obtain the bacteria to be used in the study. These isolated strains were compared with two strains of *Pseudomonas fluorescens* (PF-279 and PF-417), which were effective in biological control of several detrimental bacteria in some agronomic crops, to find beneficial bacteria to be used as biocontrol biocide during postharvest handling of gladiolus. All tested strains resulted in a similar vase life of cut gladiolus stems, which was also similar to that of stems placed in tap water. However, stems placed in solutions with PF-279 *Pseudomonas fluorecsens* had higher water uptake, while those with higher concentrations of *Herbasperillum* sp. had lowest water uptake. Use of nutrient broth to culture bacteria increased initial pH of all solutions, which might be a reason of early senescence of cut stems compared to the stems placed in tap water (control). In summary, the tested bacterial strains had no effect on controlling detrimental bacteria present in the vase solutions and had no significant effect on vase life extension of cut gladiolus.

Keywords: Pseudomonas fluorescens; Bacillus pumilus; Herbasperillum sp.; Delftia acidovorans; beneficial microbes; vase life.

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Characterization of anti-Candida activity of vaginal lactobacilli

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The female lower genital tract is an ecological niche where several aerobe and anaerobe microorganisms coexist in a dynamic balance, its homeostasis results from complex interactions and synergies among the host and such microorganisms. Healthy vaginal microbiota is generally dominated by *Lactobacillus* genus, with the prevalence of one species among *L. crispatus*, *L. iners*, *L. jensenii* and *L. gasseri*. It is well documented that lactobacilli are relevant as a barrier to pathogen infections, by preventing overgrowth of pathogenic and opportunistic organisms, such as *Candida.* Known mechanisms by which lactobacilli exert their protective functions include (i) reduction of vaginal pH by producing organic acids, specially lactic acid, (ii) production of antimicrobial substances (bacteriocins, hydrogen peroxide), (iii) competition with other microorganisms for the nutrients and for adherence to the vaginal epithelium and (iv) stimulation of the immune system. Recently, it has been reported that sodium butyrate, a metabolite produced by lactobacilli and a known histone deacetylase inhibitor, is able to reduce *Candida* growth and virulence [1].

Candidiasis is the most common yeast infection in the vaginal environment, affecting 70-75% of women at least once during their lives, and is frequently recurrent; to date, little is known about the pathophysiology of recurrent vulvovaginal candidosis.

In this work we report the isolation of vaginal *Lactobacillus* strains from healthy donors, and the study of their potential activity against *Candida* and their mechanism of action, focusing on hydrogen peroxide production, culture pH and histone deacetylase inhibitor production.

17 *Lactobacillus* strains have been isolated from mid vaginal swabs and taxonomically identified. *Lactobacillus* culture supernatants have been tested for their fungistatic and fungicidal activity against 9 *Candida* isolates, belonging to *C. albicans, C. tropicalis, C. krusei, C. glabrata, C. parapsilosis,* and *C. lusitaniae* species. All lactobacilli have been evaluated for peroxide production production and culture supernatants pH, and preliminary test have been performed on *Candida* histone acetylation profile by Western Blot.

Isolated lactobacilli belong to *L. crispatus*, *L. gasseri* and *L. vaginalis* species, and many of them inhibited *Candida* growth. *L. crispatus* culture supernatants were the most effective, in particular against *C. albicans* and *C. lusitaniae*. None of the isolated *Lactobacillus* was able to interfere with *C. krusei* and *C. parapsilosis*. *L. vaginalis* supernatants showed an intermediate antifungal activity, while *L. gasseri* did not alter *Candida* growth. All *Lactobacillus* produced hydrogen peroxide, and reduced culture pH through the release of organic acid. Some *Lactobacillus* supernatants also influenced *Candida* histone acetylation profile, suggesting the capability to interfere with histone modifying enzymes.

Lactobacillus strains selected on the basis of their health-promoting activity could be employed in probiotic formulations, that could be administered as adjuvants in the prophylaxis/therapy of *Candida* vaginal infections.

Keywords: Vaginal *Lactobacillus*; *Candida*; histone deacetylase inhibitors; probiotics

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Characterization of lactic acid bacteria multi- antagonist isolated from the maternal milk and new born feces

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The most abundant microorganisms in milk are lactic bacteria. They are involved in the production of organic acids and other antibacterial substances such as bacteriocins, which inhibit some pathogenic strains. Antagonism tests were performed in order to find inhibiting agents in lactic bacteria (Tabak, 2011).

In this context, 120 strains of lactic bacteria were isolated from maternal milk and feces of infants of different ages. The isolation was performed on three culture media, MRS-Cysteine 0.05% BSM and RB medium. Cultures were incubated for 48 hours at 37 ° C.

We proceeded in three steps, sorting, at first, the isolates by the mean of phenotypic tests. In a second step, we performed a screening of the isolates based on their ITS footprint (Internal transcribed Sequence). The strains were then identified by the sequencing of the 16S RNAr gene. They belong to the genus *Lactobacillus*, *Enterococcus* and *Streptococcus*, *Lactococcus*. In parallel, we evaluated the antibacterial activity of the isolates against pathogens, Gram positif and negatif. Antagonism tests revealed the ability of some strains to inhibit the indicator strains: *Listeria ivanovi*, *L. inocoa*, *Staphylococcus aureus*, and *Micrococcus Pseudomonasas aeruginosa*. The diameter of inhibition varied between 9 mm and 35 mm. The largest antibacterial activity was observed with *Enterococcus faecium* isolate (D8).

Key words: Maternal milk, antagonism, ITS, 16S RNAr, lactic acid bacteria

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Characterization of novel bio-active compounds in the heat resistant Streptomyces isolated from soil

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The genus *Streptomyces*, which are mainly found in the soil, are producing the secondary bioactive compounds such as antibiotics, antifungals, anti-tumors and immunosuppressive agents [1]. At least two-third of the previously known natural antibiotics was produced by this genus. Identification and production of antimicrobial compounds that are produced by this genus began at 70s and since now about 1 to 3% of them have been discovered [2].

Lut desert, Iran, as one of the hottest places in the world (with the recorded temperature of 70.7 ºC during 2003-2009) was chosen for sampling. This study was aimed to isolate the heat resistant Streptomyces from the Lut desert and detect the antimicrobial compounds from these novel bacteria. Therefore, 46 samples were collected from the surface (up to 5cm depth from external surface) and depth (5-20cm underneath) soils of three geographically identified places of Gandomberian (30.34N, 57.51E) in the Lut desert. The soil sample was mixed, and a suspension of 50 g soil in 50 ml of sterile saline solution was prepared. The suspension was then heated in water bath at 55ºC for 6 minutes in order to destroy the vegetative forms of the bacteria. Four selective culture media (humic acid-vitamin agar containing 50µg/ml cyclohexamide and 10µg/ml nalidixic acid, starch-casein agar containing 25µg/ml cyclohexamide and 25µg/ml nystatin, raffinose-histidine agar containing 25µg/ml cyclohexamide and 25µg/ml nystatin and glucose-yeast extract agar which 20µg/ml rifampicin has been added) were employed. The preparations were subsequently cultured onto the surface of the media, followed by incubation at 28 ºC for three weeks. The suspected colonies were further characterized using gram staining and PCR assay. Culture extracts from the confirmed isolates were obtained from the inoculated TSB broth after centrifugation the broth at 10000 rpm for 10 min. The well diffusion assay was performed to investigate the antimicrobial activity of the neutralized extracts (pH 6.5-7.5) on *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium* and *E. coli*. The antimicrobial activity of the effective neutralized extracts was detected again after decomposition of hydrogen peroxide by adding catalase (150 IU/ml). The extracts that were still in effect were treated with pepsin to a final concentration of 1 mg/ml and again well diffusion method was used to determine the antimicrobial activity [3].

Using the PCR assay, nine of the suspected colonies were confirmed. None of the isolates were effective against *E. coli*, whereas one and six of them reduced the growing of *S. typhimurium* and *S. aureus*, respectively, which were completely inhibited following use of catalase. Six isolates were also influenced the growing of B. cereus which Just four isolates were affected following the application of catalase. Following ingestion by pepsin, two isolates showed the antimicrobial activity against B. cereus up to 15.5 to 33.1 % of inhibition rates.

Results of the current study were confirmed the presence of a rare heat resistant Streptomyces in the soil of Lut desert, Iran which showed some in-vitro antimicrobial activities.

Key word: heat resistant *Streptomyces*; bioactive compounds; soil; Lut desert

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Different growth kinetics and their impacts on production of enterocin OS13 following by applying different purification strategies for recovering of high yield bacteriocin

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Enterococcus faecalis belongs to lactic acid bacteria which are commonly known to produce antimicrobial peptides called bacteriocins. In a previous study, the food isolated *E. faecalis* OS13 was shown to produce large amount of a narrow spectrum and highly potent bacteriocin named enterocin OS13 with activity against antibiotic resistant nosocomial *E. faecalis* and *E. faecium*. In our efforts to optimize the production and subsequently purification of enterocin OS13, several trials related to applying different growth conditions and their effects on the recovered bacteriocin were attempted. The growth kinetic curves and subsequently bacteriocin production at different incubation times; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 hr, different starting inoculums; 0.01, 0.1, 1, 10 %, and different incubation temperatures; 30, 37 °C were recorded and graphed. The optimum growth conditions with maximum bacteriocin production were obtained after growing at 30 °C for 14 hr with starting inoculums of 0.01% of producer strain. Three purification strategies were followed to compare and optimize the recovered yield of bacteriocin. The first strategy was based on the following sequence; precipitation of proteins using 40% ammonium sulphate, washing the precipitated protein with 70 % isoprobanol pH 2, ion exchange chromatography using SP Sepharose column, reverse phase chromatography using RPC1 column and finally reverse phase chromatography using Sephasil C8 column. The second strategy was based on precipitation of proteins by 40% ammonium sulphate, ion exchange chromatography using SP Sepharose column without adjusting the pH, filtration using a cut off protein membrane filter and finally applying to Mono S column. The third strategy was based on Extraction of proteins with Amberlite XAD-16 resin, ion exchange chromatography using SP Sepharose column without adjusting the pH, applying to Mono S column and finally reverse phase chromatography using Sephasil C8 column. The best purification strategy was the first one where the finally recovered bacteriocin was about 20 % of the initial amount.

Keywords: *Enterococcus faecalis*; bacteriocin; purification; growth kinetics.

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Discovery and characterisation of a novel plasmid of a probiotic strain *Lactobacillus fermentum* 3872

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Previous *in vitro* studies have demonstrated the outstanding probiotic properties of *Lactobacillus fermentum* strain 3872, which include enhanced antibacterial activities and the ability to adhere to various tissue culture cell lines (unpublished observations). The molecular basis for these properties and factors involved remained unknown until a draft genome sequencing of this bacterium became available (1). In particular, a partial sequence of a gene encoding an unusually large collagen-binding protein (CBP) has been reported (1). In this study we discovered and characterised plasmid pLF3872 carrying this and other genes that may be essential for the antibacterial properties of this microorganism. The complete sequence of the plasmid was derived in the course of a genome sequencing project using Ion Torrent PGM and 400nt sequencing kit. In addition to *cbp*, this circular 32 kb plasmid also contains thirty two other genes, including those encoding a toxin-antitoxin pair required for a stable maintenance of the plasmid within the strain. There is also a gene encoding a peptidoglycan hydrolase with a potential role in conjugation. The collagen binding protein encoded by the *cbp* gene was found to contain five repetitive 'B domains'. These domains may be involved in the formation of a 'stalk' presenting a non-repetitive 'A domain' involved in adhesin. Interestingly, 3872 is the only strain of *L. fermentum* carrying the *cbp* gene. The CBPs produced by some other *Lactobacillus* spp contain only four B domains. Such adhesins are also produced by some pathogenic bacteria, e.g. *Staphylococcus aureus* which may explain the mechanism of a probiotic action based on competitive exclusion during attachment to host cells (2). The ability to produce a collagen binding protein may also allow the probiotic to compete against pathogenic bacteria bound to the same target protein resulting in colonisation of the site of infection followed by the release antimicrobial substances (such as bacteriocins and hydrogen peroxide).

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Effect of rhizosphere bacteria on the growth of phytopathogenic fungi

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The presence of microorganisms in the root zone of plants is associated with the different kinds of interactions, both between the various types of microorganisms and between the microorganisms and plants. Plants affect the number and activity of rhizosphere microorganisms releasing into the soil secretions containing sugars, proteins, amino acids, organic acids, low molecular weight alcohols, phenolic compounds, hormones, vitamins and others. Oxygen, which is introduced through the rhizosphere of plants, has a high impact on the development of soil microbial,. Also plants benefit from advantages associated to the presence of rhizosphere microorganisms.. This is due to various interactions which take forms of symbiotic or antagonistic. Microorganisms while decomposing organic matter in the soil increase the availability of nutrients to plants by increasing their yield. In addition, some species of microorganisms produce specific metabolites which can restrict presence of other microorganisms, including pathogenic to plants. The aim of the study was to determine the effect of microorganisms isolated from the rhizosphere of willow (*Salix viminalis* L.) on the growth of selected strains of phytopathogenic fungi. On the basis of differences in the thermal requirements and colony morphology, 17 strains of mesophilic bacteria and 13 strains of psychrophilic bacteria were isolated from the rhizosphere of willow. The various bacteria were grown in liquid media for 24h then the suspension was introduced pointwise (to wells) on Petri plates containing PDA medium (potato dextrose agar), inoculated sentinel fungal strains. As an indicators the *Fusarium oxysporum* and *Fusarium graminearum* fungi were used. The co-culture of the bacterial isolates of fungal strains was conducted for 5 days at the temperature of 25°C. After this time the zones of inhibition size of fungal growth was measured. It was found, that in the rhizosphere of willow (*Salix viminalis* L.) there are strains of bacteria exhibiting antagonistic effects forward *Fusarium oxysporum* and *Fusarium graminearum* fungi. Among 30 isolated strains four sentinel mezofinych bacteria were characterized by the most antagonistic action in respect of fungi. Operatively they were designated as K5, K6, P1, Z3 and Z5.

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Keywords: phytopathogenic fungi, rhizosphere bacteria,

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Effects of fungal-bacterial consortium on hydrocarbons biodegradation efficiency - analysis of metagenomes

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An analysis of the efficiency of diesel biodegradation by microorganisms of the consortium tested with the addition of AC5 *Trichichoderma resei* fungus was carried out. The decrease of hydrocarbons was determined on an initial 5th, 10th, 15th, and 20th day of the process. Biodegradation of diesel oil by a fungal-bacterial consortium after 20 days of the experiment reached 95%. The degree of degradation was compared with:

- The result of the biodegradation of the diesel without microorganisms, which after 20 days was 6%;

- The result of biodegradation diesel containing the bacterial consortium, which after 20 days was 74%;

- The result of biodegradation of diesel oil with the filamentous fungus, which after 20 days was 23%.

On the twentieth day of the process a metagenomic analysis of the 16S rRNA gene encoding based on the V4 hypervariable region of the 16S rRNA gene was performed. To amplify the selected region-specific primers 515F and 806R were used. Sequencing was held on MiSeq sequencer. Automatic analysis of the data was carried out on the camera MiSeq using software MiSeq Reporter (IAS) v2.4 protocol 16S metagenomics. Metagenomes analysis allowed the identification of 158 species of bacteria. The bacterial consortium was dominated by *Stenotrophomonas retroflexus* 14.90 %, *Sphingobacterium multivorum* 13.94 %, *Ochrobactrum intermedium* 12.39 %, *Achromobacter xylosoxidans* 10.14 %, *Pseudomonas parafulva* 9.65 %, *Pseudomonas alcaligenes* 4.65 %, *Pseudomonas tremae* 4.50 %, other 18.56%, unclassified 14,21%. In contrast, however, the addition of the fungus caused a change in the bacterial population, where the dominant were 3 species: *Stenotrophomonas retroflexus* 47.46%, *Ochrobactrum intermedium* 25.83%, *Citrobacter freundi* 23,45% other 1.74%, unclassified 5.09%.

This work was financially supported by the National Science Centre, Poland (Project Opus 22011/03/B/NZ9/00274).

Keywords: biodegradation, metagenomic analysis, *Trichoderma resei*

Heat stable antifungal compound production by *Burkholderia cepacia* JBK9 effective against Fusarium rot disease of garlic

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Fusarium oxysporum f. sp. *lycopersici* is the causal agent of Fusarium rot disease in garlic bulb, which is one of the most common post-harvest destructive disease worldwide. A number of chemical fungicide and refrigeration methods are being exploited now a days to combate with the such diseases. Recently, bacterial antagonists have been explored for the isolation and characterization of metabolite responsible for the effective control of such fungal dieases. In the present study, bacterial isolates from garlic field were screened for thier antagonestic activities against the *F. oxysporum* f. sp. *lycopersici*. The petri dish based assay revealed that an isolate JBK9 possess strong antifungal activity. This bacterial isolate JBK9 was identified as *Barkholderia cepacia* strain through the comparison of 16S rRNA gene sequences. The antifungal compound(s) produced by JBK9 in the culture broth were extracted through solvent solvent extraction using ethyl acetate, butanol and n-hexane. The extracts were subjected to thin layer chromatography (TLC) to separate and single out the mentbolites. The bioactive fraction against the *F. oxysporum* was selected and further purified through high-performance liquid chromatography (HPLC). Nuclear magnetic resonance (NMR) with the support of gas chromatography mass spectrometry confirmed the compound as pyrrolnitrin [3-chloro-4-(28-nitro-38-chlorophenyl)pyrrole]. In conclusion, as *Burkholderia cepacia* and pyrrolnitrin showed strong activity against the economically important garlic pathogen *Fusarium oxysoprum* f. sp. *lycopersici* which may be considered as promising biopesticide.

Key words: Fusarium rot disease; Garlic; *Burkholderia cepacia*; Pyrrolnitrin

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Identification of loci associated with antimicrobial activity in *Burkholderia gladioli* strain UAPS07070

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Burkholderia gladioli strain UAPS07070 shows a strong antagonistic activity against bacteria and fungi, some them human or plant pathogens (Marín-Cevada *et al.,* 2012). The aim of this study was detecting genes associated to antibiosis by *B. gladioli* UAPS07070. The antagonistic effect of 3500 random mutants transposed with *Himar1* (Rholl *et al.,* 2008) was tested in double layer agar. Twelve of them displayed decreased inhibitory capacity against the pathogenic bacteria of pineapple, *Tatummella ptyseos* UAPS07007 (Marín-Cevada *et al.,* 2010). The sequence analyses of the mutated genes in three of these mutants corresponded to: an Acyl homoserine lactone (AHL) synthase, a LuxR regulator, and to a regulator of the family LysR. These same mutants also showed decreased inhibition in double layer agar test in LB media against the gram-negative bacterium *Acinetobacter sp*. UAPS01-69, but they exhibited a different inhibitory phenotype in other culture media. These results suggest that the metabolism may be related to the regulation of the genes implicated in antibiosis in *Burkholderia gladioli.* Besides, one of the mutants interrupted in AHL synthase gene does not produce N-acyl homoserine lactones that could be detected by the biosensor strain *Chromobacterium violaceum* CV026 (McClean *et al.,* 1997), suggesting the synthesis of an acyl-homoserine lactone short chain molecule. In conclusion, this is the first report of putative regulatory genes and quorum-sensing genes associated with a phenotype of antibiosis in *Burkholderia gladioli*.

Keywords: *Burkholderia gladioli*; *Himar1* transposon; Antibiosis; Quorum sensing.

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Isolation and characterization of acid lactic bacteria from maternal milk and newborn feces of the northwest Algerian population

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Breast milk is a rich source of nutrients. It helps to establish a good intestinal flora of infants [1]. In this context we have isolated and characterized lactic acid bacteria from human milk and infant feces of Algerian population. We also determined their sensitivity to antibiotics. The use of VITEK ® 2 Compact has allowed us to identify strains by GP cards and to test their sensitivity to antibiotics by AST-P586 cards.

Most of the strains studied are part of the genus *Enterococcus (E. faecalis, E. faecium, and E. durans)*. These strains are shared between breast milk and infant feces with predominance of *E. faecium* in breast milk and *E. faecalis* in infant feces.

All strains are susceptible to vancomycin and teicoplanin. Only one *E. faecalis* (Q) is resistant to ampicillin and benzylpenicillin; this strain and also 33% of *E. durans* have a multidrug resistance.

Keywords: Lactic acid bacteria; *Enterococcus (E)*; Breast milk; infant feces; antibiotic susceptibility.

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Lectin-type pyocin action against *Pseudomonas aeruginosa* is O serotype independent

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Lectin-like bacteriocins are antibacterial proteins constituted of two structurally similar monocot mannose-binding lectin (MMBL) domains, and are followed by a short C-terminal extension. These so-called LlpA bacteriocins have been characterized in strains of *Pseudomonas*, *Xanthomonas* and *Burkholderia*, and display highly specific intra-genus activity [1]. In contrast to typical Gram-negative bacteriocins (such as *Escherichia coli* colicins or *Pseudomonas aeruginosa* S-type pyocins), LlpAs do not require a cognate immunity protein [1,2]. These antibacterials are retrieved in soil-dwelling and plant-associated isolates, but are not widely distributed in these populations.

Recently it was demonstrated that only one of the MMBL modules of LlpAs displays sugar-binding properties. Interestingly, LlpAs seem to prefer D-rhamnose over D-mannose. The latter property enables the use of D-rhamnose-containing lipopolysaccharide as a cellular receptor, presumably for initial attachment of the bacteriocin to the outer membrane of a target cell. In contrast, the other MMBL domain of the LlpAs serves a role as a target strain specificity determinant. Presence of both lectin modules is mandatory to obtain a fully active antibacterial molecule [3,4], but the actual killing mechanism of lectin-like bacteriocins remains unknown.

We identified two highly-divergent groups of lectin-like bacteriocin genes in recently sequenced *Pseudomonas aeruginosa* genomes. The encoded proteins show only borderline sequence homology to previously identified LlpAs. Recombinant His-tagged proteins, purified via affinity chromatography, were tested against a large panel of *Pseudomonas* isolates and other Gram-negative bacteria. Antagonistic interactions were detected solely against *P. aeruginosa* strains. In addition to environmental isolates, these L pyocins also targeted multidrug-resistant clinical isolates, with minimum inhibitory concentrations down to the nanomolar range. Spectrum overlap between close homologues belonging to one of the L pyocin groups was only minimal. This is in contrast to the spectral redundancy that was observed for LlpAs in other *Pseudomonas* species. Residues differing between these close homologues, potentially responsible for the spectrum differences observed, were mapped on the L pyocin 3D-structure [4], and appeared to mainly cluster in the domain lacking D-rhamnose binding capacity.

We found no correlation between susceptibility to L pyocins and phylogenetic relatedness of the *P. aeruginosa* isolates, suggesting that a highly variable characteristic is responsible for the spectrum differences observed. Out of 15 O serotypes tested, 13 contained L pyocin susceptible strains, excluding the possibility that the highly variable and immunogenic O serotype antigen of the LPS coating would represent a susceptibility-discriminating factor [5].

Keywords: bacteriocin; lectin; O-specific antigen; *Pseudomonas aeruginosa*

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Leuconostoc mesenteroides J33 as biocontrol agent of *L. monocytogenes* in fresh goat milk cheese

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Goat's milk cheeses are very popular in the Mediterranean region. These products can be manufactured from raw and pasteurized goat's milk where the quality differences in these products are mainly due to the presence of indigenous microbiote from goat milk. Recent studies have focused on the use in dairy products of selected strains from goat's milk with a technological potential. The isolation of strains from goat's milk has the advantage that can be considered as proper source for new bacteria strain susceptible to be used as adjunct cultures in dairy products with safety and technological potentials.

Leuconostocs are heterofermentative lactic acid bacteria frequently used in dairy industry. Additionally, like the majority of lactic acid bacteria (LAB), Leuconostoc may have a role in the growth prevention of pathogenic microorganisms such as *Listeria monocytogenes*. This study had the objective to evaluate in vitro some characteristics related to anti-listeria activity of *Leuconostoc mesenteroides* J33 strain isolated from raw goat's milk in order to select an appropriate adjunct culture, which could play as a biocontrol agent role in goat dairy products.

To evaluate the in vitro antimicrobial activity, well diffusion assay were used to provide semi-quantitative measures of anti-listerial activity, and Minimum Bactericidal concentration (MBC), were determined by a micro dilution assay against differents strains of *L. monocytogenes* isolated from dairy food. Results indicated that *Leuc. mesenteroides* J33 was active against all the target strains.

Co-cultures were also performed in BHI broth of *Leuc. mesenteroides* J33 and *L. monocytogenes* CECT 4032 with different initial cell concentrations. Results showed that equal Leuconostoc and Listeria initial cell loads, allowed normal growth of the pathogenic strain. However, when *Leuc. mesenteroides* J33 initial concentration was higher than that of *L. monocytogenes* CECT 4032, partial or total growth inhibition of the pathogenic strain was observed. Specifically, at 10^6 CFU/mL Leuconostoc and 10^2 CFU/mL Listeria initial cell loads, the growth of the pathogenic strain in co-culture was maintained three log orders lower than the control throughout the experiment. Finally biocontrol capability by *Leuc. mesenteroides* J33 was evaluated against *L. monocytogenes* in a goat's milk cheese model. Viable cell counts of *L. monocytogenes* and Leuconostoc were carried out at the beginning and throughout the experience (14 days). Listeria counts remained below 10^4 CFU/g from the beginning to the end of storage at 4ºC compared to the control batches (10^7 CFU/g).

As finding, these results support the viability of novel agent biocontrol methods including the native strain *Leuconostoc mesenteroides* J33 to improve food safety of goat milk products.

Keywords: *Leuconostoc mesenteroides*; biocontrol; Goat's milk cheese; *Listeria monocytogenes*;

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Molecular cloning, expression, and purification of *Staphylococcus pseudintermedius* secreted proteases, a potential virulence factor

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Staphylococcus pseudintermedius was described in 2005 as a new coagulase-positive species and an opportunistic pathogen mostly found in dogs [1]. A recently publication of genomic sequence of *Staphylococcus pseudintermedius* [2,3] revealed a complement of genes encoding putative virulence factors and open multiple new directions of research. Those factors belong to three distinct groups of toxins, extracellular enzymes and cell-wall-anchored proteins, which together form a machinery enabling survival in different environmental niches. Among the extracellular enzymes the cluster of eight genes encoding predicted glutamyl-edopeptidase-like proteins attracted our attention. The predicted proteins share 56-75% sequence identity with each other, and all contain amino acids found in the catalytic triads of enzyme from the trypsin-like serine protease family. The enzymes show a remarkable sequence homology to substrate specific serine proteases from *S. aureus*, including epidermolytic toxins (ETA, ETB), V8 protease, and proteases from *spl* operon.

The goal of presented studies was to clone, express, and develop efficient system to produce recombinant form of *Staphylococcus pseudintermedius* secreted proteases. The genes coding proteins of interest were PCR amplified from genomic DNA of *Staphylococcus pseudintermedius* strain ED99 and cloned to pET21a expression vector. Independently, three genetically engendered *E.coli* strains BL21, Shuffle and Rosetta-gami, were tested, but only in Rosetta-gami recombinant proteins were produced in a soluble form. The proteases were purified using affinity, ion-exchange chromatography, and gel-filtration methods. In a future, the purified proteases will undergo further biochemical and structural characterization to elucidate its function in molecular pathogenesis of *Staphylococcus pseudintermedius* infection.

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Keywords: staphylococcal proteases, virulence factor, bacterial ifection

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New approach to extend shelf life of Mozzarella cheese using antimicrobial microbes

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Mozzarella cheese is a very popular Italian *pasta filata* product; its shelf life is approximately 5 to 7 days [1]. It is spoiled above all by *Pseudomonas* spp., mostly coming from water used during manufacture; *Pseudomonas* - due to its intense proteolytic activity - can grow in Mozzarella cheese modifying texture and reducing its shelf life. Another factor that reduces its shelf life is the presence of coliforms; effectively, *Escherichia coli* can grow in Mozzarella cheese reducing its safe life and, consequently, also its shelf life. Obviously, proteolytic and lipolytic reactions are of high importance in its preservation [2]; in details, the influence of the ratio of rods to cocci is one of the subjects of much interest because of its effect on proteolytic activity [3].

In order to extend shelf life of Mozzarella cheese, different options were recently evaluated: innovative active packaging systems [4,5], addition of chitosan [1], milk bactofugation [6]. However, in spite of the research carried out, at present no valid method to efficaciously inhibit the fast microbial spoilage of Mozzarella cheese can be employed. In our opinion, utilization of antimicrobial microbes can be an innovative strategy to gain this result. Recently, a strain of *Lactobacillus* was efficaciously tested to control *Pseudomonas* growth in Cottage cheese [7]. So, our aim was to select adjunct cultures of *Lactobacillus* spp. possessing antagonistic activity against both *Pseudomonas* and *Escherichia coli*.

Through a preliminary screening in Petri plate of several lactic acid bacteria belonging to the collection of our laboratory, we identified the strain L356 of *Lactobacillus paracasei* ssp. *paracasei* exhibiting antagonistic activity against both *Escherichia coli* [8] and *Pseudomonas* spp. [data not published]. Accordingly, the strain L356 of *Lactobacillus paracasei* ssp. *paracasei* was used in co-fermentation (1:1) with a commercial strain of *Lactococcus*, specifically selected to control the fermentation of Mozzarella cheese: strain Lyobac-D, MO 097, Mofin Alce Group. The milk was inoculated with 5% of a preculture in milk of the *Lactococcus* strain and 5% of a preculture in milk of the *Lactobacillus* strain. We compared the co-fermentation performance towards the standard fermentation, obtained using only the *Lactococcus* strain. The analyses were performed after the Mozzarella cheese production (0 days) and after 7 days of storage at 5°C and at 10°C. We investigated Mozzarella cheese for the following physico-chemical and microbiological parameters: A_w; pH; % of lactic acid; % of dry matter; colour, expressed as L*, a*, and b*; total coliforms, *Pseudomonas*, coccal-shaped lactic acid bacteria, and Lactobacilli. Some of these analyses were also carried out on the governing liquid.

Our preliminary results and the relevant related literature are discussed considering, above all, the advantages for dairy industry and the consequences for starter and adjunct culture selection.

This work was supported by PRIN 2012: “Long life, high sustainability. To combine the shelf life extension due to a formulation, processing or packaging innovation, with the possible increase of global sustainability of a food product from farm to fork”.

Keywords: Mozzarella cheese; shelf life extension; antimicrobial microbes; *Pseudomonas*; *Escherichia coli*; lactic acid bacteria; *Lactobacillus*; *Lactococcus*

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On the antimicrobial potential of thermophiles: Production of an antibacterial polypeptide and a siderophore by thermophilic *Geobacillus* sp. Strain ZGt-1

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The growing problems of antibiotic resistance, as well as the continuous demand for preservative-free food within the food industry are creating a critical need for finding novel antimicrobial agents. The search for a new source of antimicrobial compounds is a challenging task. Therefore, innovative approaches are required. One of the potential sources is the microorganisms themselves; bacteria produce different antimicrobial substances that defend and maintain their communities.

Extremophilic bacteria are a special group of microorganisms, which can be screened for the production of antimicrobial agents. The unique physiological and structural characteristics of extremophilic bacteria raise the probability of isolating a novel antimicrobial compound. The capability of thermophilic bacteria, a group of extremophiles, as antimicrobial-producers has not been well investigated so far. In our study, thermophilic bacilli were isolated from 4 hot springs in Jordan with the aim of investigating their bioactive potential, mainly antibacterial compounds. Water samples were collected, inoculated onto a low-nutrient agar medium (R2A) and incubated at 60°C. The purified isolates were screened for their potential to produce antibacterial compounds. Such compounds could be applied in food industry to inhibit the growth of other thermophilic bacteria that cause food spoilage, they can be used as antibacterial additives for coatings (paints, wooden surfaces, stainless steels), or they might lead to the development of a new antibiotic within the pharmaceutical industry. *Geobacillus* sp. strain ZGt-1 exhibited antimicrobial activity against *Geobacillus stearothermophilus* which causes spoilage of canned vegetables and meat. It also forms biofilms that attach to stainless steel surfaces in dairy manufacturing plants, causing problems in the manufacture of dairy products.

The ZGt-1 strain was found to secrete an antibacterial protein as well as a siderophore, an iron-chelating compound, as two possible strategies to reduce the cell density of its competitors. In order to isolate sufficient amounts of the antimicrobial molecules, we have developed a method for increasing the cell density by their immobilization in agar gel. The antimicrobial protein has a molecular weight in the range of 15-20 KD as indicated by SDS-PAGE analysis.

Mass spectrometry and *nuclear magnetic resonance* (*NMR*) are being performed in order to identify the two compounds.

Our results indicate that thermophilic bacteria isolated from hot springs in Jordan have the potential for pharmaceutical and industrial applications.

Keywords: Extremophiles; thermophiles; *Geobacillus*; antibacterial proteins; siderophore; antibiotic resistance.

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Prelimanary screening of strains from extremely area product antimicrobial secondary metabolites

Mourad Bendahou; Omar Messaoudi

18 actinomycetes were isolated from different soil samples of sebkha of Kenadsa (Bechar-Algeria). All isolates showed antimicrobial activity against at least one test microorganism used. One isolate LAM143cG3 was selected for its broad spectrum and high antimicrobial activity.

Primary characterization of antimicrobial substance of the isolate LAM1 43cG indicates the presence of single spot (Rf= 0.73) in the solvent system (water-ethanol-ammonia), while two spots are obtained in the system (ethyl acetate-methanol). Tests chromogenic reactions suggest the presence of amines and phenols. The spectral analysis by UV-VISIBLE indicates the absence of polyene peaks, while the infrared conform the presence of amine groups.

Based on morphological and chemotaxonomic criteria, the isolate LAM143cG3 can be related to the genus *Spirillospora*. The comparison between the species of this genus, *S. rubra and S. albida*, and our isolate, indicating the existence of several differences, which leads us to suppose that this is a new member in this genre.

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Probiotic bacteria as inhibitors of quorum sensing and biofilm formation upon skin pathogens

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The elucidation of cell communication systems in bacteria has been coincident with the growing appreciation of the importance of biofilms in bacterial physiology and virulence. Most bacteria in the environment reside in biofilms, as do many of those involved in human infection. The close proximity of bacteria and limited diffusion of molecules within the biofilm matrix suggest that quorum sensing (QS) may be crucial for the development of biofilm-associated infections [1]. So, bacterial biofilm activity is regulated by QS, a system used by both Gram positive and Gram negative bateria based on the secretion and/ or detection of chemical external signaling molecules called auto-inducers [2]. QS represents a novel target for the development of agents to treat or prevent bacterial infections [1].

Probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* degrade the auto-inducers of pathogenic bacteria by secreting enzymes or auto-inducer antagoniscs which block the QS among pathogens [3]. The search for inhibition of QS signals might offer evidence on the ability of probiotics to prevent growth and proliferation of pathogenic bacteria and it is an acknowledged strategy for finding and developing new antibiotics.

The aim of this study was, therefore, to evaluate whether spent culture supernants of probiotic cultures could prevent growth and proliferation of selectd skin pathogens through inhibition of their biofilm formation or break down of an established biofilm.

To achieve the proposed objective we carried out three diferent assays: biofilm inhibition assay, pre-formed biofilm destruction assay and QS inhibition assay. The supernatans from seven probiotics (*Lactobacillus delbrueckii*, *Bifidobacterium lactis*(BB12), *Lactobacillus acidophilus*(LA-5*), Lactobacillus acidophilus*(LA-10), *Lactobacillus paracasei* (L-26), *Propioniferax innocua*, *Bifidobacterium lactis* (B-94)) were used to evaluate their capactity to inhibit the biofilm formation and/or break down pre-formed biofilm from four skin pathogens (*S. aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa* and *Propionibacterium acnes*).

Based in the obtained results from anti-biofilm assays, we can conclude that when put directly in contact probiotic bacteria inhibited or decreased the biofilm formation from the pathogens. However, we observed that the tested probiotics were unable to break down the once formed biofilm. The highest inhibition percentage of adhesion (37%) was observed using for *L. delbrueckii* in the presence of *S. aureus* (MRSA). With respect to the destruction of pre-formed biofilm, *P. innocua* presented 45% of destruction of the biofilm formed by *S. aureus* (MRSA).

To elucidate the mechanism whereby the tested probiotics inhibited the biofilm formation we evaluated if this effect was through of inhibition of QS. Here the results revealed that the probiotics with the exception of *P. innocua* and B-94, showed a reduction of the violet pigment production by *Chromabacterium violaceum*. In particular, the higher inhibition zone (2.83 mm) was observed with LA-10. These results indicate that the capacity of the probiotics to inhibit the adhesion of pathogens might be related to QS inhibition.

Keywords: quorum sensing; biofilm; skin patogens; probiotic bacteria

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Purification of bacteriocin produced by a strain of Enterococcus isolated from cheese

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Because of their potential carcinogenic and allergy action, and their high residual toxicity consumers, chemical preservatives used in foods should be reduced. In addition, heat treatment, often detrimental to the organoleptic and nutritional properties of heat-sensitive foods, does not constitute a sufficient guarantee in the fight against pathogens. Also the resistance of some microorganisms vis-à-vis antibiotics has led researchers to propose other methods of conservation and the fight against these harmful microorganisms on the hygienic and technological plans. To overcome these problems, a great interest has been shown for natural inhibitors such as antimicrobial metabolites and intensive studies of bacteriocins of lactic acid bacteria in the last two decades

The aim of my work was the screening of antimicrobial substances producing by Lactic Acid bacteria bacteriocin types . From traditional fermented products of Moroccan origin and French cheeses we have isolated seven producing isolates inhibitory substances against several target organisms and includes the famous food pathogens (Listeria monocytogenes, Bacillus cereus, Escherichia coli, Salmonella enteredis and Staphylococcus aureus) . The test for bacterial antagonism was performed by different methods. The best results are obtained by testing 'spot -on-the -lawn'.' The E16 strain revealing the best spectrum of activity was identified by biochemical and molecular methods. Only the bacteriocin produced by this strain E16 identified as belonging to Enterococcus was completely purified by flash chromatography followed by cation exchange HPLC and a reverse - phase. The pure bacteriocin has been a measure of its molecular weight by mass spectrometry ESI -TOF MS , and a detailed analysis of its amino acid sequence.

Keywords: Bacteriocins; Lactic acid bacteria; Antimicrobial substances; Bio-conservation

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Streptomyces efficiency against Ascochyta foot rot in pea (*Pisum sativum*) seedlings

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Peas are highly susceptible to preemergence damping off, caused by *Mycosphaerella pinodes* in western Algerian regions. Rhizosphere Actinomycetes which were antagonistic to the growth of this pathogen were isolated from chellif soils. An isolate of *Streptomyces* (St7c5) provided superior seed protection. Increased in both the germination and plant growth were recorded following treatment of seeds with *Streptomyces* formulated with inert or organic charge when compared to control. Application of the antagonist agent resulted in a significant reduction of *Mycosphaerella* foot rot to 5% compared to untreated seeds (30.5%).

Application of *Streptomyces* resulted in a significant reduction of blight symptoms when compared to the control or when treated with talc alone. Hence, the talc formulation of *streptomyces* agent can be recommended as one of the crop strategies for the management of foot rotting and blight caused by *Mycosphaerella pinodes*.

Keywords: Talc formulation, Streptomyces, Antagonism, Mycosphaerella pinodes, Foot rot, Ascochyta blight.

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Study of antagonism from different cellular, subcellular and molecular fractions of cultured microbes against each other

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The overuse of antibiotics has contributed to the emergence and increasing prevalence of antibiotic resistant bacteria. So other ways must be sort like Medicinal plants and the bioactive molecules other than antibiotics. By keeping in view the importance of new, unique and natural antimicrobials agents this study was designed and six microbes including *Escherichia coli, Salmonella typhi, Staphylococcus aureius, Pasteuralla multocida, Lactobacillus bulgaricus,* and *Micrococcus leutius* were tested against each other for antimicrobial activity. Each microbe was cultured in LB broth and culture was divided into three parts, the first part was subjected to centrifugation and supernatant was collected. Second and third parts were also subjected to centrifugation but supernatants were discarded and pellets were dissolved in normal saline. One of them was subjected to freeze and thaw treatment for three times and other was subjected to ultrasonication treatment, before testing for antimicrobial activity against other microbes. Disc diffusion method was used and zones of inhibition were measured. Different bacterial fractions were providing different level of antimicrobial activity against different microbes and some interesting results were obtained. For example supernatant fraction of *E. coli* was positive against *Sal*. typhi and supernatant fraction of *P. multocida* was positive against *E. coli*. Most of the microbes produced antimicrobial activity through their supernatant fractions that means antimicrobial agents were secretory in nature. Sonicated fractions were stood second in order to produce antimicrobial activity. The freeze thaw method was least effective in releasing active antimicrobial compounds from bacterial bodies. In short this study has highlighted the potential of microbes as a natural reservoir of antimicrobial agents. Some of these microbes can be called as medicinal microbes as we use the term medicinal plants in case of plant producing antimicrobial activity. Isolation of new antibiotics and other antimicrobial agents from bacteria as compared to plants is easier and cheaper way of drug production especially by the help of biotechnology.

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The in vitro effects of lactic acid bacteria screened from gastrointestinal tracts of *Lates niloticus* on *E. coli* and Salmonella spp

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Biopreservation systems in foods are of increasing interest for industry and consumers. Bacteriocinogenic lactic acid bacteria and/or their isolated bacteriocins are considered safe additives (GRAS), useful to control the frequent development of pathogens and spoiling microorganisms in foods and feed. Bacteriocins are indicated to prevent the growth of undesirable bacteria in a food-grade and more natural way, which is convenient for health and accepted by the community. This study was carried out to screen lactic acid bacteria from gastrointestinal tracts of *Lates niloticus* fish and to assess their in vitro effects on *salmonella enteritidis* and *E. coli*. Lactic acid bacteria were isolated and characterized from gastrointestinal tracts of *Lates niloticus* fish samples from Lake Victoria in Kenya. Their antimicrobial activity was tested on on salmonellae (ATCC 13076) and *E. coli* (ATCC 25922). The findings suggest a potential biopreservation effect which could be of use as an intervention technology in the hurdle concept to address food safety concerns in packed fish products.

The power of microbial volatile organic compounds

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Soil microorganisms produce a range of secondary metabolites like antibiotics, toxins, biosurfactants, siderophores. Beside these metabolites soil microorganisms are capable of emitting another class of secondary metabolites so called volatile organic compounds (VOCs). Volatiles are low molecular mass compounds (100-300 Da) with high vapor pressures, low boiling points and lipophilic character. As compare to other secondary metabolites volatiles are relatively less studied.

The major aim of our study was to obtain more insight in the role of volatiles in microbial interactions. For this we performed several experiments in glass Petri dishes plates, which were designed as such that growth of different microorganisms occurred in physically separated areas within a common atmosphere. In this way we studied the role of volatiles in fungal-bacterial and bacterial-bacterial interactions. The obtained results revealed that volatiles play important role in the interaction between soil microorganisms. Here we will report on bacterial volatiles with (1) antimicrobial activity and (2) on volatiles acting as infochemicals affecting the behavior, growth, antibiotic production and gene expression in responding bacteria. Furthermore we will report on the effect of fungal volatiles on bacteria and on the importance of interspecific interactions for the production of volatile.

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The study of antimicrobial activity of *Enteroccocus spp* against two species of *Listeria (L. innocua and L. ivanovii)*

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Enterococcus (E) is a genus of lactic acid bacteria (LAB). These bacteria form part of the commensal flora and they are widespread in milk and milk products [1]. *Enterococci* can produce bacteriocins which are active against other *Enterococci* as well as other groups of bacteria such as *Listeria spp*, which are able to induce food poisoning [2]. Research of lactic acid bacteria with antagonistic activity against *Listeria innocua* and *Listeria ivanovii* was the subject of this work.

Lactic acid bacteria were isolated from breast milk and infant feces. The strains were grown on MRS medium (Man-Rogosa-Sharpe) and incubated at 37 ° C for 48 hours. The culture of these bacteria was carried out in anaerobic conditions. The phenotypic identification of LAB was performed by VITEK ® 2 Compact.

To demonstrate the inhibitory effect of these bacteria, we studied their antimicrobial activity against two species of *Listeria (L. ivanovii and L.innocua)*. Both direct and indirect methods were used. The first one is to grow both strains in the same double layer medium [3]. The second is the well diffusion test [4].

The seven strains studied belong to the genus of *Enterococcus*. Interaction studies revealed the ability of two strains tested (*Enterococcus faecium* "C1" and *Enterococcus durans* "R") to inhibit indicator strains by direct and indirect methods. This indicates that the inhibitory agent is produced by the test strains and it is present in the supernatant. The inhibition obtained by the direct method was more important than the indirect method. The inhibition exerted against both species of *Listeria* was the same for each strain tested. This explains why inhibitory agent of each strain tested has the same effect against the two species of *Listeria.*

Keywords: *Enterococci; Listeria spp*; antibacterial activity.

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The use of *Trichoderma longibrachiatum* as a biocontrol agent of *Fusarium* wilt in cucumber plant

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Cucumber is an important vegetable in the world because of its enhanced drug detoxification effect and antioxidant activity. However, the production of cucumber is hindered by pests and diseases and the need for increasing the productivity has led to the use of inorganic pesticides. These chemicals cause environmental pollution and are detrimental to consumers' health. Therefore, this research was carried out to investigate the effect of *Trichoderma longibrachiatum* NGJ167 (Rifai) as a biocontrol agent of *Fusarium* wilt disease in two varieties of cucumber (Ashley and Marketmoor). Sterilized soils were inoculated with mycelial plugs of *T. longibrachiatum* NGJ167 before transplanting while the control soil was mock-inoculated with agar plug of Potato dextrose agar (PDA). Results revealed that the *T. longibrachiatum* NGJ167-inoculated Marketmoor had significantly higher fruit weight of 220.0 g when compared with the control which had a fruit weight of 120.6 g. Moreso, the control plants had higher incidence and severity of *F. oxysporum* than the *T. longibrachiatum*-treated plants. When the extracted DNAs from cucumber fruits were run on agarose gel using universal primers, result revealed that *T. longibrachiatum* DNAs were not present in the cucumber fruits, implying that fruits from *T. longibrachiatum*-treated plants are safe for human consumption. The ability of *T. longibrachiatum* as a biocontrol agent indicates its potentials in improving plant health in agriculture.

Keywords: biocontrol, disease incidence, polymerase chain reaction, yield.

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Yeast biodiversity in oil mill waste: characterization of antifungal activities

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The traditional olive oil mills separate the decomposed pulp produced at a first stage in a two-phase centrifuge into oil and a liquid solid mixture traditionally called *alpeorujo*. The final solid waste represents about 800 kg per ton of processed olives. This *alpeorujo* still contains 3% residual oil and about 58% water. It can have an important role as a fertilizer or a food additive, provided that it can be detoxified through bioremediation by breaking down the toxic phenolic compounds. Some previous studies have been conducted to evaluate the bacteria and yeast strains involved in this process. In this study we have selected different yeast isolates from different samples dilution, after purification by triple groove, and stored at 4°C for further use. The use of molecular techniques as RFLP analysis and DNA sequencing enabled us to group and identify yeasts isolated from *alperujo*. We have firstly described the presence of *C. norvergica* and *C. molendinolei* and *C. adriatica* (both recently described) on this substrate.

The total yeast strains isolated from the three oil mills were screened for enzymes relevant in the processing of olives, oils and other food products, regarding their potential use in manufacturing to enhance the quality of products. The characterization of some isolates producing β-glucosidase, provides us with a battery of yeasts capable of attack phenolic compounds. This can be used to reduce olive oil bitterness.

The twelve representative yeast isolates, previously selected in the enzymatic characterization were used in a nutritional competition assay. This test was proposed in order to select yeast strains able to affect the co-inoculated fungi when colonizing a common ecological niche. A different inhibitory activity against *Aspergillus flavus* and *A. parasiticus* was found among yeast isolates. *Saccharomyces paradoxus*, *C. boidinii* and *C. oleophila* showed the highest inhibition and *C. molendinolei* and *Candida* sp. isolate the lowest one. Assayed yeast inhibitory activity was affected by the concentration of fungal inoculum: the growth in the lowest fungal inoculum concentration (10^3 spores/mL) was the most inhibited by antagonistic yeasts; similar growth was observed in growth when 10^5 spores/mL were used as inoculum.

The completion of this study allowed us to have a collection of more than 200 yeasts isolated from olive oil ecosystems, characterized by phenotypic and molecular form. At least ten yeast isolates have the great industrial interest, either by their ability biochemical substrates for biodegradation of waste, or for their potential as biocontrol fungus. Finally, we proceeded to perform bioassays to evaluate the interference of yeasts isolated in the growth of various phytopathogenic filamentous fungi, determining different levels of yeast-fungus competition. We have selected ten yeasts: afterwards, we have proceeded to determine the optimal conditions for use.

Keywords yeasts; olive oil; nutritional competition

Bacteriophages

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Antibacterial target discovery: Lessons learned from bacteriophages

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Lytic bacteriophages are the natural enemies of bacteria, which mostly reply on their host for reproduction. These bacterial viruses inhibit, activate or redirect the host metabolism towards efficient phage production, using protein-proteins interactions (PPIs) between phage proteins and key host proteins, which evolved through years of co-evolution. A number of host-phage PPIs have already been studied, most of them involving interactions of the RNA polymerase of the model organism *Escherichia coli* and proteins of its phages [1]. However, as several papers suggest that the elucidation of the working mechanism of these 'antibacterial proteins' may be useful in drug discovery, the investigation of other phage-host PPIs can unveil various new insights [2].

Unfortunately, the function of 70% of the annotated phage proteins of all currently sequenced phage genomes remains unknown. Especially for small proteins produced early in infection, which are believed to be highly involved in phage-host PPIs. Therefore, we set up a screen to identify unknown phage proteins binding to several key components of the multidrug resistant pathogen *Pseudomonas aeruginosa*. A *Strep*-tag® II was genomically fused to the C-terminal side of ten bacterial target proteins involved in transcription, replication, fatty acid biosynthesis, RNA metabolism and cell division. After infection with a broad variety of lytic *P. aeruginosa* phages, 38 phages proteins were identified using affinity purification combined with mass spectrometry analyses. Eight of these proteins were found to have an inhibitory effect on the growth upon expression in the host cells, revealing a first hint towards an antibacterial function.

As an example of the output generated by this screen, I will focus on the fate of the *Pseudomonas* RNA degradosome. This is a well conserved multi-enzyme complex which is mainly involved in RNA turnover and post-transcriptional control of gene expression [4]. Although it is postulated that the hallmark myovirus T4 can somehow influence the *E. coli* degradosome, no phage protein has been found to interact this complex so far [5]. This screen was able to identify a protein of the giant phage phiKZ, which is forming a direct interaction with the RNA degradosome. This interaction was confirmed by several PPI analyses and was further specified to the scaffolding domain of the ribonuclease subunit RNase E. Together with the recently discovered crystal structure, which shows that this unique protein form dimers, more experiments are ongoing to characterize the precise binding interface with the RNaseE scaffolding domain. Moreover, first functional assays suggest that the binding of the phiKZ protein to the RNA degradosome inhibits the RNA cleaving function of this complex *in vitro*. More experiments will be done to see if an equal influence on RNA turnover is found *in vivo*.

These data illustrate that this screen can unravel the function of 'hypothical proteins' and at the same time be a powerful tool in antibacterial drug discovery.

Keywords: Bacteriophage; phage-host protein-protein interactions, RNA degradosome

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Artilysins are a novel class of enzyme-based antibacterials that quickly kill (multidrug-resistant) *Pseudomonas aeruginosa* and their persisters: from concept to application.

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Artilysins represent a novel, promising class of antibacterials, exploiting the lytic power of bacteriophage-encoded endolysins. These enzymes are produced by bacteriophages at the end of their lytic replication cycle. Once the endolysins have passed through the cytoplasmic membrane, they degrade the peptidoglycan layer rapidly, causing osmotic lysis of the infected cell and liberation of the progeny. Purified endolysins have successfully been exploited to kill Gram-positive pathogens. However, Gram-negative bacteria are not susceptible due to the presence of a protective outer membrane.

Artilysins tackle this barrier. Selected polycationic or amphipathic peptides that locally destabilize the LPS layer of the outer membrane have been covalently fused to endolysins. These peptides promote the transfer of the fused endolysin to the peptidoglycan layer through the outer membrane. Time-lapse microscopy has shown that cells are killed within seconds due to active peptidoglycan degradation and subsequent cell lysis.

LoGT-008, a first-generation Artilysin combining a polycationic nonapeptide and the PVP-SE1gp146 endolysin, kills *P. aeruginosa in vitro* with a 4 to 5 log reduction within 30 minutes and rescues infected nematodes (*Caenorhabditis elegans*) to the same extent as ciprofloxacin. Infected human keratinocytes were treated with LoGT-008, resulting in a full protection of the keratinocytes against an otherwise cytotoxic dose (10^5 CFU/ml) , associated with a corresponding reduction in bacterial load [1].

A second generation Artilysin, Art-175, comprises the 29 amino acid SMAP-29 peptide and the KZ144 endolysin, and kills *P. aeruginosa* in a rapid and highly efficient way. Art-175 is refractory to resistance development during serial passaging to subinhibitory doses, while resistance mechanisms against 21 therapeutically used antibiotics do not give cross-resistance. Art-175 has a superior killing effect against persisters (> 4 log reduction), presumably because Artilysins do not require an active bacterial metabolism to exert their bactericidal effect [2].

In summary, Artilysins have a completely novel mode-of-action, are highly bactericidal within a few minutes, irrespective the presence of drug resistance mechanisms, show a low probability of resistance development, and have unprecedented bactericidal activity against persisters.

Keywords: Artilysin; endolysin; bacteriophage; persister

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Bacteriophage endolysins to detect *Clostridium* species associated with cheese spoilage

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Butyric acid fermentation, also known as late blowing defect (LBD), is a major cause of spoilage in semi-hard and hard cheeses. It results in the appearance of texture and flavour defects that generate severe economic losses for the cheese industry. *Clostridium tyrobutyricum*, an anaerobic Gram-positive spore-forming bacterium, is considered the primary cause of LBD in cheese, but other clostridial species such as *C. sporogenes*, *C. beijerinckii* and *C. butyricum* have also been shown to contribute [1].

Bacteriophage endolysins represent potential antimicrobials via their ability to lyse Gram-positive cell walls when applied externally. Endolysins are small proteins which break down peptidoglycan and commonly consist of two domains: the N-terminal domain contains the catalytic activity of the enzyme which specifically cleaves the major bonds found in the peptidoglycan and the C-terminal domain binds to a specific substrate found in the cell wall of the host bacterium. Bacteriophages or their endolysins may provide a biological control method for eliminating undesirable organisms without affecting other microbiota. In addition, the specific binding of endolysins can act as a basis for a detection system [2].

In this study, we have investigated the use of the endolysin CTP1L [3] as a method to detect *Clostridium* spp. in cheese. The use of green fluorescent protein (GFP)-labelled proteins produced by translational fusion demonstrated that while the catalytic domain GFP-CTP1L$_{1\text{-}197}$ only showed weak binding, the whole enzyme GFP-CTP1L and the C-terminal domain, GFP-CTP1L$_{196\text{-}274}$, bound clearly and strongly to cells of *C. tyrobutyricum, C. beijerinckii, C. butyricum* and *C. sporogenes.* Thus, detection systems based on GFP-labelled endolysins could have the ability to determine whether spoilage organisms like these dairy-related *Clostridium* species are present in cheese.

Keywords: endolysin; *Clostridium*; Late Blowing Defect, GFP-labelled proteins, bacteriophage.

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Control of *Escherichia coli* O157:H7 on fresh-cut iceberg lettuce by immersing in bacteriophage mixture (STP-1, STP-2, and EP-6)

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Background *Escherichia coli* O157:H7 outbreaks associated with fresh-cut vegetables have become a major concern on public health [1]. For elimination or reduction of *E. coli* O157:H7 in foods, the bio-control method using bacteriophage was recently proposed [2, 3]. In this study, lytic effects of bacteriophages against *E. coli* O157:H7 on fresh-cut iceberg lettuce were examined by immersion method.

Materials and methods Three *E. coli* O157:H7 specific bacteriophages (STP-1, STP-2, and EP-6) (Figure 1) isolated from wastewater environment were obtained from iNtRON Biotechnology, Inc., and cocktail of these bacteriophages was used in this study. Three strains of GFP-expressing *E. coli* O157:H7 (ATCC 35150, 43889, and 43890) were individually inoculated on fresh-cut iceberg lettuce (initial concentration = 2.79 ± 0.10 log CFU/g). Samples were compeletely immersed in bacteriophage cocktail solution (MOI 10^3-10^4) or 0.85% saline with no bacteriophages, and then maintained at room temperature with 100 rpm of agitation. Survived cells in both lettuce samples immersed with and without bacteriophages were enumerated quantitatively after 30 min and 60 min treatment.

Results and discussion *E. coli* O157:H7 on all fresh-cut iceberg lettuce samples treated with bacteriophage mixture were reduced compared to the untreated sample (control). In case of 0.85% saline treated samples, populations of viable cells were slightly increased (up to 3.30 CFU/g) after 60 min. Whereas the levels of *E. coli* O157:H7 in bacteriophage treated samples were gradually decreased according to the treatment time except for *E. coli* ATCC 35150. Population of *E. coli* ATCC 43889 showed the greatest decreases after 30 and 60 min treatment compared with other two strains and reduced under detection limit (2.30 and 3.11 log reduction for 30 min and 60 min, respectively). This result might be relevant to the different susceptibility on phage mixtures among bacterial strains.

Significance and impact Results from this study highlight the potential of bacteriophage as an antimicrobial agent applicable to washing process of fresh-cut iceberg lettuce. Bacteriophage can be used in a bio-control method for *E. coli* O157:H7 on fresh-cut vegetables without quality deterioration unlike other antibacterial treatments using chemical agents.

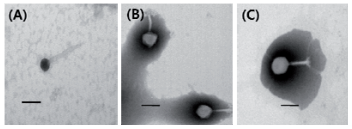

Figure 1. Transmission electron microscopy images of bacteriophages: (A) STP-1, (B) STP-2 and (C) EP-6

Keywords: *Escherichia coli* O157:H7; Bacteriophage; Fresh-cut lettuce; Post-harvest treatment

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Effectiveness of phage-based probiotic dietary supplement in the prevention of E.coli traveler's diarrhea: a small-scale study

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Introduction. More than 60% of traveler's diarrhea (TD) cases are caused by a variety of bacterial enteropathogens: diarrhea-producing E.coli accounts for the majority of infections (15-40%), although Campylobacter (3-20%), Salmonella (3-15%), Shigella (3-10%) and some other bacteria have also been isolated from travelers. It is known that probiotics may offer a safe and effective method for TD prevention. We have developed a phage-based probiotic dietary supplement for prophylaxis of TD caused by E.coli (including O157 and non-O157 STEC-strains), Shigella flexneri and sonnei or Salmonella enterica. We have evaluated its effectiveness against infection caused by the non-pathogenic Lac (-) strain of E. coli in animal and human trials.

Materials and methods. Model experiment was performed at first on white Mus musculus mice (16-18 g). Twenty animals were divided to make a study group (10 mice) and a control group (10 mice). Experimental infection in both groups was initiated by per os administration of 0.5 ml E. coli K12 C600 (5×10^7 CFU/ml) daily for three days (2nd-4th day of experiment). Mice from study group took 0.5 ml of phage-based probiotic dietary supplement daily for five days (1st-5th day of experiment) while mice from control group took 0.5 ml of PBS. In the small-scale human trial were included 10 volunteers, healthy persons 24-42 years old. They were infected by per os administration of 20 ml the same non-pathogenic Lac (-) strain of E. coli daily for three days (2nd-4th day of experiment). Five persons from study group took 50 ml of phage-based probiotic dietary supplement twice a day for five days (1st-5th day of experiment) while 5 volunteers from control group took PBS in the same dose. We evaluated E. coli K12 C600 strain in the mice and human feces one day before and one day after the experiment using both microbiological and PCR methods.

Results. We didn't find Lac (-) E. coli in the feces of any participants in both trials before the experiments using the microbiological method and we didn't observe the specific orf264 gene of E. coli K12 C600 using PCR-analysis. On the 6th day of both animal and human trials E. coli K12 C600 strain was detected in up to 3rd dilution of mice feces (10^4 CFU/g) and up to 5th dilution (10^6 CFU/g) of human feces in the control groups, while it was not detected in the samples of either of the study groups (there were no Lac (-) E. coli in microbiological test and negative PCR-analysis). At the same time in both mice and human feces of study groups two coliphage strains included in phage-based probiotic dietary supplement were detected in 100% of cases on the 6th day of the trials and in 20% of cases on the 7th day.

Conclusion. In these animals and human small-scale trials we have demonstrated the possibility to use the phage-based probiotic dietary supplement for prophylaxis of a non-pathogenic E. coli infection. These results have great significance because two original coliphages included in this cocktail have a broad host-range including O26, O55, O103, O104, O121, O125, O127, O128, O145, O146, O157 E. coli strains which cause severe cases of TD.

Keywords: traveler's diarrhea; phage-based probiotic dietary supplement; phage prophylaxis; O157 and non-O157 STEC strains

Emergence of bacteriophage-resistant *Salmonella* cells in broilers during phage therapy

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Salmonellosis is the second most common zoonosis in humans in the European Union (EU) and was responsible for 91,034 confirmed cases of human non-typhoidal salmonellosis in the EU during 2012 [1]. Poultry and their derived products are the principal sources of *Salmonella* in the food chain. Thus, the EU promotes control programs in fowl (*Gallus gallus*) populations in order to reduce the *Salmonella* prevalence in farms. In this sense, bacteriophages appear as interesting agents for biocontrol of food-borne pathogens due to their characteristics: target specificity, rapid bacterial killing and self-replication in presence of their bacterial host. Nevertheless, during bacteriophage therapy, bacterial mutants resistant to bacteriophage may be selected preventing an effective treatment [2].
In previous works, a bacteriophage cocktail composed by three lytic bacteriophages (UAB_Phi20, UAB_Phi78 and UAB_Phi87) that produced a significant decrease of *Salmonella* concentration in the intestinal tract of SPF White-Leghorn chicken and in foods was developed by our group [3,4]. Herein, the emergence of *Salmonella* cells resistant to those bacteriophages was studied in broilers during bacteriophage therapy.
Considering that the loss of the bacteriophage receptors or their mutation are the main cause of resistance to bacteriophages [5], the attachment site of the bacteriophages UAB_Phi20, UAB_Phi78 and UAB_Phi87 was identified. An analysis of the genomic sequences of these bacteriophages revealed that all of them attach to the LPS of *Salmonella* at different regions, which was confirmed by infecting *Salmonella* strains with different mutations at the LPS. Results showed that bacteriophage UAB_Phi78 attaches to a deeper region of the LPS. Therefore, it would be expected that *Salmonella* cells resistant to this bacteriophage would be also resistant to the others. In attention to this, the bacteriophage UAB_Phi78 was selected as the model for studying the emergence of resistance to bacteriophages during phage therapy in *Salmonella* infected commercial broilers. *Salmonella* was recovered by plating methods from cecum of two groups of 24 animals, one of them treated with bacteriophages and the other untreated. Afterwards, 360 colonies from plates of each group were isolated and resistance to UAB_Phi78 bacteriophage was determined. Any colony from untreated broilers was resistant to UAB_Phi78, and only the 0.3% of colonies from treated animals was resistant to this bacteriophage.
As it is known, LPS plays an important role in *Salmonella* colonization and virulence [6] and the bacteriophage-resistant mutants are probably not able to persist in the cecum of broilers because their fitness can be lower than that of cells with a wild type LPS. Therefore, the emergence of bacteriophage-resistant *Salmonella* in phage therapy is minimized.

Keywords: bacteriophage therapy; resistance; *Salmonella*; cell receptor; LPS

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Epidemiological and clinical efficacy of bacteriophages in the treatment and prevention of infectious diseases

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The bacteriophage Preparations highly effective in the prevention and treatment of acute intestinal infections and septic and enteric diseases of different localization.

Preparations on the basis of bacteriophages are the FSUE "NPO Microgen" and widely used in Russia and CIS countries since the end of 30 years. These drugs are used for prevention and treatment of acute intestinal infections, dysentery, typhoid and Salmonella, as well as for treatment of purulent - septic and enteric diseases of different localization - surgical infections, diseases of ear, throat, nose, lungs and pleura, urogenital diseases, gastroenterocolitis, dysbiosis, an infectious disease of newborns and children in the first year of life. There are 10 drugs, containing bacteriophages Shigella, Salmonella, Pseudomonas, Escherichia, Klebsiella, Proteus, Staphylococcus, Streptococcus.

Among the most universal of polyvalent drugs for indications include dysentery bacteriophage containing phages Sh.flexneri(1, 2, 3, 4, 6) and phages Sh. Sonnei, and salmonelosis bacteriophage phage groups a, B, C, D, E and bruchnotifosny bacteriophage phage S.typhi. A generic drug is eubacteria polyvalent cleared. The drug is highly active against 89,0% of strains of E.coli bacteria; 85,0% - Staphylococcus; 88,0 % - Streptococcus; 72,0% - Proteus; 81% - p aeruginosa; 89% of K. pneumoniae. Clinical effectiveness - 82,3 - 97,6% in the treatment and prevention of septic and enteric diseases of different localization. Bacteriophage Klebsiella polyvalent cleaned for the first time in the world effectively used for the treatment of chronic klebsiellezny infections, including ozeny (92%efficacy) and scleroma - (80%). Active against 88 - 93% of the bacteria Klebsiella.

Polyvalent therapeutic and preventive the bacteriophage preparations, unlike most antibiotics, broad spectrum antibacterial activity and a high degree of clinical efficacy, have an immunostimulating action, does not affect normal or bowel activity of antibiotics and probiotics, cleared bacterial metabolites and toxins, and therefore, harmless not cause toxic and allergic reactions, do not have contraindications to the use and effectively use them in pregnant and lactating women and newborns and children of early age.

Keywords - bacteria, bacteriophages, treatment and prevention of infectious diseases.

In vitro efficacy of Eliava phage preparations against clinical strains of *S. aureus*, *P.aeruginosa* and *E.coli* isolated in Austria

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For the past 70 years antibiotics have been successfully used to treat patients with various bacterial infections. At present a worldwide increase of antibiotic resistance, related to wide uncontrolled use of antibiotics in human and animal health care represents one of the major public health concerns. Bacteriophages are considered as effective alternative treatment for infections caused by antibiotic resistant bacteria. The multi-decade history of phage research and phage therapy at the Eliava Institute in Tbilisi, Georgia has proved high specificity, safety and effectiveness of phage preparations.

The aim of the presented research was to demonstrate effectiveness of Eliava phages *in vitro* on a set of clinical isolates of *S.aureus* (mainly MRSA*), E.coli, and P.aeruginosa* collected in a different geographical region-Austria, and to compare it with the effectiveness of commonly used antibiotics.

The material for investigation comprised 300 strains, including 100 isolates each of *MRSA, E.coli,* and *P.aeruginosa*, collected though processing and analysis of different clinical samples collected at the Innsbruck Medical University (IMU). Four commercial preparations – Pyophage, Intestiphage, SES and Staphylococcal phage ("Eliava Biopreparations"), as well as 12 phages from the Eliava research collection were used in the parallel screenings by two versions of the phage spot test. The antibiotic susceptibility profiles of the tested clinical isolates were studied by disc diffusion method. The work was implemented in the labs of the Division of Hygiene and Medical Microbiology at the IMU, Innsbruck, Austria.

The obtained results demonstrated high antibacterial efficacy of Eliava phages against Austrian clinical isolates. Especially high antibacterial activity (up to 100%) was registered for *S.aureus* (MRSA) strains. As to *E. coli* and *P. aeruginosa* strains, polyvalent commercial Eliava phage preparations showed efficacy of about 80% of isolates, while individual phage clones lysed more than 90% of isolates. This indicated the possibility of adjustment of lytic activity of therapeutic phage preparations for a particular set of strains through introduction of new well characterized phage clones. The comparative analysis showed that antibacterial efficacy of phage preparations was in the same range as the commonly used wide spectrum antibiotics or even higher (e.g. in case of MRSA isolates). The variability in phage susceptibility profiles can provide an option for additional, quite informative, strain subtyping.

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Membrane fusion in the final step of phage lysis

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Considering the increasing inutility and unavailability of small molecule antibiotics, the tailed dsDNA bacteriophages, or Caudovirales, are likely to be important as targetable antibacterials in the coming years. Accordingly it will be important to understand common mechanisms by which these phages accomplish successful infection cycles. For Gram-negative hosts, the final step of the infection cycle is overt lysis of the host, releasing the progeny virions to the medium. It has been long established that lysis is a programmed event independent of virion morphogenesis and requiring two phage proteins, a cytoplasmic membrane protein called the holin and a muralytic enzyme, the endolysin(*1*). At an allele-specific time, the holins suddenly aggregate to form lethal holes in a membrane-potential sensitive fashion, a process called "triggering". This allows the degradation of the peptidoglycan cell wall by endolysins, enzymes that have activity against the glycosidic, amide or peptide links of the bacterial murein (*2*). Holins are extremely diverse in terms of sequence and membrane topology and also are found in two distinct functional types, based on the size of the membrane lesion(*3*). Canonical holins form variable, micron-scale holes that allow fully folded endolysins to escape from the cytoplasm(*4*). Pinholins form small heptameric channels of ~2 nm diameter(*5*). Pinholins function with secreted muralytic enzymes called SAR endolysins that are secreted in an inactive membrane-tethered form(*6*). Pinholin triggering depolarizes the host membrane, causing release and activation of the SAR endolysins. In some phages, triggering is sensitive to environmental conditions via signal transduction through a phage-encoded antiholin, a holin-specific holin inhibitor(*7*).

Unexpectedly, we have recently found that destruction of the cytoplasmic membrane and cell wall is insufficient for host lysis in Gram-negative bacteria(*8*). Instead, a third functional class of lysis proteins, the spanins, is required for lysis, specifically to destroy the outer membrane (OM)(*9*). In the absence of spanin function, the infection cycle terminates in spherical cell forms bounded by the OM, within which the progeny virions are trapped(*8*). Most phages encode two-component spanins, with an inner membrane protein (i-spanin) and OM lipoprotein (o-spanin) that form a complex spanning the periplasm(*9*). Others encode unimolecular spanins (u-spanins) that are OM-lipoproteins at the N-terminus but have a C-terminal transmembrane domain embedded in the inner membrane. Evidence from fluorescence microscopy experiments will be presented indicating spanins function by fusing the cytoplasmic membrane and the OM, in a manner akin to SNARE-mediated fusion. Moreover, genetic and biochemical experiments providing clues to the molecular basis of the fusion events in the two different spanin systems will be described.

Keywords: lysis; fusion; conformation; lipoprotein

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Phage-based cocktail to control hospital-acquired pathogens

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In view of the multidrug-resistance, the application of lytic bacteriophages is considered as a potential approach to control of nosocomial infections.

We describe experimental composition including lytic bacteriophages against *Pseudomonas aeruginosa, Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Acinetobacter baumannii* which are the most important pathogens associated with hospital-acquired infections of intensive care and burn units. On the basis of microbiological, genomic and bioinformatic analyses several different phages against each nosocomial pathogen were chosen for inclusion in this phage cocktail.

Bacteriophages were isolated from sewage, clinical materials, and in-hospital environmental samples.

Selected phages belong to the *Myoviridae* and *Podoviridae* families.

The biological properties of the phages including phage host range, specificity, infection parameters determination, phage stability under the influence of physical and chemical factors were characterized.

Bacteriophages are lytic for 75 % of 85 *A. baumannii*, 85 % of 40 *S. aureus*, 50 % of 35 *P. aeruginosa*, and all of 35 *K. pneumoniae* genotype-varying multidrug-resistant clinical strains obtained from hospitalized patients in 2008-2014.

The phage double-strand DNA genomes were sequenced and analysed. Bioinformatic analysis of the genomes confirms that there are no genes coding toxins or known factors of virulence. Besides there are no genes responsible for lysogeny.

The phage cocktail has no toxicity for laboratory mice in a case of intraperitoneal injection and considered as a potential therapeutic preparation to control nosocomial infections.

Keywords: hospital-acquired infections; phage-based cocktail; phage therapy; multidrug-resistance*; Pseudomonas aeruginosa; Staphylococcus aureus; Klebsiella pneumoniae*; *Acinetobacter baumannii*

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The past, present and future of phage therapy: experience of the Eliava Institute

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Emergence and re- emergence of life- threatening drug- resistant bacterial infections in last two decades gave a new insight at the potential of bacteriophages as alternative to antibiotics. The vast experience accumulated at the Eliava Institute of bacteriophages is considered as one of the most valuable.

The Eliava Institute of Bacteriophages, Microbiology and Virology, established in 1923 by George Eliava, is a world renown institution in the field of phage research and phage therapy. The main activity of Eliava reseechers has been focused on isolation, characterization and selection of effective bacteriophages against bacterial pathogens of humans, animals and plants and elaboration of phage- based biocontrol technologies. Throughout its 90 years history the largest phage collection has been gathered at the institute.

The presentation will cover:

- Short history of the Eliava institute; Felix D'Herelle's idea to create World Phage Center in Tbilisi.
- Main research activities of the Eliava Institute
- Basics of phage research and phage therapy
- Phage preparations elaborated at the Eliava Institute
- Phage applications in different fields of medicine and veterinary
- Phage-based products
- Present and future of phage therapy

Biofilms

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Adhesion property of the highly adhesive bacterium *Acineotbacter* sp. Tol 5 mediated by a new trimeric autotransporter adhesin

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Acinetobacter sp. Tol 5 exhibits an autoagglutinating nature and noteworthy adhesiveness to various abiotic surfaces from hydrophobic plastics to hydrophilic glass and stainless steel [1)]. Electron-microscopy revealed that Tol 5 cells have at least three types of peritrichate bacterionanofibers [2)]. These are type 1 fimbria, Fil fimbria, and the novel trimeric autotransporter adhesin (TAA) designated AtaA (*Acinetobacter* TAA). Among them, AtaA is responsible for the adhesive nature of Tol 5 [3)].

AtaA consists of 3,630 amino acid residues, which makes it one of the largest TAAs known to date. Although AtaA follows the general N-terminus-head-stalk-membrane anchor-C-terminus organization of TAAs, an additional head domain localizes in the C-terminal region. The stalk domain of AtaA is notably longer than that of other TAAs and contains peptide repeats that are mosaically arranged. TAAs have been reported to mediate bacterial adhesion to host cells and/or extracellular matrix proteins, invasion of host cells, serum resistance, autoagglutination, and biofilm formation [4)]. However, there has been no report about such nonspecific, high adhesiveness to abiotic surfaces as AtaA mediates. This unique adhesion property can be conferred to other Gram-negative bacterial species by transformation of the host cells with *ataA* gene.

Unlike adhesion of usual bacteria forming biofilms, AtaA mediates adhesion of resting cells, independent of cell growth. Detail analyses of the adhesion process under optical microscopes suggested that autoagglutination of bacterial cells mediated by AtaA greatly contribute to cell immobilization onto material surfaces. Analyses using an atomic force microscope (AFM) revealed that adhesion forces of bacterial cells for any tested surfaces (from negatively or positively charged surfaces to hydrophobic surfaces) through AtaA are much higher (3~7 nN) than those of other biofilm-forming strains, such as *Pseudomonas aeruginosa* PAO1 (2 nN only for a hydrophobic surface). The stepwise adhesion force curves were observed for Tol 5 cell adhesion, implying detachment of the multiple AtaA molecules from the cantilever probe one by one. Adhesion force of a single molecule of AtaA to a silicon nitride probe recorded over 300 pN.

For characterization of the AtaA passenger domain (PSD), which is translocated and displayed at the cell surface through the C-terminal anchor domain, a HRV3C protease recognition site was inserted at the base of the PSD, which was cut down for separation and purification. The fibrous structure of the PSD was observed under a transmission electron micrograph (TEM). The stability profiles of the PSD against heat and pH shift were analyzed by circular dichroism (CD) spectrometer and TEM. Affinity of the AtaA PSD was also measured for various modified surfaces using a quartz crystal microbalance (QCM) apparatus.

Keywords: adhesion; autoagglutination; trimeric autotransporter adhesin; abiotic surfaces; *Acinetobacter*; biofilm

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Adsorption and biodegradation of reactive orange 16 by *Funalia trogii* 200800 in a biofilm reactor using activated carbon as a supporting medium

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Synthetic dyes were widely used in paper-printing, color photography, medicine and cosmetics etc. Approximately 90% reactive dyes flowed into receiving river from wastewater treatment plants because the reactive dyes were not easy to be degraded by an activated sludge process. Conventional methods to deal with textile wastewater mainly included physical and chemical treatment methods. Although, these methods were effective to treat textile wastewater, however, these methods have some disadvantages such as high cost, high energy requirement, and easy to produce chemical waste sludge for post-disposal. In this study, biological activated carbon was used to treat reactive orange 16 (RO 16) in a continuous-flow reactor to meet a requirement of discharge standard set by the government of Taiwan for textile wastewater effluent. A non-steady-state mathematical model system for the kinetics of adsorption and biodegradation by *Funalia trogii* (*F. trogii*) cells on activated carbon was also derived. The batch kinetic tests were conducted to determine biokinetic and adsorption parameters. The yield coefficient of *F. trogii* cells obtained from a batch kinetic test was equal to 0.204 mg cell/mg RO 16. The maximum specific growth rate (μ_m) of *F. trogii* cells was 0.63 day^{-1}. The maximum specific utilization rate (k) of RO 16 was 3.1 mg RO 16/mg cell-day. The half-saturation constant of RO 16 was 107.3 mg RO 16/L. The decay coefficient of *F. trogii* cells was 0.024 day^{-1}. Freundlich iostherm tests were conducted to evaluate the adsorption capacity of activated carbon for RO 16. The values for Freundlich isotherm coefficients K_q and n were 0.271 $(g/g)(L/mg)^{1/n}$ and 1.755, respectively. A continuous-flow fixed-biofilm reactor using activated carbon as a supporting medium was conducted to evaluate the removal efficiency of reactive orange 16. The effluent concentration of RO 16 was 0.01-0.02 mg/L while the influent concentration of RO 16 was maintained at 10 mg/L. Once the influent concentration of RO 16 increased to 50 mg/L, the removal efficiency was about 97-98%. Moreover, the removal efficiency reached at 97-98% when the influent concentration of RO 16 was maintained at 100 mg/L. The kinetic model system of biological activated carbon for adsorption and biodegradation of RO 16 can predict the experimental results well. The approaches of experiments and kinetic model presented in this study can be employed for a design of pilot-scale or full-scale biological activated carbon process for RO 16 decolorization by *F. trogii* cells in textile wastewater to meet a discharge standard.

Keywords: Reactive orange 16; *Funalia trogii* cells; biological activated carbon; fixed-biofilm; kinetic model

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Analysis of Activity of Blood Serum and IgG for the Ability to Destroy Biofilms microorganisms

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Background. Microorganisms are capable of forming a kind of community - a biofilm, which increases their resistance to the adverse effects of environment.

An extracellular matrix is the most important protective element of biofilm. Since extracellular matrix greatly influences resistance of microorganisms to protective factors of macroorganism, influence of antibiotics and antiseptics, is important to study their ability to destroy the biofilm.

Methods. Blood serum was taken from patients with different forms of surgical infection (group 1: extensive cellulitis, ulcers, large bedsores, group 2: panaritiums, boils, small abscesses) and donors.

Isolation of IgG-preparations were produced from sera by rivanol-sulfate method using affinity chromatography on protein A of Staphylococcus.

Formation biofilm conducted by incubating suspensions of *Staphylococcus aureus* strain ATCC 6538 or *Pseudomonas aeruginosa* strain ATCC 9027 on polymer membrane for 72 hours ($T=37^0C$). The received biofilms, labeled with the solution of Congo red that was used as a substrate for determining activity of blood serum. After incubation the solution was centrifuged.

Blood serum (or solution of IgG) and labeled solution of biofilms were mixed and incubated (24 hours, $T = 37^0C$). If serum destroyed biofilm Congo red goes into solution and changed its color from colorless to red with a maximum range of absorption at a wavelength of 495 nm. Undissolved particles of Elastin-Congo red were besieged by centrifugation.

Absorbance of the solution was measured in a multichannel spectrophotometer in the wavelength of 492 nm.

Results. The ability of blood serum to destroy biofilm in group 1 (252 AU; 25-75 percentiles - 204-333 AU) was lower than in group of donors (315; 298-356 AU; $p<0,05$) and in group 2 (364; 340-399 AU; $p<0,05$).

The ability of IgG to destroy biofilms in group of patients with surgical infection (6; 0-13AU) was lower than in group of donors (16; 11-20 AU; $p<0,05$)

Conclusions. The technique allows to determine activity of blood serum for ability to destroy extracellular matrix of biofilms.

It was found that the ability of blood serum, received from patients with more heavy forms of surgical infection was lower than activity of serum from patients with light forms of surgical infection and from donors. The ability of IgG to destroy biofilms in group of patients with surgical infection was lower than in group of donors.

We assume that the destruction of the biofilm matrix is an important antibacterial protection factor macroorganism.

Keywords: Biofilm, Staphylococcus aureus, Pseudomonas aeruginosa, Congo red, blood serum, IgG

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Anti-biofilm Activity of *Lactobacillus mucosae* Extracellular Extracts against *Staphylococcus aureus* from ovine mastitis

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Background. *Staphylococcus aureus (S. aureus)* is a major pathogen responsible for mastitis in dairy herds. The multidrug resistance and/or formation of *S. aureus* biofilm, considered as a one of the virulent factor, may explain why mastitis is difficult to treat and persists in herd as chronic or recurrent disease. These factors often results in the frequent failure of antibiotic therapy and economic lost in dairy industry [1]. In this context, the development of new strategies, as alternatives or complements to antibiotic therapy for the management of mastitis, is particularly appealing. One sustainable alternative to treat or prevent mastitis with avoiding intra-mammary biofilm formation is the use of lactic acid bacteria (LAB) or their extracellular metabolites as mammary probiotics.

Methods. In present *in vitro* study, we evaluated the ability of CFCSs (Cell Free Culture Supernatants) from two ovine *Lactobacillus mucosae (L. mucosae)* isolates to prevent *S. aureus* (three ovine isolates from acute mastitis) biofilm formation using by molecular approaches as Fluorescence in situ Hybridization (FISH) and Flow Cytometry method combined with 16S rRNA fluorescent labelled probes (FISH-FCM) as well as spectrophotometric crystal violet assay (SCVA).

Results. The strongest biofilm forming ability determined was shown by *S. aureus* 91 (FISH: 8,37±0,19; FISH-FCM: 8,35±0,11, both given as $\log_{10}$ of biofilm forming bacteria numbers per well ± SD (standard deviation); SCVA: 0,524±0,13, given as average values of absorbance measurement in λ=570 nm (A_{570} ± SD) in comparison with other two isolates 20 (FISH: 8,26±0,07; FISH-FCM: 8,32±0,03; SCVA: 0,381±0,03) and 203 (FISH: 8,21±0,10; FISH-FCM: 8,20±0,13; SCVA: 0,479±0,06). The CFCSs of *L. mucosae* strains affected *S. aureus* biofilm formation in a strain-dependent manner. The most significant anti-biofilm effect was caused by *L. mucosae* 14K CFCS on *S. aureus* 203 (percentages of *S. aureus* biofilm formation after treatment ranged from 1,74 to 3,76%, detected by three methods). *L. mucosae* OV6 mediated approximately 10-fold diminishing of *S. aureus* biofilm formation, with exception of *S. aureus* 203 with higher discrepancy results achieved by FISH method and *S. aureus* 20 in results obtained by SCVA. Pearson´s R correlation test showed positive correlation of used methods with correlation coefficient (r) ranging within 0,719 - 0,923 and probability p<0,0001.

Conclusions. The ovine *L. mucosae* 14K CFCS appears to be an efficient alternative to the use of commonly prescribed antibiotics for the prevention of *S.aureus* biofilm formation, which may be responsible for troubles in treatment of infection mastitis. Antimicrobial metabolites from CFCS of *L.mucosae* 14K were effective in repressing the *S. aureus* biofilm. The future of our study will be concentrated on determination of antimicrobial compounds types secreted by *L. mucosae* 14K although seeing that, the *L. mucosae* isolates CFCS inhibited *S. aureus* biofilm formation without affecting bacteria growth, thus, the way in which these extracellular products influence bacterial-surface interaction seems to be more closely related to changes in surface tension and bacterial cell-wall charge.

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Keywords: *Staphylococcus aureus*; *Lactobacillus mucosae;* biofilm formation

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Anti-biofilm peptide combinations against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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Today, we are facing a major challenge regarding the development of new strategies and the discovery of new compounds with effective antimicrobial outcomes. The emergence of resistance is a preoccupant health threat and conventional antibiotics are being rendered ineffective [1]. Specifically, biofilm related infections are becoming a serious threat, being highly related to chronic infections but also nosocomial and biomaterial related infections, and they are considered the major cause of dissemination of antibiotic resistance in the nosocomial scenario [2].

Researchers are now focusing in alternatives, such as the discovery of new antimicrobials with different modes of action, and the combination of agents potentiating their efficacy. AMPs are an example of new antimicrobials with promising applications, since they have different and sometimes unspecific mechanisms of action compared to traditional antibiotics, reducing the chance of acquired resistance, and are showing promising results in the biofilm area [3].

A growing interest has been emerging for the use of antimicrobial combinations as a strategy to increase the antimicrobial spectrum, prevent the emergence of resistance, reduce toxicity and side effects and provide synergistic activity. Because of this, in this work we analyse AMP combinations against major pathogenic bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, currently great contributors for resistance development and responsible for chronic infections, such as cystic fibrosis pneumonia.

We present a screening of combinations of the AMP antibiotic colistin with the AMPs temporin A, citropin 1.1 and tachyplesin I against these pathogens, including references and clinical isolated strains. Planktonic and biofilm mode of growth were implemented and results show that most combinations have addictive and synergetic activities, including total inhibition of biofilm formation for some of the combinations tested. This means that AMP combinations should be a viable way for the development of new antimicrobial treatments, thus reducing their toxicity and side effects, while maintaining efficacy.

Keywords: antimicrobial peptide combinations; synergism; *Pseudomonas aeruginosa*; *Staphylococcus aureus*

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Antibacterial and antibiofilm activities of cyclolipopeptides produced by a marine bacterium *Pseudoalteromonas* sp. h*Cg*-6

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Since the last century, development of multi-resistances to antibiotics by bacteria has become one of the main public health issues. Faced to this, special attention is paid to the under-explored marine biodiversity for new bioactive compounds discovery. We first created a marine bacterial collection isolated from bivalve hemolymph and screened it for the isolate ability to produce antibacterial compounds [1,2]. Experiments focused then on a strain of the *Pseudoalteromonas* genus named h*Cg*-6 (according to its origin: the hemolymph of *Crassostrea gigas* oyster) that exerts an antibacterial activity against Gram negative bacteria. *Pseudoalteromonas* sp. h*Cg*-6 produces a set of antibacterial compounds (at least 12 active compounds) with molecular weight ranging between 926 and 1004 Da. NMR and MS-MS analyses showed that those compounds were cyclolipopeptides (CLP). They share the same heptapeptidic ring linked by an amide bound to diverse fatty acid chains which vary in length, saturation and/or hydroxylation. Minimal Inhibitory Concentrations (MICs) of the four main CLPs are in the µM range. Since bacteria are more resistant to antibacterial compounds when they are forming biofilms, which constitute their preferred lifestyle, we investigated the potential antibiofilm activity of CLPs against three *Pseudomonas aeruginosa* strains (the laboratory strain PAO1 and two clinical strains, including one mucoid and one non-mucoid strain) which are all sensitive to CLP antibacterial activity, and against *Vibrio tapetis* CECT4600, which is a marine bacterium pathogenic towards the Manila clam *Ruditapes philippinarum* and is not sensitive to CLP antibacterial activity. The CLP impact on bacterial attachment onto the glass substratum and subsequent biofilm formation were investigated at sub-lethal CLP concentrations under dynamic conditions using confocal scanning laser microscopy. While CLPs had no effect on *Vibrio tapetis* CECT4600 biofilm development, they significantly reduced biofilm formation by all tested *P. aeruginosa* strains when added during the attachment step. This antibiofilm activity towards clinical strains of the human pathogen *P. aeruginosa* is of interest for potential medical applications.

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Keywords: *Pseudoalteromonas*; cyclolipopeptides; antibacterial activity; antibiofilm activity; *Pseudomonas aeruginosa*

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Antibacterial and antioxidant efficacy of chitosan edible films added with *Thymus vulgaris* and *Thymus mastichina* essential oils obtained from organic growth.

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At present there is an interest in biodegradable edible chitosan films due to the excellent biodegradability, biocompatibility, edibility and their potential applications. Furthermore, biodegradable chitosan films (CH) are excellent vehicles for incorporating a wide variety of additives such as essential oils (EOs). The purpose of incorporating antioxidant or antimicrobial compounds, such as EOs, into an edible film instead of applying them directly onto the food surface by spraying or dipping is to extend delivery of the bioactive compounds during food storage rather than delivering them in a single massive dose. The aim of this work was to evaluate chitosan edible films incorporated with the *Thymus mastichina* (TMEO) and *Thymus vulgaris* (TVEO) essential oils obtained from organic growth for (i) the inhibition growth of some pathogenic microorganism indicators or spoilage microorganism such as *Serratia marcescens*, *Listeria innocua* and *Alcaligenes faecalis* (ii) their total phenolic content (TPC), and (iii) their antioxidant activity, to define if the chitosan edible films incorporated with these EOs could be used as natural active films for food use.

The agar disc diffusion method was used to determine the antibacterial activities of chitosan edible films incorporated with TVEO (CH+TVEO) or TMEO (CH+TMEO) at different concentrations (1 and 2%). A suspension (0.1 mL of 10^6 CFU/mL) of each microorganism was spread on medium plates. CH+TVEO or CH+TMEO edible film discs, 10 mm in diameter, were aseptically obtained and placed on the inoculated plates. The antioxidant activity was determined by means of two different antioxidant tests: DPPH radical scavenging assay and Ferric reducing antioxidant power (FRAP). All tests were performed in triplicate. Inhibition zone diameters yielded by chitosan edible film disks with various concentrations (0, 1 and 2%) of *T. vulgaris* and *T. mastichina* EOs against test organisms are shown in Table 1. Films containing only chitosan (control) were not effective against any of the three tested bacteria. CH+TVEO or CH+TMEO showed an inhibitory effect, at all concentrations, on all bacteria assayed. CH+TMEO was more effective ($p<0.05$) against *S. marcescens*, *A. faecalis* and *L. innocua* than CH+TVEO at all concentrations assayed.

Table 1. Antibacterial effect of chitosan edible films incorporated with the organic *T. vulgaris* and *T. mastichina* EOs at different concentrations against several bacteria, by disc diffusion method.

Films	Diameter of inhibition zone (mm) including film (10 mm)		
	Serratia marcescens	*Listeria innocua*	*Alcaligenes faecalis*
Control	¥N.A.	N.A.	N.A.
CH+TVEO 1%	18.49 ± 0.05^{aD}	12.19 ± 0.06^{cD}	14.35 ± 0.11^{bD}
CH+TVEO 2%	26.49 ± 0.08^{aB}	16.49 ± 0.06^{cC}	21.23 ± 0.18^{bB}
CH+TMEO 1%	21.15 ± 0.04^{aC}	17.92 ± 0.08^{cB}	18.42 ± 0.12^{bC}
CH+TMEO 2%	32.36 ± 0.05^{aA}	25.51 ± 0.04^{cA}	28.29 ± 0.04^{bA}

For a same essential oil, values followed by the same lower case letter are not significantly different ($p>0.05$) according to Tukey's Multiple Range Test. For a same bacteria, values followed by the same upper case letter are not significantly different ($p>0.05$) according to Tukey's Multiple Range Test. ¥N. A.: Non active.

Chitosan films containing *T. mastichina* EO (CH+TMEO) had higher TPC ($p<0.05$), at all concentrations assayed (1 and 2%), than chitosan films containing *T. vulgaris* EO (CH+TVEO). Films containing only chitosan (control sample) showed slight values of TPC. As regards the antioxidant activity, CH+TVEO and CH+TMEO showed ($p<0.05$) antioxidant activity, at all concentrations and methods assayed. The antioxidant activity was concentration dependent. For both antioxidant methods, CH+TMEO had higher antioxidant activity than CH+TVEO.

The results obtained showed that chitosan edible films incorporated with organic *Thymus vulgaris* or *Thymus mastichina* essential oils could be used as active films due to its excellent antibacterial and antioxidant activities.

Keywords: Essential oil, organic, *Thymus*, antibacterial, chitosan films

Antibiotic resistance and biofilm formation of *Staphylococcus aureus* clinical isolates

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Staphylococcus aureus is an important pathogenic bacterium that cause nosocomial infection. The pathogen causes abscesses, furuncle, cellulitis and other infections such as bloodstream infection or bone and joint infection. Treatment of staphylococcal infections is difficult due to antibiotic resistance related to biofilm formation. In this study, antibiotic resistance pattern of 83 clinical isolates of *S. aureus* was determined by agar disc diffusion method. 64.8% of clinical isolates were demonstrated to resist to oxacillin. About 40-60% of the isolates were resistant to ciprofloxacin, clindamycin, erythromycin, gentamicin, rifampin, teicoplanin, and tetracycline. On the other hand, more than 90% of the isolates were susceptible to chloramphenicol and trimethoprim-sulfamethoxazole. To evaluate biofilm formation of 83 *S. aureus* isolates, microtiter plate method was used in this study. Interestingly, 99% of the isolates exhibited biofilm formation ability. 56.6% of tested pathogen demonstrated strong biofilm formation while 38.6% of them revealed moderate biofilm formation. Only 1.2% of the clinical isolates were non-biofilm producers. The results showed high prevalence in biofilm formation of *S. aureus*. This finding may help physicians to evaluate bacterial virulence in order to select a suitable treatment for their patients and reduce the risk of severe diseases and the rate of antibiotic resistance.

Keywords: *Staphylococcus aureus*; biofilm; antibiotic resistance

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Antimicrobial resistance of *Staphylococcus aureus*: Importance of 2D aggregates on the subsequent resistance of biofilms

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The development of implantable devices is an issue of continuous interest in medicine, since their help in the treatment of diverse diseases and also in the replacement of parts of the body. One of the most important risk associated to invasive medical devices is their predisposition to chronic infections[1,2] either by damaging or invading epithelial or mucosal barriers, as well as serving as support and reservoir for microbial growth. Among opportunistic human pathogen related to biomaterials, *Staphylococcus aureus*[3] are frequently isolated from metallic implantable devices, while *Staphylococcus epidermidis* are commonly found on polymeric implants[4]. In the pathogenesis of medical device-associated infections[5,6], the formation of biofilms is a fundamental factor, being its eradication difficult to achieve by conventional antibiotic therapy. In the analysis of the antimicrobial resistance of these biofilms, several factors, such as adhesion, high bacterial density, aggregation and induction of persisters are some of the factors that must be considered.

The aim of this work is to provide an alternative approach to the understanding of these issues by using a specially designed experimental set up that includes the use of microstructured (MS) gold surfaces (potential inhibitors of bacterial aggregation) in combination with conventional antimicrobial agents (streptomycin and levofloxacin) against *sessile S. aureus*. Smooth surfaces were also used as plain controls (PC).

S. aureus biofilms were formed on MS gold surfaces with patterned features that tune with bacterial diameter and on plain controls (PC, smooth randomly nanostructured gold) for 2 h at 37 ºC. Our results demonstrated that the formation of two dimensional aggregates were inhibited on MS, where a great amount of isolated sessile cells were found. In contrast, biofilm grown on the PC substrate formed a dense 2D ramified pattern (2D network distribution). We had also evaluated the effect of streptomycin (1 - 4 mg/L) and levofloxacin (0,25 - 1 mg/L) on the biofilms formed on these substrates Results showed that bacteria are persistintly resistant to both antibiotics applied to PC-biofilms. The antimicrobial activity of streptomycin and levofloxacin was enhanced when bacteria were attached on MS, where single cells or small aggregates were eradicated by the antibiotic treatment. Therefore, the formation of dense 2D aggregates of bacteria seem to be crucial as a previous stage in the development of the antimicrobial resistance.

Keywords: Biofilm, antibiotic, persister, surface topography, Staphylococcus, bacterial density

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Antimicrobials in salivary concentration modify oral multispecies biofilm

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Biofilm formed on different sites in the oral cavity are responsible for the majority of oral diseases. The effect of antimicrobials in concentration commonly found in saliva on the oral biofilm is poorly understood. Therefore, the aim of this study was to investigate the effect of antimicrobials in salivary concentration on microbial population and on exopolysaccharide matrix of a multispecies biofilm model. The multispecies biofilm model was composed by five bacteria (*Streptococcus oralis*, *Streptococcus mutans*, *Actinomyces naeslundii*, *Veillonella dispar* and *Fusobacterium nucleatum*) and one yeast (*Candida albicans*) [1]. Two antibiotics, azithromycin and metronidazole, and one antifungal, fluconazole, were evaluated. Mature biofilms (64.5 h development) were exposed to azithromycin, metronidazole or fluconazole at concentrations found in saliva of 2.12 μg/mL [2], 15.15 μg/mL [3] and 2.56 μg/mL [4], respectively, for 24h. After this period, the biofilm was removed by ultrasonic waves (7 watts, 30 sec), plated on selective agar media and CFU counts of each microorganism were calculated. Soluble extracellular polysaccharides (S-EPS), insoluble extracellular polysaccharides (I-EPS), and intracellular polysaccharide (IPS) were extracted [5] from the biofilm and the total carbohydrate was estimated by the phenol sulphuric method [6]. Scanning electron microscopy and confocal scanning laser microscopy were used to assess the biofilm organization and structure. Statistical analyses were performed with significance level set at 5%. The counts of each microorganism for each antimicrobial treatment were analyzed by independent-samples t test or Wilcoxon-Mann-Whitney nonparametric test. The polysaccharide analyses were analyzed by two-way ANOVA followed by Tukey's test. All antimicrobials evaluated were able to change the microbial population ($p<0.05$), however none of the antimicrobials was able to eliminate a specific microorganism from the biofilm. Azithromycin reduced *A. naeslundii* and *V. dispar* population while increased *C. albicans* ($p<0.05$). Metronidazole reduced all the microorganisms evaluated, with a great reduction for *V. dispar* and *F. nucleatum* ($p<0.001$). Fluconazole reduced *C. albicans* and *F. nucleatum* population and increased *S. oralis* and *V. dispar* counts ($p<0.05$). Only metronidazole reduced the concentration of S-EPS ($p<0.05$). No significant difference was observed on the concentrations of I-EPS and IPS for all antimicrobials evaluated. It can be concluded that the antimicrobials in salivary concentrations can change the microbial population and also modify the exopolysaccharide matrix.

Keywords: Biofilm, Hydroxyapatite, Titanium, Polymethylmetacrylate, Azithromycin, Metronidazole, Fluconazole.

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Bacterial Surface Sensing: Proteome and subsequent Virulence of bacteria depend on inorganic surface properties

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Bacterial adhesion on inorganic surface is a key issue in biotechnology and medicine because it is the first step in the development of a potential pathogenic event. New anti-biofilm and anti-bioadhesive engineered surfaces are subjected to extensive study but do not take into account any potential physiological adaptation of bacteria triggered by the contact with the surface. While it has been demonstrated that the biofilm development induces a modification of the bacterial proteome[1], the effect of surface properties on bacterial phenotype has not been investigated. In our knowledge, only one study showed that surface softness induces change in protein expression of adherent cells, prior to the biofilm formation[2]. Here we examined the impact of inorganic surfaces with similar softness but different energetic properties on the growth, virulence and proteome composition of adherent *Pseudomonas aeruginosa* PAO1, a model opportunistic pathogen.

Adhesion of *Pseudomonas aeruginosa* PAO1 in physiological water onto three materials of different wettability (polyethylene terephthalate, stainless steel and glass) were performed and followed by virulence evaluation on a vegetal model, growth rate quantification and total proteome analysis by liquid chromatography coupled with tandem mass spectrometry.

Virulence activity evaluation on a plant model (*Cichorium intybus)* showed impaired infection capacity from bacteria detached from stainless steel, compared to both planktonic and bacteria detached from other surfaces (polyethylene terephthalate or glass) while no differences were found in bacterial proliferation. Proteomic study revealed that 89 proteins were differentially produced depending on the substratum properties. These proteins are distributed in diverse cellular functions such as transcription, translation, cellular efflux, carbon metabolism, , protein routing to membranes and appendage biosynthesis. Adherent bacteria on stainless steel exhibited important loss of transmembrane proteins such as porins that could be related to modified resistance to antibiotics and could explain the reduced infection on chicory leaves.

We showed that adhesion to inorganic surfaces induced major surface-dependent physiological modifications in adherent bacteria. Bacteria are indeed capable of "sensing" the surface and mechanisms underlying such abilities should be investigated in order to identify involved surface parameters. These data suggest that sensing processes of bacteria adhering to surface should be explored in order to design efficient innovative biomaterials with anti-microbial activities.

Keywords: biofilm; bacterial attachment; physiology; surface sensing; proteomics; virulence

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Bioactive Plant Metabolites Reverses Resistant of MRSA Biofilms to Ampicillin

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The ability to form biofilm is a vital factor in device-related infections caused by MRSA as a result from reduced effectiveness of current chemotherapeutics. This initiates the development and screening for biofilm inhibitors particularly from natural products. The tropical plants *Acalypha wilkesiana* and *Duabanga grandiflora* traditionally have been used to cure ailments associated with bacterial infections subsequently suggesting presence of biofilm inhibiting metabolites in the extracts. In an early study, fractions of these plants were investigated for anti-MRSA activity and synergistic activity in combination with ampicillin. The study revealed that two fractions labeled as AW and DG (isolated from *A. wilkesiana* and *D. grandiflora* respectively) showed modest antibacterial activity against MRSA. However, synergy experiments indicated that presence of sub-minimum inhibitory concentration (MIC) AW or DG lowered MIC ampicillin to 1.56 µg/ml and 0.78 µg/ml correspondingly from initial MIC of 50 µg/ml. The enhanced antibacterial effect in combination treatment is suspected to be due to interference with MRSA resistant factors, such as biofilm formation. Hence, the present study examined effects of combination treatment on MRSA biofilm to gain mechanistic insight of the improved antibacterial effects. The appropriate concentrations for combinations of AW or DG with ampicillin were determined via checkerboard method from an earlier study. Ability of these combinations in preventing MRSA biofilm was investigated using microtiter attachment assay and inhibition of biofilm formation assay. In both assays, quantification of cell attachment and biofilms were done by measuring optical density (OD) of crystal violet staining using an enzyme immunosorbent assay reader. In microtiter attachment assays, combinations of sub-MIC AW (0.75 mg/ml) or DG (0.19 mg/ml) with sub-MICs ampicillin (1/2 to 1/64 x MIC) resulted in 60-80% reduced MRSA cell attachment to surface upon treatment. Furthermore, the combination treatment 1/64 x MIC ampicillin + 0.75 mg/ml AW and 1/64 x MIC ampicillin + 0.19 mg/ml DG significantly ($p < 0.001$) inhibited biofilm formation as high as 65.7% and 71.0% respectively. Figure 1 below shows a lower % of biofilm formation recorded in combination treatments. Results from this study evidently demonstrated that ampicillin in combination with AW or DG inhibits MRSA biofilm production. Therefore, a plausible mechanism for restoration of ampicillin's therapeutic efficacy as demonstrated by increased antibacterial effects in synergy experiment is suggested to be related to this phenomenon

Keywords: MRSA; biofilms; synergism; *A. wilkesiana*; *D. grandiflora*

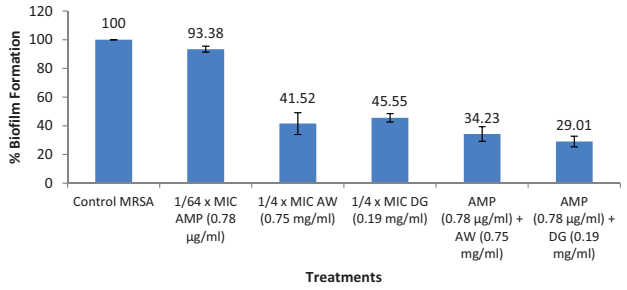

Figure 1 Biofilm formations in studied treatments. Three wells were used for each treatment. Experiment is representative of 3 independent tests, and error bars indicate the standard deviation All difference between control MRSA and treated MRSA were statistically significant ($p < 0.001$) (MIC= minimum inhibitory concentration, AMP= ampicillin).

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Biofilm formation and detection of *ica AD* gene in Staphylococcus spp isolated from urinary catheters at the University Hospital of Tlemcen, Algeria

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Staphylococci are most often associated with chronic infection of implanted medical devices [1]. Urinary tract infections can also be caused by these organisms and occur preferentially in patients carrying indwelling urinary tract catheters [2]. Urinary catheters have become the second most frequently used medical inserted into the human body. 40% of nosocomial infections involve the urinary tract, especially in catheterized patients [3]. The predominant species isolated in these infections are *Staphylococcus epidermidis* and *Staphylococcus aureus*. It was found that the major pathogenic factor is the ability to form biofilm on polymeric surfaces to which it adheres and colonizes artificial materials [4].

The aim of our study was to determine the biofilm forming capacity of microorganisms isolated from urinary tract catheterized patients and the occurrence of *icaA* and *icaD* genes in biofilm producing in a collection of staphylococcal isolates.

200 strains of staphylococci were isolated from urinary catheter at the University Hospital of Tlemcen with a predominance of *Staphylococcus epidermidis* species followed by *Staphylococcus aureus*. The studies of antibiotic resistance showed significant resistance to β lactam antibiotics. Studies of bacterial adhesion and biofilm formation revealed that the majority of strains produced a bacterial slime by RCA technique (55,5%), 49,5% of the strains formed biofilm by the TCP technical . Among the 44 strains selected for molecular study 38% present *ica A/Ica D* genes responsible for the synthesis of polysaccharide (PIA) and 29,5% were found to be positive for toxin genes which demonstrate the virulence of staphylococci responsible for catheter urinary tract infection.

Staphylococci isolated from catheter segments showed a higher extent of biofilm production, all biofilm producing staphylococci were positive for *icaA and icaD* genes, which indicates the important role of ica genes as virulence marjers in staphylococcal infections with urinary catheterization .

Keywords: Staphylococcus spp; urinary catheter,biofilm ;slime; ica operon

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Characterization of a Trimeric Autotransporter Adhesin from a highly adhesive bacterium *Acinetobacter* sp. Tol 5

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The bacterial adhesion to material surfaces or host cells is mediated by cell surface proteins called adhesins. The trimeric autotransporter adhesin (TAA) is one of the major non-fimbrial adhesin widely distributing in Gram-negative bacteria, and has been reported to mediate autoagglutination and adhesion to host cells or ECM proteins [1]. TAA forms homotrimeric fibrous structure comprised of the N-terminal passenger domain and the C-terminal membrane anchor domain which exports its own N-terminus peptide chain [1]. The passenger domain is exposed onto the cell surface and exhibits its original function.

Acinetobacter sp. Tol 5 shows autoagglutinating nature and high adhesiveness to various abiotic material surfaces from hydrophobic plastics to hydrophilic glasses and metals [2]. Our previous study showed that Tol 5 adheres rapidly through its TAA, namely AtaA, independing of biofilm formation [3]. The rapid and high non-spesific adhesion to abiotic surfaces have not been reported on other TAAs. AtaA consisting of 3630 amino acids is one of the largest TAAs known to date. The long fibrous passenger domain was predicted to have various motifs which often appear in TAAs family.

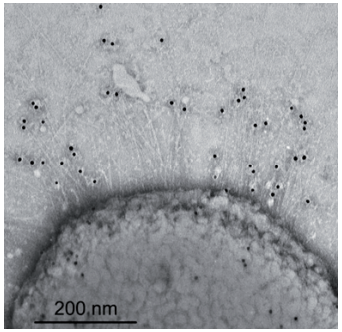

Figure 1. Immuno-electron microscopy of Tol 5 cell with anti-AtaA antibody.

In this study, we established a method for purifying the AtaA passenger domain from the cell surface. The purifyed AtaA passenger domain was subjected to biochemical and biophysical analyses. The fibrous structure of the AtaA passenger domain was obserbed by transmission electron microscopy and its adhesion property was analysed by quartz crystal microbalance (QCM). This purification method might be applied to purification of other TAAs or cell surface proteins.

Keywords: adhesion, autotransporter, adhesin, Acinetobacter,

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Chitosan effect upon biofilm formation of multiresistant *Staphylococcus aureus* strains

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Antibiotic resistance within biofilms is higher than in planktonic cells with antibiotic concentrations around 1000-fold higher than those registered for planktonic growth. This higher resistance is thought to be the underlying reason as to why treatment with antimicrobial agents fail and it is estimated that *ca* 65-80% of all infections are biofilm related. Furthermore, antibiotic development pipelines rarely test the susceptibility of recalcitrant biofilm cells or utilize animal models in which bacteria form biofilm infections.

In later years, in what was thought would be the answer to the growing antimicrobial resistance problems, new sources of antimicrobials were sought with natural compounds being the preferred answer. Among the explored compounds was chitosan, a polysaccharide with confirmed antimicrobial activity against planktonic cells, who has gained a particular interest due to its biocompatibility and wide spectrum of activity.

In order to properly assess the potential effect of chitosan upon multiresistant microorganisms, three *Staphylococcus aureus* strains – two multiresistant clinical isolates, one MRSA and one MSSA, and a control MSSA strain (ATCC25923) were used in the present study. To fully comprehend chitosan's effect upon these microorganisms a two pronged approach was undertaken: first the effect of two chitosan molecular weights (MW) (624 kDa and 107 kDa) in a planktonic setting was assessed via determination of MICs, MBCs. Having established this baseline, the effect of the same chitosans upon biofilms was assessed via determination of Minimal Biofilm Inhibition Concentrations (MBIC), inhibition at sub-MIC concentrations, of biofilm formation and mature biofilms, and through the effect upon celular metabolism via the XTT assay.

The results showed that in the planktonic phase, chitosan was active at low concentrations, however no significant differences were found between the tested strains, with both chitosans presenting an average MIC of 0.5 mg/ml. On the other hand, when analysing the results obtained for biofilms, several differences were observed. First when analysing the MBIC results it was possible to see that, contrary to most antimicrobials, chitosan was still effective at relatively low concentrations with MBICs varying between 0.6 mg/ml (MRSA) and 1 mg/ml (both MSSA strains). A similar behaviour was observed for biofilm formation and mature biofilm assays at sub-MIC concentrations, presenting inhibition percentages between 50 and 75% and higher inhibition percentages being observed for both multiresistant isolates, particularly MRSA. Lastly, the results observed in the XTT assay showed a similar trend with the multiresistant clinical isolates reflecting higher levels of cellular metabolism impairment, particularly for the 107 kDa chitosan.

In conclusion, chitosan displayed an evident strong effect against the tested *S. aureus* strains in planktonic and sessile state. While the results observed showed no differences between strains in planktonic phase the biofilm results showed that the multiresistant microorganisms were more sensitive to chitosan than the control strain. In all biofilm related assays both chitosans at sub-MIC concentrations, exhibited high inhibition percentages preventing biofilm formation, disrupting mature biofilms and cellular metabolism. Furthermore, and contrary to traditional antimicrobials, chitosan's MBIC values were not 10 to 1000 fold superior to those registered for the MICs, with only a 2 fold increase (0.5 to 1 mg/ml) being registered in the worst case scenario. Overall, these results show the potential of chitosan as a means to control the rapid growth of antibiotic resistances among microorganisms and as a possible treatment to multiresistant bacterial infections.

Keywords: chitosan; *S. aureus*; MRSA; multiresistant microorganism

Conventional antibiotics in form of nanospheres prevent biofilm formation and provide infection control

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Bacterial biofilms are formed when unicellular organisms come together to form a community that is attached to a solid surface and encased in an exopolysacharide matrix. When growing in the biofilm phenotype, bacteria are able to survive in hostile environments and acquire increased antibiotic tolerance and resistance to clearance by the host immune system. Currently there is an urgent need for new antibacterial agents with low susceptibility to resistance development. In this study, two conventional antibiotics, to which pathogenic bacteria such as Pseudomonas aeruginosa and Escherichia coli are resistant, were processed into nanospheres using a one-step, environmentally friendly sonochemical technology. The penicillin G and vancomycin nanospheres were shown to possess improved antibacterial and antibiofilm activity compared to the non-processed antibiotics. Their effect was further related to the enhanced membrane permeability of the spheres, studied by their interaction with cell membrane models - Langmuir monolayers and liposomes - allowing minimum diffusion limitations and maximum surface area per unit mass. Importantly, the studied nano-structured materials selectively killed bacteria, without imparting toxicity to human cells. It is believed that bacteria do not recognize antibiotic nanospheres as a threat, hence their efficiency is improved and a delay in the development of bacteria resistance mechanisms probable

Keywords: bacterial biofilms; sonochemistry; nanoantibiotics; model cell membranes; antimicrobial; antibiofilm

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Crowning, novel *Escherichia coli* colonizing behaviour: implications for the development of new anti-biofilms formation drugs.

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Biofilms are multicellular communities of tightly interacting microorganisms colonizing living or non-living surfaces encased in a self-produced extracellular matrix [1]. The development of drugs capable to inhibit the formation of biofilms is an important goal of antimicrobial research [2]. The biofilm extracellular matrix in *Escherichia coli* is typically made of extracellular polymeric substances (EPS) composed of flagella, adhesins, amyloid fibers (curli), and exopolysaccharides (cellulose, β-1,6-*N*-acetyl-D-glucosamine polymer-PGA-, colanic acid) forming biofilms and colonies of intricate morphology [1,3]. However, many aspects of the *E. coli* biofilm formation remains poorly understood. Thus, recently, it has been described a new colonizing behaviour in *E. coli* called "Crowning" because to the ability of *E. coli* "crowner "cells to generate a self-organized corona over plastic surface inside semisolid agar [4]. By using different *E. coli* K-12 mutant strains it was determined that the corona formation neither require of the biofilm master regulator CsgD, chemotaxis sensory system nor of the adhesiveness of the major components of *E. coli*'s EPS matrix. However, it was discovered that its generation is under glucose repression, but intriguingly this repression is not mediated by cyclic AMP (cAMP)-cAMP receptor protein (CRP) complex [4]. Hence, crowning shows unique phenotypical and genetic traits that must be considered in the search and design of novel drugs able to interfere with formation of *E. coli* biofims.

Keywords *E. coli* Biofilms; Crowning behaviour; anti-biofilm formation drugs.

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Current approaches to reduction marine biofilm formation

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Biofilm formation is the initial step of the complex marine biofouling process with own negative impact on the performance of submerged surfaces in numerous applications, such as underwater sensors, water colectors, piping, desalination systhems, vessels, etc.

No technology is known up to now capable to stop the marine biofilm development even on biocidal pains coated surfaces. Since their ban during 2008, a number of non-toxic approaches for control over marine biofilms are under investigation, based on physical, chemical or/and biological methods, most of them inspired by the nature or copping it.

Current knowlege about the biofilm composition and development, adhesive strategies of fouling microorganisms and impacting their initial adhesion surface characteristics, protein adsorption as mediator of biofilm formation and possibilities for its minimisation are presented here as a theoretical base of the current approaches to reduction marine biofilm formation.The most promissing regarding the control over multi species biofilms seems to be the combat with the initial microbial adhesion establishment by employment of combinations of diferent strategies as it is at many naturally non-fouling surfaces.

In the scope of this presentation are anti-biofilm strategies, such as surface patterning, electro-assisted approaches, surface modifications and coatings, enzyme alternatives of toxic biocides and nature derived anti-biofilm agents (natural biocides, bacteria in antifouling strategies, bio-surfactants/dispersals and quorum sensing inhibitors) any one with its own advantages and disadvantages. Future prospects for combat with multi species marine biofilms formation are outlined.

Keywords: reduction marine biofilm formatin; theoretical basis; anti-biofilm strategies; surface patterning and electro-assisted approaches, enzyme alternatives, nature derived anti-biofilm agents.

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Effect of 16S rRNA methyltransferase RmtD on biofilm formation and pyocyanin production in *Pseudomonas aeruginosa* PAO1

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Pseudomonas aeruginosa is one of the leading causes of life-threatening nosocomial infections. The problem of antibiotic resistance in *P. aeruginosa* is highlighted by the emergence of multi-drug resistant strains. One of the pathogenic potential of *P. aeruginosa* is based on formation of biofilm during chronic infections. The regulation of many virulence associated factors, such as pyocyanin production and as well biofilm formation in *P. aeruginosa*, are under the control of quorum sensing system. RmtD is a methyltransferase that confers resistance to aminoglycosides, and was identified in an *P. aeruginosa* clinical isolate. In this study, we investigated the link between 16S rRNA methyltransferase RmtD and biofilm formation and production of virulence factors in *P. aeruginosa*. Expression of RmtD in *P. aeruginosa* PAO1, demonstrated that RmtD positively affected biofilm formation in *P. aeruginosa* PAO1. Furthermore, linking its role in regulating virulence factors production such as pyocyanin, revealed that RmtD negatively affected the production of this virulent factor, regulated by quorum sensing system. Our further interest was to investigate the effect of RmtD on biofilm formation in *P. aeruginosa* mutants defective in production of RhlI/R quorum sensing system. We observed positive effect of RmtD on biofilm formation in these mutant strains. These results indicate that 16S rRNA methyltransferase RmtD exerts both positive and negative effect on quorum sensing system in *P. aeruginosa* PAO1 and further studies will focus on deciphering the link between 16S rRNA methyltransferase RmtD and quorum sensing.

Keywords: *Pseudomonas aeruginosa* PAO1; 16S rRNA methyltransferase RmtD; biofilm; pyocyanin

Effect of Chilean propolis on metabolic activity and architecture in *Streptococcus mutans* biofilm

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Streptococcus mutans (*S. mutans)* has the ability to form a bacterial biofilm on the tooth. The biological potential of propolis is related to its high content of polyphenols and can act with sinergical effect in reducing dental caries. The aim of this study was to identify and to quantify the main polyphenols present in Chilean propolis and to evaluate the antimicrobial activity, changes in the architecture (size) and metabolic activity of the biofilm when treated with an ethanolic extract of propolis (EEP), and four commercial polyphenols (Pinocembrin, Apigenin, Quercetin and Caffeic Acid Phenethyl Ester (CAPE) alone and within a mixture. Minimal bactericide concentration (MBC) and minimal inhibitory concentration (MIC) were determined by dilution in microplates following the CLSI Guidelines. The biofilm was incubated at 37 ºC for 72 hrs in a 5% CO_2 atmosphere and analyzed by confocal microscopy using Film Tracer™ calcein green biofilm stain. Metabolic activity was quantified using a stain LIVE/DEAD® BacLight Bacterial Viability Kit by flow cytometry. All results were compared with chlorhexidine 0.2% as positive control. Statistical analysis was carried out using an ANOVA test with a significance level of $p< 0.05$. The most abundant polyphenol in EEP was pinocembrin (80.5 mgL^{-1}). The MBC and MIC of EEP were 1.9 and 0.9 $\mu g\ mL^{-1}$. Using the mix including four polyphenols was obtained the highest antimicrobial activity (0.23$\mu g\ mL^{-1}$of MBC) suggesting a sinergical effect between these compounds. Moreover, significative differences were observed in the biofilm size and metabolic activity ($p<0.01$) when applied 6.25$\mu g\ mL^{-1}$of mix. With these results, we can conclude that Chilean propolis inhibits the *S. mutans* biofilm formation, which might give benefit to these natural products.

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Effectiveness of 'Ya-Sa-Marn-Phlae' on *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* biofilms

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Biofilms formed by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* have been recognized as an important factor in the pathophysiology of chronic wounds. The biofilm protects these pathogens from antibiotic therapy as well as host immune response. Therefore, identification of a possible biofilm eradicating strategy has revolutionized the research approach in medical, pharmaceutical, and biosciences. The aim of this study was to explore anti-biofilm properties of a traditional Thai herbal formulation namely, Ya-Sa-Marn-Phlae on *P. aeruginosa* and *S. epidermidis*. The component responsible for its antibacterial property, *Garcinia mangostana* (pericarp) was additionally tested. Ya-Sa-Marn-Phlae was prepared by hot oil extraction method from fresh (F-YSMP) and dried herbal components (D-YSMP). Hot oil extractions of fresh (F-GM) and dried *Garcinia mangostana* pericarp (D-GM) were also included. Biofilm development inhibition of the tested agents was evaluated by crystal violet assay and scanning electron microscopy (SEM), while thereof mature biofilm eradication effects were determined by MTT reduction assay. The results revealed that all of the tested oils (0.78-50 %v/v) could inhibit the biofilm development of *P. aeruginosa* and *S. epidermidis*. Remarkable reductions in biofilm formation of the pathogens were found after treatment with D-YSMP and D-GM (50 %v/v; $P<0.05$). Moreover, treatment with D-YSMP significantly destroyed the preformed biofilms of the pathogens. Promising anti-biofilm activity was displayed by D-YSMP suggesting further investigation in order to explore the possible utilization of the polyherbal formulation as an anti-biofilm agent, especially for wound treatment.

Keywords: anti-biofilm activity; polyherbal formulation; traditional medicine

Engineering *E.coli* to visualise antibiotic resistance in biofilms

Elli Amanatidou, James McEvoy & Ben Raymond

Biofilms, which are the preferred natural state of being for bacteria, have been implicated in recurring nosocomial infections and higher mortality rates when at the same time they have been shown to be particularly resistant to commonly used antibiotics. The mechanisms conferring this enhanced resistance are currently under investigation and one of the most popular theories amongst the scientific community is that bacteria resistant to beta lactams can confer protection to their susceptible counterparts. In order to investigate further this theory a pair of resistant and susceptible isogenic bacteria expressing different colour fluorescent proteins was created using commercially available fluorescence vectors and the Expanded Spectrum Beta Lactamases gene CTXM-14.

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Enhancing the efficiency of the methylene blue-induced lethal photosensitization of some biofilms of wound-associated bacteria using gold nanoparticle

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Due to the increased biofilm antibiotic resistance and subsequently the requirement of high doses and long-term use of antibiotics for its treatment, there is an urgent need for development of alternative therapeutic approaches.

Antimicrobial Photodynamic Therapy (aPDT) may be an effective alternative strategy to treat biofilms.

In recent years, various nanotechnology platforms were used to enhance the photodynamic therapy efficacy.

Gold nanoparticles (GNPs) with good biocompatibility have surface plasmon resonance property which can results in excitation of more photosensitizer and subsequent more generation of single oxygen.

The objective of this study was to determine whether gold nanoparticles could enhance the efficiency of antibiofilm Photodynamic Therapy against methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Acinetobacter baumanni* through electrostatic interaction with methylene blue (MB).

At first, anionically charged gold nanoparticles (4 nm in diameter) were synthesized by the citrate reduction method. Aqueous suspensions of gold nanoparticles at 45 ppm concentration were mixed with aqueous MB at (at 25, 50, 100, and 200 μM concentration) and stirred for 10 min, then ultraviolent (UV)-visible absorption spectra of MB, the gold nanoparticles, and each mixture was measured.

Biofilms were developed in a 96-well flat bottom polystyrene microtiter plate for 24 h, then were treated with the best mixture (based on the absorbance peak) for 5 min and subsequently exposed to laser light (630 nm) for 15 min. also, appropriate controls were included. Finally, the number of surviving bacteria was determined by viable counting on nutrient agar plates.

The best absorbance spectrum of the tested mixtures observed when the MB concentration was 100 μM. Gold nanoparticle was unable to enhance the methylene blue-induced lethal photosensitization of *P. aeruginosa* biofilm but a significant reduction (4 log10) in viability was observed for MRSA biofilm. Also, a 2 $\log_{10}$ reduction in viable cells was observed for A. *baumannii* biofilm.

PDT with MB alone led to approximately <1 $\log_{10}$ reduction for all of the biofilms tested.

In conclusion, gold nanoparticles increase the kinetics of oxidation processes during the methylene blue-induced lethal photosensitization of biofilm. So, the intended mixture can be a very promising therapeutic option to fight biofilm-related infections especially wound infection.

Keywords: Gold nanoparticle; Lethal photosensitization; Oxidation processes; Biofilms of wound-associated bacteria

Epidemiology of alteration types of medical implants in ICU

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The diagnosis of catheter-related candidiasis is difficult; however, the differentiation between catheter infection (or other medical implant devices) and a simple contamination is essential to establish an antifungal treatment. Though, the methodology used for this type of studies is that of Brun-Buisson (1987); however, it was examining catheters alterations caused by bacteria. So, we wanted to evaluate this method so as to the yeast cells of *Candida* spp.

To evaluate the various alterations types of catheters (contaminations, colonization and infections) and their corresponding rates, as well as the responsible yeast species, we conducted our study between February 2011 and Jun 2011 in the ICU of Sidi Bel Abbes University Hospital Center (Algeria)

We realized this study in the ICU between February 2011 and Jun 2011. The sampling was performed from implanted catheters and intubations for 48 hours and more. Candidemia was considered nosocomial when it first occurred more than 48 hours after hospital admission. Other samples were taken by swabbing patient's tongue. For the samples transport to the laboratory, a refrigerant bag was used.

A total of 63 samples were taken from ICU during the study period, 12 strains (19,04%) of *Candida* spp. were isolated (three *C. albicans* and nine *C. glabrata*). We reveal that the proportion of non-albicans *Candida* species, especially *C. glabrata* was increased in nosocomial infections.

On the other hand, three different types of alteration of Implanted Medical Devices were observed; likewise in our study, six (9.52%) contaminations, two (3.17%) colonizations and three (4.76%) infections of Implanted Medical Devices.

To conclude, the present study is the first survey in an ICU population in Algeria; it demonstrated that Brun-Buisson's method seems to be appropriate for these studies.

Keywords: *Candida* spp, ICU, medical implants, alteration types.

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Evaluation of biofilm formation of *Klebsiella pneumoniae* isolated from medical devices at the University Hospital of Tlemcen, Algeria

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Klebsiella pneumoniae is an opportunistic pathogen that infects immunocompromised patients who are hospitalized or suffering from severe underlying diseases, such as chronic pulmonary or diabetes mellitus) [1]. *Klebsiella pneumoniae* is a major cause of community-acquired and nosocomial infections, infections due to *K .pneumoniae* are particularly devastating with a mortality rate between 25 and 60% [2]. This germ is responsible for acute and chronic infections, most of which are due to is ability to adhere to medical implants and from a biofilm. Biofilm development is a dynamic multi-step process, from the initial adhesion of bacteria to support the maturation of aggregates.

The objective of this work is to study the interaction between clinical isolates *of Klebsiella pneumonaie* and antibiotic surfaces (medical devices) and some factors influencing biofilm formation. Over a period of 2 years, 115 strains of *K. pneumoniae* were isolated from medical devices CHU Tlemcen, most of which had a high level of resistance to cephalosporins 1, 2 and3 generation . According to 3 techniques studies (TCP, TP, RCA) strains of *K.pneumoniae* isolated from urinary catheters have proved very good forming the biofilm to those isolated from medical devices. 24 of 115 isolated strains showed a clear difference in antibiotic susceptibility between planktonic populations and biofilm populations they were 10-2O times higher. All strains presented a highly hydrophilic character and adhesion 2-10 times greater in PVC with respect a glass support. The MrkD gene (detected by PCR) responsible for biofilm formation was found in 22 strains *of K. pneumoniae* which may explain their adhesion and therefore their pathogenicity.

Keywords: *Klebsiella pneumoniae*; biofilm; medical devices

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Have motility behavior and biofilm formation a specific link with antibiotics resistance in *P. aeruginosa* and *P.fluorescens*?

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Knowledge about the biofilm formation and antibiotic resistance are resulting in identification of new targets for therapeutics against *Pseudomonas* infection. These one generally persist despite the use of long term antibiotic therapy. The ability of growing within a biofilm enhances their chances to protect themselves from host defences, antibiotic therapies, and biocides. To our knowledge, few studies had been undertaken to compare the implication of swimming-swarming and biofilm in antibiotics resistance with *P.fluorescens*. The relationship between this formations is debated in some Pseudomonas species literature. *P. aeruginosa* has been well-studied as a model organism for the study these interaction. However, with *P.fluorescens* biofilms formation and motility behaviour has not been extensively analysed. Our data demonstrate that our isolates exhibited an important biofilm mass and were categorized as slime-producers. Phenotypically, *P. aeruginosa* (S8) seems to be most adherent with a smooth, mucoid appearance, which is attributed to the production of alginate slime. The morphological and microscopic analysis of biofilm formation in these isolates revealed a very complex, dynamic, and biologically exciting view about the architecture, and function of the EPS matrix. Till date, the biofilm life cycle is summarized in three steps: initial attachment events, the growth of complex biofilms, and detachment events. Thus it is unlikely that our data are in discordance with previous observations. The results indicate that biofilm formation and swarming and swimming motility exhibited a significant effect of resistance toward The AMC, AMP and ATM antibiotics.

Keys words: biofilm, *Pseudomonas* infection, antibiotics resistance, swarming, swimming

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Identification and Characterization of a Putative Mega Polysaccharide Gene Cluster in *Enterococcus faecium*

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Enterococcus faecium is an emerging multi-resistant nosocomial pathogen increased dramatically worldwide and causing bacteremia, endocarditis, urinary tract and surgical site infections in immunocomprised patients. Due to the complex structure of cell wall, these microbes interact with their environment and providing the factors required for progression in their specific ecological niche. The *enterococcal* cell wall contains in addition to the peptidoglycan surface proteins and lipids also a capsular polysaccharides, a crucial contributors to the virulence which also allow these microbes to escape detection and clearance by the host immune system. Recently, we found a large locus in *E. faecium* which contains 32 genes. This was confirmed by using genome comparison of already sequenced strains that has no homology to known capsule genes and the epa-locus (*Enterococcal* Polysaccharide Antigen Gene) but except some genes coding for glycosyl transporters. Also, we create one novel knock out mutant from that locus and characterized it by *in-vitro in* cell lines and *in-vivo in* animal model. The aim of the study is to identifying and examined putative mega capsule polysaccharide locus of *E. faecium* and its functions by creating knock-out mutants using homologue recombination method. This study will prime a way for the development of novel vaccine for treatment of enterococcal infections in near future.

Identification of compounds that inhibits bacterial diguanylate cyclases involved in biofilm formation from theraputics drugs

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Cyclic dimeric guanosine monophosphate (c-di-GMP) is a common, bacterial second messenger that regulates cellular processes in bacteria. High concentrations of c-di-GMP usually implies in biofilms formation, which are highly resistant to treatment with antibiotics and represent the predominant phenotype in most chronic infections. The c-di-GMP is synthesized from two GTP molecules by enzymes diguanylate cyclase (DGC) belonging to GGDEF family, these enzymes are attractive anti-biofilm targets for drug design. A drug repositioning strategy was applied in order to select potential diguanylate cyclase inhibitors from FDA-approved drugs. Using consensus scoring of docking, shape and electrostatic similarities ten compounds were selected for biochemical assay resulting in the discovery of antinflamatory and antihypertensive drugs as DGC inhibitors at micromolar range. Mass spectrometry was used to confirm the compounds binding to DGC and probe the GTP site. The approved drugs identified as DGC inhibitors showed anti-biofilm activity and are excellent starting compound for DGC potency optimization.

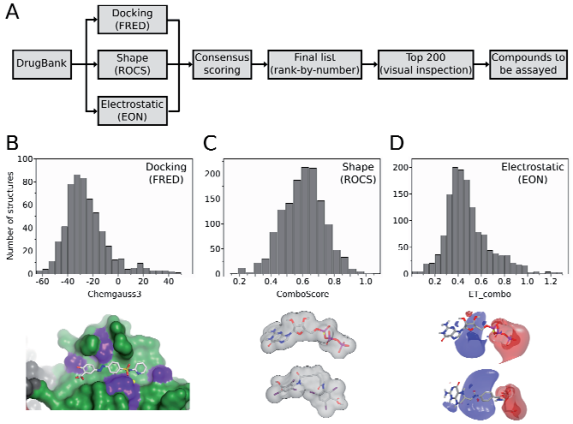

Figure 1. (A) The flowchart of virtual screening strategy employed for selecting the compounds for biochemical assay and histograms of (B) calculated binding energy by FRED program and Docking pose of sulfasalazine in the DGC PleD structure, (C) shape similarity calculated by ROCS program and shape comparison between the query GTP-α-S and iodipamide, (D) electrostatic similarity calculated by the EON program and electrostatic comparison of the query GTP- α-S and folic acid.

Keywords: drug design; anti-biofilm, drug repositioning

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In vitro activities of new cationic steroid antibiotics against *Legionella pneumophila*

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Legionella pneumophila is a bacteria that settles on the plumbing systems of the large buildings such as school, hospital, hotel and spreads to humans by the respiration of the contamined water drops that go out from the outsources of the plumbing system such as taps and shower heads. They cause legionnaires' disease which is an important lung infection. Effective treatment must be started as soon as possible to be succesful in the cure, because of the delays in the diagnosis of the disease. Cationic steroid antibiotics have a wide effect spectrum like Gram-positive and Gram-negative bacteria, fungi, enveloped viruses and including some parasites. The aim of our study is to investigate in vitro activities of cationic steroid antibiotics that effective on gram negative bacteria against *L. pneumophila* strains isolated from water systems. Also, *L. pneumophila* is a strong biofilm forming bacteria We investigated the in-vitro activities of antibiotics (erythromycin and doxycylin) and new cationic steroid antibiotics (CSA-8, CSA-13, CSA-44, CSA-131 and CSA-138) against *L. pneumophila* biofilm. The minimum inhibitory concentrations (MIC) and minimum biofilm eradication concentrations (MBEC) were determined by microbroth dilution technique.

The MIC values of CSAs were within the 0.25 - 8 mg/L range, (CSA-8=13<131=138<44) were active against standard and environmental *L. pneumophila* strains. When we considered the anti-biofilm activities of these antibiotics and CSAs, MBEC values ranged between 80 - >2560 mg/L. According to these results, antibiotics and CSAs were active against *L. pneumophila* biofilms.

In vitro biofilm formation by uropathogenic *Escherichia coli* and their antimicrobial susceptibility pattern in various hospitals of Tehran, Iran

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Background:
Bacterial biofilms play an important role in urinary tract infections (UTIs), being responsible for persistence infections causing relapses and acute UTI and allow UPEC strains to endure killing by antibiotics and immune responses. The main aim of this study was to survey of relationship between biofilm formation and antimicrobial susceptibility pattern in uropathogenic *Escherichia coli* strains in Tehran, Iran.

Materials and Methods:
A total of 103 clinical strains of uropathogenic *Escherichia coli* collected from various hospitals of Tehran between March 2013 and February 2014. We used Microtiter plate method to study biofilm formation and antimicrobial susceptibility test was performed by Kirby Bauer-disk diffusion method.

Results:
Of the 103 UPEC strains, 74 (71.8%) strains displayed a biofilm positive phenotype under the optimized conditions in the Microtiter plate and 24 (32.4%) of the strains were classified as highly positive, 14 (19%) as moderate positive and 36 (48.6%) as weakly positive strains. The rates of antibiotic resistance of biofilm producing *E. coli* were found to be 100% for cotrimoxazole, ampicillin, amoxicillin and ceftriaxone, 81% for ceftazidime and ciprofloxacin, 74% for nalidixic acid, 68% for gentamicin, cefotaxime and cefepime, 67% for tetracycline and cefixime, and 28% for imipeneme, ofloxacin and nafcillin.

Conclusions:
Our results indicated that biofilm formation was common phenomena among UPEC strains. Also there is a relationship between biofilm formation and antimicrobial susceptibility pattern. More over multiple drug resistance is dominant character among the strains. On the basis of this results we recommend combination therapy for the treatment of biofilm associated infections.

Keywords: Biofilm formation, uropathogenic *Escherichia coli*, Antimicrobial susceptibility pattern

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Inhibition of pre-formed or formed *Pseudomonas aeruginosa* biofilms by antibiotics and antimicrobial cationic peptides

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Pseudomonas aeruginosa is one of the major nosocomial pathogen that can causes a wide variety of acute and chronic infections *P. aeruginosa* is a dreaded bacteria not just because of the high intrinsic and acquired antibiotic resistance rates but also the biofilm formation and production of multiple virulence factors. We investigated the in-vitro activities of antibiotics (ceftazidime, tobramycin, ciprofloxacin, doripenem, piperacillin and colistin) and antimicrobial cationic peptides (AMPs; LL-37, CAMA: cecropin(1-7)-melittin A(2-9) amide, melittin, defensin and magainin II) alone or in combination against biofilms of standard and 4 clinical strains of *P. aeruginosa*. The minimum inhibitory concentrations (MIC), minimum bactericidal concentration (MBC) and minimum biofilm eradication concentrations (MBEC) were determined by microbroth dilution technique. The MBEC values of antibiotics and AMPs were 80 - >5120 and 640 - >640 mg/L, respectively. When combined with the LL-37 or CAMA at 1/10 x MBEC, the MBEC values of antibiotics that active against biofilms, were decreased up to 8-fold. All of the antibiotics, and AMPs were able to inhibit the attachment of bacteria at the 1/10 x MIC and biofilm formation at 1x or 1/10xMIC concentrations. TKC studies showed 3-$\log_{10}$ killing against biofilms in 24 h with almost all studied antibiotics and AMPs. Synergism were seen in most of the studied combinations especially CAMA/ LL-37 + ciprofloxacin against at least one or two strains' biofilms. Since biofilms are not affected the antibiotics at therapeutic concentrations, using a combination of antimicrobial agents including AMPs, or inhibition of biofilm formation by blocking the attachment of bacteria to surfaces might be alternative methods to fight with biofilm associated infections.

Keywords: Biofilm; Antimicrobial cationic peptides; *P. aeruginosa.*

Interactions between bacteria of a marine benthic biofilm: antibiofilm activity of a *Pseudomonas* bacterium against a *Flavobacterium* strain

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In the marine environment, most solid surfaces are covered with microbial biofilms. Biofilms are multi-cellular systems in which many complex interactions, competitions and cooperation occur, which are poorly understood and that we wish to explore. The biofilm model we are interested in is particularly original: it is a benthic biofilm, taken from the intertidal mudflat of the French Atlantic coast, mainly composed of microalgae (diatoms), extracellular polymeric substances (EPS) and bacteria. The peculiarity of this type of biofilm is that microalgal cells migrate to the surface of the sediment at each diurnal emersion where they constitute an almost continuous temporary biofilm. Previous researches have shown that, in intertidal benthic ecosystems, diatoms can stimulate or inhibit the growth of bacteria by secreting EPS [1, 2]. However, no study has been conducted on bacterial interactions in this kind of biofilms, prompting us to focus our work on these interactions. One of the bacteria that we isolated from the mudflat biofilm (II2003), belonging to the *Flavobacterium* genus, was able to form a stable biofilm in vitro both in microtiter plates (PVC) and in a flow cell system on a glass surface. We used this strain as a model, and we tested the effect of the culture supernatants of the other isolated mudflat strains on the biofilm formation and the growth of the *Flavobacterium* strain II2003. Several supernatants were shown to display activities against this strain. In particular, one supernatant, produced by a bacterium of the *Pseudomonas* genus (strain IV2006a), was shown to contain a proteinaceous molecule that inhibits biofilm development by the *Flavobacterium* strain II2003. The effects of the *Pseudomonas* IV2006a culture supernatant on the bacterial attachment and the subsequent biofilm formation were characterized under dynamic conditions in flow-cell chambers.

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Keywords: Marine biofilm; bacterial interactions; antibiofilm activity; *Pseudomonas*; *Flavobacterium*

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Linalool: a natural strategy to control biofilms of *Acinetobacter baumannii*

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Acinetobacter baumannii is an opportunist microorganism capable of causing nosocomial infections and responsible for high mortality and morbidity rates, mainly in intensive care units. One of the *factors leading to the* virulence and pathogenicity of *A. baumannii* is the ability to adhere to biotic and abiotic surfaces, and to form biofilms. Biofilm increases the ability of bacteria to resist antimicrobial treatments, disinfectants and other environmental stresses. In sum, the increasing incidence of hospital-acquired infections caused by *Acinetobacter baumannii*, coupled with the low efficacy of available drugs and disinfectants has increased the interest in the potential antimicrobial properties of natural products, such as linalool.

The main objective of this work was to determine the potential of linalool to inhibit biofilm formation by *Acinetobacter baumannii* strains and its effect on the ability of *A. baumannii* to adhere to different surfaces.

The minimum inhibitory concentration (MIC) of linalool against two reference strains and three clinical isolates of *A. baumannii* was determined using a microdilution broth susceptibility assay. The ability of *A. baumannii* to form biofilms, as well as, the effect of linalool on biofilm formation on different surfaces (stainless steel, polystyrene , polyvinyl chloride, latex and anodized aluminum) was evaluated by the sessile drop contact angle measurements and by the quantification of biofilm biomass using the crystal violet assay (CV). The contact angle measurements were used to calculate the free energy of adhesion between the *A. baumannii* strains with and without linalool treatment and the different surfaces. Furthermore, for a quantitative analysis, we used the CV assay to evaluate the biofilm biomass of *A. baumannii* biofilms after 24h of treatment with linalool.

The antibacterial activity of linalool against the five tested strains was determined and the minimum inhibitory concentration values ranged from 2 to 8 µL/mL. With the contact angle measurements, it was found that after linalool treatment, the adhesion of two strains (LMG 1025 and AcB24/10) to the surfaces was less thermodynamically favourable (lower ΔG) when compared to cells without treatment. However, for the other strains (LMG 1041, AcB10/10 and AcB23/10) it was found that the adhesion was thermodynamically most favored after treatment with linalool. Since the reference strains (LMG 1025 and LMG 1041) showed a distinct behavior in the contact angle measurements, these two strains were selected for further assessing not only their ability to form biofilms, but also test the effect of linalool on their ability to form biofilms in the abiotic surfaces. It was observed that the two tested strains showed similar ability to form biofilm on the various tested surfaces and, in general, after the treatment with linalool, the biofilm biomass decreased, meaning that biofilm formation was inhibited.

In conclusion, linalool is a promising antimicrobial agent for controlling the formation of biofilms of *A. baumannii* on abiotic surfaces, particularly those used in this study, which are common surfaces in equipments in hospitals and are susceptible to colonization by *A. baumannii*.

Keywords: *Acinetobacter baumannii* ; linalool ; biofilm inhibition ; abiotic surfaces

Low adhesive surfaces significantly reducing biofilm formation

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Biofilm formation is the initial step of the complex marine biofouling process. No technology is known that is capable to stop its development. Biofilm forms even on biocide paint coated surfaces subjected on high underwater share stress. Several non-toxic approaches to the marine biofilm control are currently under search based on physical, chemical and/or biological methods, most of them inspired by the nature. Unfortunately any one combines its advantages with some disadvantages. The control over marine biofilm formation remains still a challenge.

On a point of view of the praktical application, the most promissing aproaches to reduction multi species biofilm formation are focussed on the initial attachemnt of the microfoulers, based on similar for all of them mechanisms, namely secretion of adhesive proteins and polysaccarides at the first contact with the submerged surface followed by cross-lincking ensuaring durable irreversible adhesion. The creation of low energy, low adhesive antifouling surfaces atrack lately increasing interest as an alternative of the toxic, biocids based, antifouling paints [1,2].

We are trying to improve the performance of developed by us low adhesiv, biocides-free siloxane antifouling coatings [3,4] by reducing the biofilm formation on their surface employing some low toxic additives such as food antioxidants, surfactants, oils and their combinations. Superior biofilm reduction, 3-10–fold, depending on the evaluated parameter, additive type and its amount was demonstrated by some combinations of additives. Very scare, easily detaching biofilm developes on the coated surfaces in their presence. Presumable explanation of this effect is presented.

Keywords: marine biofilm reduction; low adhesive surfaces; low toxic anti-biofilm additives

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Medical and epidemiological impact of candidal biofilms. Tridimentional architecture and resistance

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The hospital can be considered as an ecosystem where the patient is found in contact with the microbial world and faces the risk of contracting an infection that is termed the nosocomial. Some opportunist pathogens yeasts parts of this universe, *Candida* sp. is responsible for more than 75% of systemic fungal infections. These infections are primarily related to medical devices such as catheters that promote the formation of biofilms. That structures set up a nidus for disease because is not easily amenable to conventional antifungal therapy. The diagnosis of catheter-related candidiasis is difficult; however, the differentiation between catheter infection and a simple contamination is essential to establish an antifungal treatment.

In this context we conducted our study between February 2011 and January 2012 at the Hospital University Center of Sidi Bel Abbès (Algeria), which is to assess the responsible yeast species, then, to check their power to form biofilms and to test their resistance against amphotericin B and fluconazol. From 457 samples, 37 strains of *Candida* sp. were isolated, along with the dominance of *C. glabrata*. Nevertheless, 31 strains were able to form biofilms, in addition, it appears from this study that the antifungal tests show clearly that sessile cells of *Candida* sp. were much more resistant than their planktonic counterparts (thirty two (32) times higher towards AmB and one hundred twenty eight (128) times to fluconazol. Moreover, images of electron microscopy show the formation of biofilms on the internal surfaces of catheters.

Keywords: *Candida* sp, biofilms, resistance, SEM

Medical biofilms easily simulated in 96-well microtiter plates

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Biofilms are a serious problem for public health because of the increased resistance of biofilm-associated microorganisms to antimicrobial agents and their potential to cause infections in patients with indwelling medical devices[1]. In order to develop strategies to prevent or eliminate these biofilms, there is a need to reproduce them *in vitro*. It is crucial that these *in vitro* biofilms resemble the "real" biofilms and this can only be achieved if they are formed in the same hydrodynamic conditions that are found inside the human body.

Microtiter plates (MTPs) with 96 wells have been intensively used for biofilm studies due to their high throughput, low cost, easy handling and easy application of analytical methods to evaluate different biofilm parameters. The main aim of this work is to evaluate if shaking 96-well MTPs are adequate to reproduce medical biofilms and define the operational conditions to be used in the simulation of different biomedical scenarios.

Computational fluid dynamics (CFD) was used to determine the shear strain rate distribution inside a 96-well MTP for different shaking frequencies (50-200 rpm) and orbital diameters (25-100 mm). A differential crystal violet (CV) staining method and scanning electron microscopy (SEM) were used to assess the biofilm distribution in the vertical wall of 96-well MTPs.

The CFD results indicate that the shear strain rates under which the biofilms develop change drastically along the vertical wall. This result was corroborated by the differential CV staining method and SEM, both showing that higher cell adhesion occurred closer to the air-liquid interface. The higher shear strain rates closer to the air-liquid interface are associated with the formation of dispersed cell aggregates, while lower shear strain rates near the bottom result in a homogeneous distribution of single cells on the wall[2,3].

The 96-well MTP is ideally suited to mimic the shear strain rates that are found in several biomedical systems as long as the operating conditions (shaking frequency and orbital diameter) are carefully set[2]. Moreover, the uneven distribution of the shear rates in 96-well plates captures the natural strain rate variation that occurs in different parts of the human body unlike other bioreactors where the hydrodynamics is constant.

Keywords: biofilm; microtiter plate; computational fluid dynamics; shear strain rate; crystal violet; scanning electron microscopy

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Novel enzymatic antimicrobial and anti – biofilm system

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Microbial biofilms are a major source of infections accounting for >80% of nosocomial infections in hospitals and the cause of many indwelling medical device associated infections e.g. in the urinary tract (1). Catheter associated urinary tract infections (CAUTI) are frequently occurring healthcare associated infections which are caused by biofilm forming pathogens colonizing the catheter surface. One of the main reasons for the resistance of microorganisms living in biofilm communities is a protective network of exopolysaccharides, contributing to its stability and protecting it from harmful effects (2).

In an effort to overcome some of these challenges, a novel enzyme based antimicrobial and anti-biofilm system is presented here. The oxidoreductase Cellobiose Dehydrogenase (CDH) has the ability to reduce molecular oxygen to the effective antimicrobial agent hydrogen peroxide (H_2O_2) using a broad range of di-, oligo- and polysaccharides as electron donors. *In vitro* liquid studies showed that 0, 3 U of CDH in combination with 1 mM of cellobiose completely inhibit biofilm formation of *E. coli* and *S. aureus*. These findings led to the testing of the CDH/ cellobiose system against 9 clinical isolates commonly found in catheter biofilms. *Acinetobacter baumanii* and MRSA were amongst those most affected by the treatment with 10 mM of cellobiose completely inhibiting growth of both strains.

Another interesting finding of this study was the ability of CDH to use *E. coli* and *S. aureus* exopolysaccharides as substrates for the production of H_2O_2 in the additional presence of amylase. This effectively means that during biofilm formation with concomitant production of bacterial EPS CDH is "turned on" leading to the release of anti-microbial H_2O_2. As shown by LIVE/DEAD staining images incorporation of both CDH and Amylase into catheter lubricant is an effective and easy strategy in order to apply the system presented here as an antimicrobial and anti-biofilm agent for the prevention microbial colonization of biomedical materials.

Keywords: urinary catheters, extracellular polysaccharides

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Photodynamic Antimicrobial Chemotherapy (PACT) decreases the viability of biofilm produced by *Candida albicans*

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Candida albicans is an opportunistic fungal able to produces both superficial and systemic infections in immunocompromised patients. It has been demonstrated that biofilms produced by *C. albicans* are resistant to different antifungal drugs and, that, the infections related to biofilm formation are, frequently, refractory to the conventional treatments. Photodynamic antimicrobial chemotherapy (PACT) is a potential antimicrobial therapy, which combines visible light and a nontoxic dye, known as a photosensitizer, producing ROS, which can kill the treated cells. In this work, we investigate the effects of PACT, using Toluidine blue (TB), as a photosensitizer on 24 hour old biofilms produced by *C. albicans*. It was observed that PACT, using TB was able to decrease the viability of the 24 hour old biofilms produced, in a TB concentration dependent manner. The inhibition promoted by PACT (0.1 mg/ml TB) was in the order of 30%, 40% and 50% in 24 hour old biofilms submitted to incubation times of 1, 2 and 3 hours, after PACT, respectively. At the same time, the increase in the ROS production was observed after PACT. Our results suggest that the inhibition observed in the viability of the 24 hour old biofilms, by PACT, can be related to the increase in ROS production, increasing the cell permeability and leading to the damage in 24 hour old biofilms produced by *C. albicans*.

Keywords: *Candida albicans*; Photodynamic antimicrobial chemotherapy; PACT; biofilm

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Polymicrobial biofilms in cystic fibrosis – the role of atypical bacteria in the consortia and impact in antibiotic treatment

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Cystic Fibrosis (CF) is characterized by high rates of morbidity and mortality caused by pulmonary microbial infections. *Pseudomonas aeruginosa* is typically the prevailing pathogen in the airways of CF patients. However, an emergent and diverse microbial community inhabiting CF lungs has been disclosed, but how it interacts and contributes to the polymicrobial consortia with CF-common pathogens is still to be revealed.

The mail goal of this study was to address the behavior of two CF-atypical bacteria, *Inquilinus limosus* (IL) and *Dolosigranulum pigrum* (DP), when associated to *P. aeruginosa* (PA) under oxygen-atmospheres resembling CF airways. For this, those bacteria were grown in dual- and three-species populations with *P. aeruginosa* in variable oxygen conditions and biofilms were thoroughly characterized for biomass, activity, CFU numbers, antibiotic resistance profiles and relative distributions of bacterial populations.

Dual-species consortia were of difficult eradication, with most antibiotics being ineffective in reducing biofilm-bacteria, particularly under low-oxygen atmospheres. Regarding microbial composition, these biofilms presented similar bacterial proportions, whereas *P. aeruginosa* and *D. pigrum* dominated the three-species consortia, with *I. limosus* being the smallest representative population. In general, biofilm compositions changed as a result of antibiotic treatment, with alterations being dependent on the antibiotic, concentration and oxygen condition implemented. *P. aeruginosa* and *I. limosus* dual-biofilms exhibited higher antibiotic resistance, with *I. limosus* persisting and occupying a significant portion together with *P. aeruginosa* in the overall biofilm after antibiotic treatment. Interestingly, the three-species biofilms displayed higher sensitivity, with *D. pigrum* and/or *P. aeruginosa* dominating and *I. limosus* populations declining in most cases. This suggests that the preponderance of *D. pigrum* in the biofilm was decisive to decrease *I. limosus* and lead to an increase in overall sensitivity of the biofilm to a large number of antibiotics. PNA FISH allowed the direct observation of the location and distribution of the three-species species within the biofilms, corroborating the dominance of *D. pigrum* and *P. aeruginosa* within the mixed-species consortia and facilitating the understanding of the real complex interactions among the bacterial species.

Data highlighted that emergent species are able to establish polymicrobial consortia with common pathogens in the airways of CF patients, modulating different social activities into those communities and impacting the CF therapeutics.

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Keywords: cystic fibrosis; polymicrobial infection; biofilm; *Inquilinus limosus*; *Dolosigranulum pigrum*

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Post-antibacterial effect of two cationic peptides on staphylococcal biofilm

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In view of the high antibiotic-resistance of bacteria in biofilm cationic peptides are becoming more and more promising. The effect of the two peptides warnerin and hominin on the development of *Staphylococcus epidermidis* biofilms was studied.

Strain *S. epidermidis* 33 was cultivated in LB medium to the mid-log phase and twice washed with NaCl (0.14 M, pH 7.2). Peptides were obtained from the culture media of collection strains *S. warneri* KL-1 and *S. hominis* KLP-1 according to the method described previously [1]. The bacterial cells (10^7 CFU/ml) were suspended in solutions of antibacterial peptides warnerin or hominin (1xMIC) and incubated into polystyrene Petri dishes statically at 37°C for 30 min. The peptide-free bacterial suspension was used as the control. Then liquid with unbounded cells was carefully aspirated, adherent cells twice washed by phosphate-saline buffer (10 mM, pH 7.2) or NaCl (0.5 M, pH 7.2), fresh medium LB (2 ml) was added in each Petri dishes with following incubation statically at 37°C for 0, 1, 2, 3, 4 and 24 h. The number of adherent cells was evaluated after their staining with gentian violet (0.1%) using the direct counting.

At start time (0 h) the number of adsorbed cells in each case was approximately equal (Table 1). During the first hour there was a noticeable increase in the number of adherent cells in the control groups, in the case of treatment by the cationic peptides no change occurred. After 2 h the number of adherent cells adsorbed in the warnerin presence and washed with PBS remained constant, whereas in warnerin with NaCl-rinsing group amount of adherent cells increased by two times, but still remained 1.5 times less than in the control groups. In hominin groups the situation was similar. The trend was continuing for the next two hours. After 24 h there was no difference between control and experimental groups, the amount of the adsorbed cells was similar.

Table 1. Biofilm formation by *S. epidermidis* 33 after adhesion in the presence 1xMIC cationic peptides (cells/field-of-view, mean ± SD)

Medium / Time, h	Control		Warnerin		Hominin	
	PBS-rinsing	NaCl-rinsing	PBS-rinsing	NaCl-rinsing	PBS-rinsing	NaCl-rinsing
0	34 ± 8	48 ± 8	24 ± 5	30 ± 9	21 ± 6	31 ± 6
1	50 ± 12	89 ± 15	22 ± 8	40 ± 9	16 ± 4	37 ± 8
2	136 ± 35	166 ± 45	36 ± 18	64 ± 20*	23 ± 10	55 ± 24*
3	207 ± 49	234 ± 62	22 ± 9	86 ± 32*	31 ± 11	91 ± 22*
4	243 ± 90	167 ± 88	36 ± 17	99 ± 46*	40 ± 24	87 ± 29*
24	1100 ± 245	1260 ± 239	1054 ± 218	1341 ± 157	1321± 212	1145 ± 198
Biomass doubling time, min	77 ± 1	77 ± 2	495 ± 34	130 ± 10*	217 ± 26	141 ± 9*

* $p<0.05$ in pair PBS-rinsing – NaCl-rinsing

The constancy of the number of cells adsorbed in the peptides presence and washed with PSB during four hours result in the existence of post antibacterial effect of these peptides. Rinsing by NaCl negates bacteriostatic effect of these peptides and allows the cells to divide, so after four hours the number of attached cells threefold increases compared to the cells rinsed with PSB. The doubling time of attached cells biomass was calculated (Table 1). Washing by 0.5 M NaCl shortened the generation time by 1.5 and 3.8 times for cells treated with hominin and warnerin respectively and made them almost equal.

Keywords: biofilm; adhesion; staphylococcus; cationic peptide.

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Regulatory Role of GntR type Transcriptional Factor LutR in Biofilm Formation of *Bacillus subtilis*

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[2] Molecular Genetics Group, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands Abstract

The lutR gene, encoding a product resembling a GntR-family transcriptional regulator, has first been identified as a gene required for the production of the dipeptide antibiotic bacilysin in *Bacillus subtilis* [1]. The genome-wide effects of a lutR null mutation were studied by combining DNA microarray, RT-qPCR and lacZ fusion analysis with the aim of understanding the regulatory roles of LutR in *B. subtilis* PY79. Gel mobility shift assays to identify the direct targets of LutR. at least 161 genes in 61 transcriptional units had altered expression levels in lutR mutant cells, as compared to $lutR^{+}$ wild type cells in early-stationary phase.

B. subtilis is able to form multicellular aggregates known as biofilms, which consist of an extracellular matrix that enables the cells to hold together and behave as a multicellular community of cells in their natural habitat. The main structural components of *B. subtilis* biofilm matrix are an exopolysaccharide (EPS) polymer produced by the products of the 15-genes *epsA-O* operon and TasA amyloid fibers synthesized by the products of the *tapA-sipW-tasA* operon. Strikingly, in our study, expression of the *tapA* operon was found to be directly up-regulated by the LutR protein while the expression of three *eps* genes: *epsD*, *epsE* and *epsK* were found to be indirectly up-regulated by LutR. Consistently, the biofilm related *lutABC* operon was previously shown to be under the dual control of LutR and SinR [2]. In addition, in our study, two DegU-regulated genes required for making complex colony architecture were also found to be up-regulated by LutR via direct binding. These were the *yvcA* gene encoding a putative membrane-bound lipoprotein and the *bslA* gene encoding a small amphiphilic protein that forms a hydrophobic layer on the surface of biofilms [3] suggesting a protective function for BslA. Taken together, all these results strongly suggest that as a regulator, LutR might affect the complex colony and/or pellicle architecture. To test this possibility, we constructed a *lutR* mutant NCIB3610 strain and then we monitored it's complex colony and pellicle formations on MSgg medium (with glycerol as a carbon source). Although the *lutR* mutant exhibited a reduced colony size, there was no drastic defect in complex colony formation, but the colony architecture of the *lutR* mutant was significantly altered: the thickness of the wrinkled structure is considerably increased and the quantity of the wrinkled structures is relatively reduced. Consistently, *lutR* mutation did not affect the initiation of pellicle formation, however, the *lutR* mutant formed a very thick and smooth pellicle which lacks a distinctive macroscopic architecture. Subsequently, we confirmed that as in the case of domesticated strain PY79, the expressions of *bslA, yvcA* and the *tapA* operon were found to be significantly reduced in the *lutR* mutant of NCIB3610 strain grown on MSgg medium by performing RT-qPCR analysis, supporting the regulatory role of LutR in biofilm development.

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Keywords: Biofilm formation,; *B. subtilis*; GntR type regulator LutR

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Risks of *Candida* spp. biofilms in nosocomial infections

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Hospital can be considered as an ecosystem where the patient is in contact with the microbial world and faces the risk of contracting an infection that is termed the nosocomial. Some yeasts parts of this universe, like *Candida albicans* and *C. glabrata*, are opportunist pathogens. These strains evade antifungal treatments when they form biofilms that constitute a nest for them.

For this framework, five strains of *Candida* were selected to test their biofilms resistance to amphotericin B (AmB), four strains were isolated from implanted catheters at Sidi Bel Abbes University Hospital Center (Algeria), and one reference stain. Testing antifungal AmB showed clearly that *Candida spp* sessile cells (in biofilms) are much more resistant than their planktonic counterparts (suspended cells) that the resistance increases during the different phases of biofilm formation until it reaches its threshold at the maturation phase (48 hrs).

On the other hand, SEM of catheter surface revealed biofilm formation by an isolated *C.albicans*.

Keywords: *Candida albicans*, *C. glabrata*, Nosocomial infections, Biofilms, Amphotericin B.

Screening assay to identify *Acinetobacter baumannii* biofilm inhibitors from a microbial natural products collection

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Acinetobacter baumannii is known for its ability to develop resistance to multiple classes of antibiotics, its ability to develop biofilm, its persistence in healthcare facilities and its inherent hardiness. Moreover, it is currently estimated that more than 65% of bacterial infections and 80% of chronic infections are caused by biofilm-forming bacteria. These infections are related to indwelling medical devices and, are actually a serious concern since once the device is colonized, infection is almost impossible to eliminate. Therefore, there is an urgent need to find successful anti-biofilm compounds against this pathogen and to do so, good, standardized and compatible with high-throughput methods for determining drug susceptibility of bacterial biofilms are required.

The goal of the present work was to set up a rapid, reliable, sensitive, reproducible and simple screening method to identify microbial natural products extracts belonging to the Fundación MEDINA collection with activity against *Acinetobacter baumannii* biofilm. It is a well known fact that natural products isolated from microorganisms have been the source of most of the antibiotics on the market today and there are still underexplored microbial sources of novel compounds. Thus, natural products offer unlimited opportunities for new drug leads due to the unmatched availability of chemical diversity. In addition, data and results obtained from the assessment of the assay as well as from a preliminary screening of 320 extracts will also be shown.

Keywords: *Acinetobacter baumannii*; biofilms; microbial natural products, screening assay

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Synergic interactions between *Candida albicans* and oral bacteria in a three-species biofilm model

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Oral biofilms are formed by well-attached bacteria that still interact with a variety of microorganisms present in the flora. The way species communicate with others and which species are involved determine how the biofilm will be formed. The presence of *Candida albicans* in a mixed bacteria-fungal biofilm contribute to bacteria improvement and bacteria are suggested to protect *Candida* against antifungal treatment in cases of candidiasis. So, the aim of this study was to develop a three-species biofilm composed by *Actinomyces naeslundii (An), Streptococcus oralis (So),* two first colonizers of prosthetic surfaces associated with *Candida albicans (Ca)* to evaluate the interactions in this model. For that, a salivary pellicle was formed for two hours on PMMA resin discs and three different combinations between species generated biofilms onto coated-discs: I (*An* + *Ca*), II (*So*+ *Ca*) and all three species. A mono-specie biofilm of *Candida albicans* was used as control. The biofilms were developed in YNBPT medium (YNB, peptone and triptone in a phosphate buffer) supplemented with 40 % glucose for 1.5 (adhesion), 24 and 48 hours. After each time point, the total amount of cells and the proportion of each species in combinations were determined in colony-forming units (CFU/mL). The interactions present in each combination during the development were evaluated planktonically using fluorescence microscopy in which *An*, *So* and *Ca* were labelled with the dyes FITC, TRITC and Calcofluor respectively and also investigated by Scanning Electron Microscopy (SEM) in biofilms. The data were evaluated by one and two-way ANOVA set at 5 % of significant level. The biofilm composed by *Candida albicans* and *S. oralis* generated the greatest amount of total cells ($p < 0.001$) and the total counts suggested no prevalence between species in three-species biofilm ($p > 0.05$). Also, the biggest amount of cells in each I and II combinations are bacteria ($p < 0.001$) although *An* counts were similar in 24 and 48 hours ($p > 0.05$). *Candida* best grown in those biofilms than as mono-specie ($p < 0.001$). The planktonic and biofilms images showed synergic interactions and bacteria well-attached to *Candida* in each step of development. In conclusion, oral bacteria synergically interacted with *Candida* and well-formed this three-species biofilm model.

Keywords: Biofilms; *Candida albicans*; Oral bacteria; Model; Interaction.

Syngonanthus nitens extract in precursor systems of liquid crystalline: action against biofilm of *Candida albicans*

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Liquid crystals are an attractive nanotechnology platform for the delivery of medicinal plant products to cells due to their interaction with membranes and ability to stimulate pharmacological reactions with eukaryotic organisms, such as *Candida albicans. C. albicans* infections start with colony formation but lead to the creation of a biofilm with subsequent resistance against antifungal agents and a fall in the host's defense [1]. *Syngonanthus nitens* (Bong.) Ruhlland (Eriocaulaceae) has shown potential with antimicrobial and anti-ulcer actions, so it seems interesting to search for new properties and optimize known therapeutic properties. The aim of this study was to evaluate the activity of the methanolic extract of *S. nitens* against *C. albicans* biofilms by comparing delivery in its naturally occurring state and incorporated into a precursor system of liquid crystalline phase (PSLC). The PSLC used was oleic acid (40%) as oil phase, PEG-5 Ceteth-20-Procetyl (40%) as surfactant and a polymer dispersion Carbophol 974P + policarbophyl-PP (pH 7.0) + Milli-Q™ water (20%) as aqueous phase. Different concentrations of artificial vaginal mucus (AVM) were used (5, 10, 30, 50 and 100%) to assess the activity [2]. The yeast suspension (1 x 10^8 cells/mL) was added to 96-well plates and incubated under rotation at 37°C for 2 hours for pre-adhesion and biofilm formation. The overlying liquid was discarded and RPMI-1640 was added; this was replaced after 24 hours of incubation [3]. The extract (naturally occurring form and incorporated in PSLC) was added after 48 hours at concentrations of 0.6 to 20 mg/mL and the microplate was incubated for a further 24 hours. Amphotericin B (0.006 to 1.6 ug/mL) was used as a positive control. The stain 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[carbonyl(phenylamino)]-2H-tetrazolium hydroxide (XTT) was used at the end of the incubation period with antimicrobial activity being evaluated by polarized light microscopy (Figure 1 and Table 1). The use of PSLC as a delivery system of the methanolic extract of *S. nitens* to treat *C. albicans* infections is promising. However, treatment using *S. nitens* in its natural state was not effective against the biofilm. This improved antifungal activity using the PSLC may be due to the oleic acid increasing the permeability of the fungal membrane.

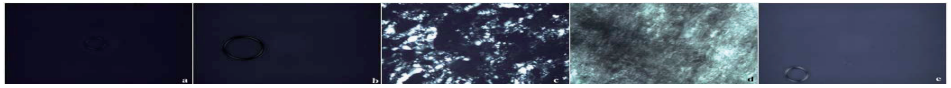

Figure 1: Antimicrobial activity against *C. albicans* biofilm using a precursor system of the liquid crystalline to deliver methanolic extract of *S. nitens* with artificial vaginal mucus at different concentrations: a (5%); b (10%); c (30%); d (50%); e (100%). Analysis by polarized light microscopy.

Table 1 - Results of biofilm assay of the metanolic extract of *S. nitens* free and incorporated in PSLC.

Strain	Extract in free form (DMSO 20%)	Extract incorporated into PSLC	Solvent Control-DMSO 20%	PSLC (pure)	amphotericin B
ATCC 102321	No inhibition	625 µg/mL	No inhibition	No inhibition	200 µg/mL
CAV1	No inhibition	2500 µg/mL	No inhibition	No inhibition	400 µg/mL
CAV2	No inhibition	5000 µg/mL	No inhibition	No inhibition	400 µg/mL
CAV3	No inhibition	625 µg/mL	No inhibition	No inhibition	100 µg/mL
CAV4	No inhibition	5000 µg/mL	No inhibition	No inhibition	100 µg/mL
CAV5	No inhibition	10,000 µg/mL	No inhibition	No inhibition	0.5 µg/mL

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Keywords: Biofilms; Nanotecnology; *Candida albicans*; *Syngonanthus nitens*

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The effect of diacetyl rhein on biofilm formation of *Staphylococcus aureus*

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Staphylococcal infections can cause severe human infections which can be even more complicated when the bacteria are able to form biofilms. Bacteria in biofilm formations are known to possess higher degree of resistance to antibiotics and thus development of new effective preparations are needed. Diacetyl rhein is a drug currently used for the treatment some inflammatory diseases. Interestingly, the diacetyl rhein have been found to possess also antimicrobial effect against various bacteria [1,2]. Moreover, there have been reported its synergistic anti-staphylococcal interactions with some antibiotics recently [3]. Although the anti-biofilm effect of rhein (the active metabolite of diacetyl rhein) have previously been reported [4], the anti-biofilm activity of diacetyl rhein itself remains unexplored. Therefore, in this study, the *in vitro* effect of diacetyl rhein was tested against biofilm formations of various *Staphylococcus aureus* strains including resistant forms. The MBEC Assay ™ was used for the experiments and the results were expressed as minimum biofilm eradication concentrations (MBEC). In addition, the effect of selected concentrations was examined by confocal microscopy using fluorescent staining.

Diacetyl rhein showed to be effective against three out of four strains tested whereas the MBECs ranged from 32 to 256 µg/mL. The strongest activity was exerted towards standard methicillin-resistant strain ATCC 43300. Surprisingly, the diacetyl rhein was effective at 16-fold lower concentrations than gentamicin towards this strain.

The obtained results revealed another antimicrobial action of diacetyl rhein which, as a currently registered anti-inflammatory drug, offers a promising alternative for the treatment of some *S. aureus* biofilm infections.

Keywords: antimicrobial; bacterial biofilms; diacetyl rhein; quinones; *Staphylococcus aureus*

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The potential application of vanillin for control of *Cronobacter sakazakii* and its biofilm formation in the reconstituted infant formula

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Background *Cronobacter sakazakii* is opportunistic foodborne pathogen, which has been detected in reconstituted infant formula (RIF) causing diseases such as meningitis and bacteremia [1, 2]. The major property of *C. sakazakii* is forming biofilm, which plays a pivotal role in bacterial survival under various external stresses as acting like a barrier. Vanillin, a major component of vanilla beans, has been generally recognized as safe (GRAS). Its antimicrobial and antioxidant properties have been tested, and many researchers suggested its potential as food additives [3, 4]. In this study, we investigated the potential of vanillin for an antimicrobial or antibiofilm agent to *C. sakazakii* and for an inhibition of *Cronobacter* biofilm formation.

Materials and methods *C. sakazakii* ATCC 12868 and 29004 were used in this study.
Antimicrobial effect. Bacterial cell cultures were subjected to the sterilized RIF containing 10, 20, and 40 mM of vanillin (treatments) or 2% of ethanol (control) at 45°C, and survived cell population was measured after 10 and 30 min to test continuous effect of vanillin.
Antibiofilm effect. Cronobacter biofilms were formed in RIF on the stainless steel coupon (SS 304, 1.5 x 2.0 cm) (SSC) at 22°C. After 5 days, biofilms on SSC were dipped in 10, 20, and 40 mM of vanillin solution (treatments) or distilled water containing 2% of ethanol (control) at 45°C. Remaining cells on SSCs after 10 min were measured.
Inhibition of Cronobacter biofilm formation. Ten and twenty millimol of vanillin were added in RIF and stored at 22°C. The level of *Cronobacter* biofilm formation were measured after 5 days.

Results and discussion There were no significant reductions in bacterial cell population in 10 and 20 mM of vanillin for 30 min at 45°C ($p > 0.05$). In the RIF containing 40 mM of vanillin, however, more than 3 log CFU/ml of bacterial cells were reduced (initial cell population of : 7.01 – 7.20 log CFU/ml). In the test for antibiofilm effect of vanillin, biofilms treated with 10 and 20 mM of vanillin at 45°C for 10 min did not show any bacterial reduction compared to control or inactivated less than 2 log CFU/ coupon (biofilm cell population of control: 4.00 - 5.43 log CFU/coupon). On the other hand, biofilms were reduced to below the level of detection when they were dipped in 40 mM of vanillin. Based on these results, we added 10 and 20 mM of vanillin, showing no either bacterial reduction or biofilm decontaminating effect, in RIF to test their biofilm inhibiting ability. *Cronobacter* species exposed to 20 mM of vanillin during 5 days did not disfigure RIF; it maintained without producing glutinous substance or becoming lumpy. Also, no cells were detected on the SSCs. These results represent that a small amount of vanillin can inhibit *C. sakazakii* from forming biofilm in the RIF. It might be explainned as its interrupting role of quorum sensing which is needed to form biofilm [5].

Significance and impact Vanillin can be applied to control *C. sakazakii* cells as well as biofilm. Also, small amount of vanillin which can attenuate *Cronobacter* cells (losen biofilm forming ability) could be prevent ubiquitous *C. sakazakii* cells from forming biofilm on the surface.

Keywords: *Cronobacter sakazakii*; Biofilm; Reconstituted infant formula; Vanillin

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The time profile of cell adhesion of the highly adhesive bacterium *Acinetobacter* sp. Tol 5

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AtaA (*Acinetobacter* trimeric autotransporter adhesin) is a highly adhesive and autoagglutinating bacterionanofiber expressed on the cell surface of *Acinetobacter* sp. Tol 5[1)]. Trimeric autotransporter adhesins (TAAs), which are produced by Gram-negative bacteria, are responsible for its pathogenicity or biofilm formation through the adhesive and autoagglutinating properties of these fibers[2)]. To date, the commonly-used adhesion assay is the plate attachment test in which cells are placed into well plates and cells adhering to the inner walls of the well are stained with crystal violet, and the adhesion property is mesured as the stain intensity[3)]. In this method, there is an issue that the adhesion property is not measured precisely when the cells show autoagglutination feature because the intensity is correlated with cell density. Therefore, other feasible approaches are to be addressed for analyzing the adhesion process of cells showing autoagglutination property.

The molecular properties of AtaA have been analyzed and its unique features have been unveiled. However, the cell level adhesion mechanism of how AtaA mediates the adhesion has not been elucidated yet. In this study, to reveal this adhesion mechanism, we assumed that autoagglutination is involved in the adhesion of cells expressing AtaA because AtaA also mediates it. As an approach to elucidating the mechanism, the time profile of cell adhesion was analyzed through confocal laser scanning microscopy (CLSM) observation and flow cell analysis.

In CLSM observation, it was revealed that Tol 5 wild-type strain (WT) formed a thick cell layer (> 30 μm) which has a void structure while Δ*ataA* strain showed only a single cell layer (< 5 μm). Moreover, in a flow cell experiment, in which the flow rate was 60 μl/min and the samples were observed using upright microscope, the result showed that autoagglutinated WT cells well attached to the glass plate surface, while its single cells hardly attached.

We emphasize the following two points in this presentation; 1) the feature of a cell clump formation represented by CLSM analysis suggest that autoagglutination mediated by AtaA exhibits a cluster-cluster agglutination and 2) the flow cell analysis shows the autoagglutination greatly contributes to the highly adhesiveness of cells expressing AtaA through extending the surface attachment area.

Keywords: adhesion; autoagglutination; nanofiber; biofilm

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Trimeric Autotransporter Adhesins (TAAs) and strategies for their inhibition

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Adhesion of bacteria to surfaces is essential for many aspects of microbial life: surface recognition and attachment, biofilm formation, and pathogenesis. Adhesion to host tissues is frequently among the first steps in host colonization by pathogens, and adherence is often an essential property for virulence. Adhesins must reach the bacterial cell surface to fulfill their function. Numerous, sometimes highly specific export systems exist for this purpose. In autotransporter proteins of Gram-negative bacteria, export through the outer membrane is mediated by a C-terminal membrane pore domain that auto-exports the N-terminal passenger domain of the same polypeptide chain [1]. Trimeric autotransporter adhesins (TAAs) comprise a widespread family of adhesive molecules in Gram-negative bacteria, many of which are important virulence factors [2]. The are also called Type Vc autotransporters (Figure 1). The prototypical trimeric autotransporter is the Yersinia adhesin YadA from *Yersinia enterocolitica*, which mediates attachment to collagen and other extracellular matrix (ECM) molecules, promotes serum resistance and mediates autoagglutination.

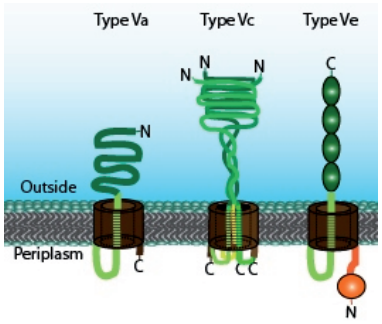

Figure 1. In type Va (monomeric) and type Vc (trimeric) autotransporters, the C-terminal β-barrel translocates the passenger domain across the outer membrane. In type Ve secretion (inverse autotransport), the passenger is located C-terminally to the translocating β-barrel [1].

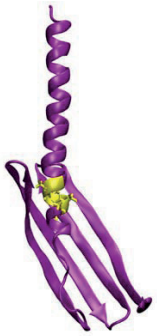

Figure 2. A flexible region in TAAs was identified from NMR data [3]. This region is targeted by point mutations.

In recent work, we showed that the structure of the autotransport domain of YadA contains a conserved region of small residues [3], that we are currently exploiting for mutational studies. We have constructed mutant YadA variants stalled in autotransport that will help us to elucidate the export mechanism through the bacterial outer membrane. Moreover, we are currently working on small-molecule inhibitor screens based on bacterial adhesion in microwell plates, with the aim to find inhibitors that target either the autotransport process (adhesin biogenesis) or the adhesion to ECM molecules (adhesin function).

Keywords: bacterial adhesion; autotransport; type V secretion; small-molecule inhibitors

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TTO and Terpinen-4-ol inhibit biofilm resistant clinical isolates of *Candida albicans*

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Tea tree oil (TTO) is the essential oils steam distilled from *Melaleuca alternifolia*. It is a complex mixture of terpene hydrocarbons and tertiary alcohols, and the main compound responsible for the antimicrobial activity is terpinen-4-ol. The aim of this study was to evaluate the efficacy of TTO and Terpinen-4-ol against clinical isolates (genotypes A1, A2 e B1, B2) of *Candida albicans*. This study was conducted in three phases: 1- Identification of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (CFM) of TTO (0.25 to 2%) and Terpinen-4-ol (0.11 to 0.95 %) on *C. albicans* in planktonic form; 2- Analysis of different concentrations of oil on *C. albicans* biofilm single species by evaluation of the metabolic activity of cells by XTT colorimetric method; 3- Analysis of the inhibitory action of TTO and Terpinen-4-ol solutions on *C. albicans* formed in specimens of resin acrylic microwave previously coated with human saliva, by XTT test. The nystatin (Sigma) was used as a positive control. The results showed that isolates of *C. albicans* planktonic were sensitive to TTO 1%, Terpinen-4-ol 0.47% and Nystatin 8 mg/mL. The fungicidal concentrations for the isolates were TTO 2%, Terpinen-4-ol 0.95% and Nystatin 16 mg/mL. When in biofilm, the concentrations TTO 2% and Terpinen-4-ol 0.95% were effective when compared to the control, and genotyping of samples A2 and B1 were more resistant. The results of XTT showed that all biofilms developed in disks of acrylic resin were similar to the action of Nystatin. The extracts showed antifungal action for the clinical isolates and can be considered an alternative treatment for patients with candidiasis.

Keywords: Oil of Melaleuca; Candida albicans

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Antimicrobial materials

science and surface chemistry

Antimicrobials in consumer products

A new highly antimicrobial bio-inspired protein-based polymer designed for medical devices

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Increasing antimicrobial resistant and the lack of new antibiotics poses a major public threat. Furthermore, conventional medical devices fail to avoid microbial colonization resulting in healthcare associated infections. These threats have triggered the search for new antimicrobial materials that have the ability to kill microorganisms in a general way and, especially considering those directed for medical devices, without damaging human tissues. With advances in synthetic biology approaches, it is now possible to reengineer novel functionalities and structures of protein-based materials, taking advantage of their extreme versatility and applicability. By combining the antimicrobial activity of naturally occurring antimicrobial peptides (AMPs) with recombinant protein-based polymers (rPBP), it is possible to create novel materials that can be explored for the development of advanced antimicrobial medical devices.

In this work we have constructed a novel antimicrobial rPBP by combining a synthetic cationic AMP fused in frame with an elastin-like recombinamer consisting of 200 repeats of the structural unit VPAVG, for improved biocompatibility and processing. The new polymer construction, named CM4-A200, was biologically produced in *Escherichia coli* and purified by exploring the thermoresponsiveness property of poly-VPAVG.

The antimicrobial activity of the hybrid polymer CM4-A200 was studied in the form of free standing films produced by solvent casting, especially thinking in downstream processing for application as thin film coating of medical devices. The CM4-A200 films showed high growth inhibition against a wide range of bacterial species, both Gram positive and negative as well as against fungi. The antimicrobial activity proved to be time-dependent, by direct contact and, in the case of *A. nidulans*, was inclusively inhibitor of spore germination. Remarkably, in some cases, almost 100% of cell death was detected after 30 minutes of contact with the cast films. Furthermore, we demonstrate that cell death is a consequence of irreversible cell damaging and disruption. Finally, we describe the development of an *ex vivo* assay using pig skin, that avoids the use of glued components that may interfere with antimicrobial activity. *Ex vivo* assays in pig skin sustained the high antimicrobial activity of this material, whereas no cytotoxic effect was found for a normal human fibroblast cell line.

These results significantly support the potential of this genetically engineered polymer for application as antimicrobial medical devices or coatings, representing a major breakthrough in the development of protein-based materials and demonstrates the high versatility of these materials.

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Keywords: elastin-like recombinamers; antimicrobial; solvent cast; antimicrobial peptides; ex vivo

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An alginate lyase functional coating catalysis-independent to prevent *P. aeruginosa* adhesion

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Bacterial colonisation of indwelling devices remains a serious threat in clinical field as it is commonly associated to persistent infections, called biomaterial-associated infections (BAI). *Pseudomonas aeruginosa* is the most common gram-negative bacillus associated with BAI and its emergence as a nosocomial pathogen is a growing concern. This opportunistic pathogen can produce a capsule-like polysaccharide called alginate that contributes to mucoid biofilm structure and persistent nature of infections. Given alginate's contribution to bacterial virulence, it has long been considered as a promising target for interventional therapies. Alginate lyase, an enzyme able to degrade alginate, has been shown to detach mucoid biofilms from abiotic surfaces and increase their antibiotic susceptibility.

In this work, a new approach for alginate lyase was explored. Instead of using this enzyme for the treatment of pre-established mucoid biofilms, the ability of alginate lyase to prevent *P. aeruginosa* adhesion to a surface was investigated. For that purpose, a polydopamine (pDA) dip-coating strategy was applied for functionalization of biomaterials with alginate lyase. Polycarbonate (PC) substrates were immersed in an alkaline solution of dopamine to form a thin layer of pDA and then transferred into a solution of alginate lyase. Surface characterization was performed with XPS, contact angle measurement and SEM. Two reference strains of *P. aeruginosa*, a mucoid strain (ATCC 39324) and a non-mucoid (27853) as well as four clinical isolates, were used to assess the anti-adhesive properties of the functional coatings.

Surface characterization confirmed the successful and efficient grafting of alginate lyase onto pDA-coated PC substrates. Untreated PC substrates allowed the adhesion of both reference strains and most of bacteria were found alive on these surfaces. Polydopamine-coated substrates had no significant effect on bacterial adhesion compared to the unmodified substrates. Substrates functionalized with alginate lyase exhibited anti-adhesive properties, causing a significant inhibition of the mucoid strain adhesion. Interestingly, substrates immobilized with this enzyme also proved to inhibit the adhesion of the non-mucoid strain and pDA-coated PC substrates immobilized with heat-inactivated enzyme also prevented the attachment of both bacterial strains. These results suggested that alginate lyase immobilized on pDA-coated substrates was able to impair *P. aeruginosa* adhesion regardless its mucoid phenotype and therefore it could be applied in a different context than cystic fibrosis. For instance, this enzyme could be used to develop functional coatings able to prevent *P. aeruginosa* infections associated to biomaterials. To confirm this hypothesis, the attachment of 4 clinical strains of *P. aeruginosa,* isolated from peritoneal dialysis catheters, on alginate lyase functional coatings were also evaluated. Alginate lyase immobilized on the substrates impaired the attachment of the clinical strains with the exception of one as its adhesion to the unmodified PC was already low.

In this work, the versatile chemistry of polydopamine was successfully exploited to functionalize biomaterial surfaces with alginate lyase to impart them with anti-adhesive properties. The antibacterial performance of these alginate functional coatings was catalysis-independent which highlights the importance of further studies to better understand its mechanism of action against *P. aeruginosa* strains.

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Keywords alginate lyase; antibacterial coating, *Pseudomonas aeruginosa*; biomaterial-associated infections

Anti-*Campylobacter* activity of resveratrol and its inclusion complex with hydroxypropyl-γ-cyclodextrin: a potential preservative for the food industry

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Campylobacter spp., among which *C. jejuni* and *C. coli* are the most relevant species, are considered the major cause of bacterial gastroenteritis in humans worldwide. The major source for human *Campylobacter* infections is the consumption or handling of contaminated food products. This pathogenic organism is increasingly resistant to antibiotics and is able to survive in hostile environments due to its capacity to form biofilms. Many outbreaks have been associated with biofilms and it has become a major problem in food industries since these biofilms are resistant to antimicrobial agents and disinfectants. Given that, one of the main goals of the food industry is the development of controlled-release active packaging systems with antimicrobial activity in order to control and/or eradicate foodborne pathogen transmission to humans. Resveratrol is a phytoallexin present in some foods and possesses several biological properties such as antioxidant, antimicrobial, anti-inflammatory and anticarcinogenic. However, resveratrol shows very poor aqueous solubility and stability, as it can suffer cis-isomerization and be easily oxidized. For these reasons, complexation of resveratrol with molecules such as cyclodextrins, could improve resveratrol solubility and stability.

The purpose of this study was to evaluate resveratrol (RV) use in the control of various *Campylobacter* strains, either as single compound or in the form of an inclusion complex (IC) with hydroxypropyl-γ-cyclodextrin (HP-γ-CD), exploiting the potential use of these compounds as food preservatives.

The first step was to evaluate the susceptibility of two *Campylobacter* spp. (one *C. jejuni* isolated from poultry meat and one *C. coli* isolated from a human presenting acute gastroenteritis) to RV and IC, by broth microdilution method. In order to better clarify the possible mode of action of these two compounds, time-kill studies and flow cytometric assays were performed. Since the colonization of poultry products by *Campylobacter* spp. is thought to be related with biofilm formation, the effect of the RV and IC on this process as well as on mature biofilms dispersion was assessed by the crystal violet staining method.

Both RV and IC presented antibacterial activity against the two tested strains. The minimum inhibitory concentration (MIC) values of RV were 50 and 100 µg/mL to *C. Coli* and *C. jejuni*, respectively and the IC presented MIC values of 64 and 256 µg/mL. Time-kill assays revealed an inhibitory effect of RV and IC against the two tested strains at 6 h of incubation, with a total growth inhibition at 24 h, revealing the bactericidal action of this compound against *Campylobacter*. Flow cytometry assays showed that this bactericidal action resulted from a decrease in metabolic activity as well as alterations in the membrane potential of cells, with this effect being dose-dependent. Concerning biofilms, RV totally inhibited biofilm formation by *C. coli* and *C. jejuni* when present at high concentrations (4×MIC), and caused an inhibitory effect on biofilm formation even at sub-inhibitory concentrations. The ability of IC in inhibiting biofilm formation was also observed. Regarding the dispersion effect on a mature *Campylobacter* spp. biofilm, RV showed an inhibitory effect with all the tested concentrations, however only high IC concentrations had some effect on the dispersion of the biofilm.

In conclusion, our results showed that both RV and RV IC inhibited *C. jejuni* and *C. coli* planktonic cells. The inhibition on biofilm formation, as wells as the reduction of the biomass cells on a mature biofilm was also observed. This work shows that the resveratrol complexation with HP-γ-CD, in addition to increasing resveratrol solubility, also maintained the biological properties of resveratrol, encouraging the incorporation of this IC in several materials to be used in food and pharmaceutical industries.

Keywords: *Campylobacter* spp. ; resveratrol ; HP-γ-CD inclusion complex ; antibacterial activity ; biofilm inhibition.

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Antibacterial Activity of Surface Coated Versatile Substrates from Catechol Conjugated Polyquaternary

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We have prepared an antimicrobial polymeric materials composed of catechol conjugated polypropylene oxide grafted poly(dimethylaminoethyl methacrylate) (PPO-g-PDMA). To prepare antimicrobial polymer, the adhesive property of 2-chloro-3′, 4′-dihydroxyacetophenone (CCDP) and antimicrobial agent of 1-bromooctance or 1-bromododecane (CX) were quaternized to PPO-g-PDMA [(CCDP/CX)-q-(PPO-g-PDMA)].[1] The synthesized antimicrobial polymer are capable to apply by using adhesive property of CCDP in a reduced environment. As a results this antimicrobial adhesive materials are possible utilized as substrate independent coating agents.[2] Moreover the antimicrobial activity could further improved through immobilization of silver nanoparticles (Ag NPs) over this modified surface. X-ray photoelectron spectroscopy (XPS) investigation shows the successful quaternization of CX and deposition of Ag NPs onto these coated surfaces. As a results the coated antimicrobial polymer shows efficient antimicrobial activity against Gram-negative bacteria (*E. coli*) at lower alkyl chain belong to octane chain where the Ag NPs deposited coating surface clearly shown antimicrobial activity both octane and dodecane alkyl chain. Therefore the Ag NPs deposited antimicrobial adhesive material [(CCDP/CX)-q-(PPO-g-PDMA)] promise a great potentiality to apply wide range of substrate.

Keywords: antimicrobial; coating; alkyl chain; ag nanoparticles; reduction;

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Antibacterial and fungicidal plastics by dendritic hyperbranched polymer-copper-hybrids

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As a result of the rapid development of antibiotic resistant bacteria strains, the research for new antimicrobial agents increased during the last years. Considering the progress in nanoparticle technology especially silver has become prominent and is highly capable to render material surfaces antimicrobial [1]. Beside silver other metals like copper and zinc also show antimicrobial properties, known as the oligodynamic effect [2]. Owing to the higher raw material price of silver and the controversial existence of silver-resistant bacteria strains [3], copper is becoming more and more attractive for antimicrobial applications.

In order to ensure a nearly constant, high antimicrobial effect, the internal developed, amphiphilic, dendritic polymer with core-shell-architecture was chosen as template and carrier [4] for copper-nanoparticles. Innovative microwave assisted synthesis of copper hybrid polymers was developed. The synthesis process has been refined so that one could reach a monomodal particle size distribution of a nanoscale product while increasing the yield of the product in conjunction with a low reaction time. Furthermore, we could demonstrate in numerous application tests that this copper-hybrid-polymer could be used as high effective antimicrobial surface active agent in coating and bulk applications. Plastic composites modified with the copper hybrid polymer showed high bactericidal activity against *Staphylococus aureus* (Figure a) and high fungicidal activity against *Candida albicans* (Figure b).

Keywords: dendritic polymer template; antimicrobial copper

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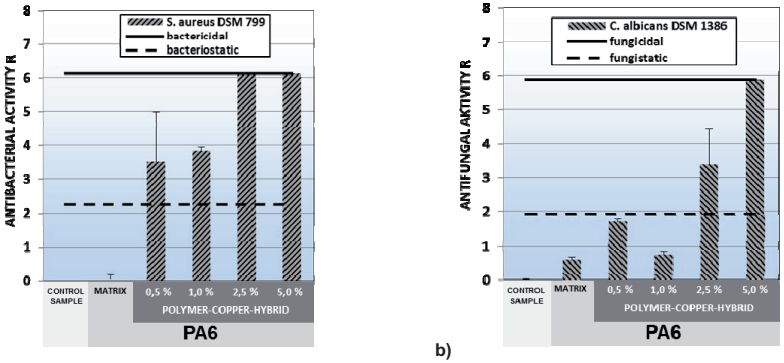

a)

b)

Figures/ Tables

Figure a) Results of the measurement of antibacterial activity of polymer-copper-hybrid-containing PA6 compounds and their reference material based on ISO 22196

Figure b) Results of the measurement of antifungal activity of polymer-copper-hybrid-containing PA6 compounds and their reference material based on ISO 22196

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Antibacterial Application of Functionalized Soluble Graphene

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To formulate soluble chemically reduced graphene oxide (RGO) in solvent media, certain functionalization of graphene plates by charges, small molecules, or polymers must be performed through either covalent or noncovalent chemistry because pristine graphene or RGO itself is not readily soluble in any solvent media due to its extremely high van der Waals interaction (or van der Waals force) between 2D graphene plates. Among noncovalent chemistry, π–π interaction is the most frequently attempted noncovalent interaction to formulate soluble RGO/π-conjugated polymer assembly. Recent theoretical simulations for the anchoring of certain amino acid molecules or cholesterols on ideal graphene plates have revealed that aliphatic molecules with various molecular weights can be spontaneously anchored on graphene plates through van der Waals interaction. But the detailed interaction parameter between RGO plates and aliphatic polymers was not fully unveiled and therefore further theoretical and experimental researches to elucidate the feature of noncovalent interaction between RGO plates and aliphatic polymers are required. Soluble graphene/polymer assembly structure was formulated through noncovalent interaction between RGO and various aliphatic polymers. The interaction parameter between RGO plates and aliphatic polymer chains was estimated mainly as σ–π interaction, a kind of van der Waals interaction between them. Several graphene/polymer assemblies having certain activities will be demonstrated and detailed studies on the noncovalent interaction between graphene plates and aliphatic dendrimers will be discussed. Especially bioapplications of graphene-based nanomaterials such as antimicrobial activity will be demonstrated. [1,2]

Keywords: graphene; chemically reduced graphene oxide; noncovalent interaction; bioapplication;

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Antibacterial performance of bovine lactoferrin-fish gelatine electrospun nanocomposites

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The alarming increase of antibiotic resistant microorganisms urged the development and synthesis of novel antimicrobial biomaterials, to be employed in a broad range of applications, ranging from food casings to medical devices [1 – 3]. This work describes the processing and characterization of an innovative fully biobased eletrctrospun nanocomposite material displaying antibacterial properties. Its composition is exclusively comprised of proteins, with fish gelatine as the structural matrix and bovine lactoferrin as the antimicrobial agent.

Mainly obtained from the inedible components of the fishery processed catch, fish gelatine (FG) represents a viable alternative source for this highly demanded protein [4]. Electrospun fish gelatine possesses highly interesting properties, such as resilience, biocompatibility, and is stable in aqueous solutions after crosslinking through exposure to glutaraldehyde or genipin atmosphere [5, 6]. Bovine lactoferrin is a wide spectrum antimicrobial protein, exerting its action in numerous virus, bacteria and prokaryotic parasites. Moreover, lactoferrin bears immunoregulatory properties and anti-tumour activity. Specifically, the antibacterial activity of lactoferrin consists of several mechanisms, namely through deprivation of environmental iron, destabilization of Gram negative lipopolysaccharide outer membrane via calcium chelation, and surface charge disruption of Gram positive [7]. In order to confirm the bovine lactoferrin bactericidal efficiency, the minimal inhibitory concentration was determined using clinical isolates of *Escherichia coli* and *Staphylococcus aureus*, through microtitre broth dilution test.

Two distinctive methods were used to incorporate lactoferrin into the fish gelatine nanofibers: i. as a filler in the electrospinning formulation using concentrations of 2, 5 and 10 (%wt), and ii. through adsorption in a solution with 40 mg mL^{-1} of lactoferrin.

Fourier transform spectroscopy analysis revealed that the structure of both nanocomposite proteins remained intact through the electrospinning blending and crosslinking procedure. The increase in the concentration of lactoferrin as a filler diminished in approximately 50% the size of the fibres when compared to pristine gelatine.

The electrospun material with adsorbed LF displayed an antimicrobial activity similar to the fish gelatine fibres without LF, most likely due to the low uptake of LF. The nanocomposites bearing 5% of LF as a filler showed a bacterial reduction of approximately 90% when compared to the control (electrospun FG). In addition, films containing 10% of LF revealed a notable antibacterial performance, with 100% of contact killing capacity, representing above 6 log reduction in *E. coli* and *S. aureus* bacterial populations.

Keywords: electrospinning; fish gelatine; lactoferrin; bactericidal; nanocomposite

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Antimicrobial activity of self-assembled carboxylic acid crystals on graphite

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Bacterial infections have remained a global issue for a long period of time due to resistance to chemical-based sterilization methods. Therefore the scientific community has been pushed to the edge to find alternative methods for controlling bacterial infections. Nature which has provided insight for many modern high-technology applications, has also developed numerous strategies for coping with bacterial infections. One of which can be found in insect wings. Cicada and dragonfly wings were recently reported to possess bactericidal activities via physical means [1, 2] . The surfaces of these wings are covered by a layer of nano-pillars made of mostly aliphatic hydrocarbons and their derivatives [3, 4]. With their hydrophobic nature, they play a significant role in superhydrophobicity and self-cleaning, and have already inspired several biomimetic applications. Palmitic acid and stearic acid, the two of the major components found on the surfaces of *Hemianax papuensis* and *Hemicordulia tau* dragonfly wings, were self-assembled on highly ordered pyrolytic graphite (HOPG), to produce ordered, three-dimensional structured surfaces. The fatty acids oriented epitaxially with the underlying graphite, which resulted in the formation of ordered micro-crystals. These crystals were also found to be able to inactivate bacterial cells through mechanobiocidal mechanisms. These surfaces were made using facile synthesis method and inexpensive materials, which will be of great benefit in a variety of antibacterial applications.

Keywords: Antimicrobial; self-assembly; graphite; mechanomechanism; carboxylic acids

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Antimicrobial activity of whey protein isolate edible films incorporating carvacrol and eugenol

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Edible films and coatings have a high potential to act as carriers of different antimicrobial agents with the aim of extending food product shelf-life and enhance safety. Application of antimicrobials directly onto the food surface may have limited benefits because the active substances could be neutralized and diffused from the surface into the food product, thus avoiding the effect of the antimicrobial compound [1-3]. Transparent, odourless and tasteless whey protein isolate (WPI) based edible films have good mechanical properties and are excellent oxygen, lipid and aroma barriers at low relative humidity. These films have been used as carriers of different antimicrobial compounds with the aim of extending food products shelf-life and safety [4,5].

The main objective of this work was to incorporate 2 different antimicrobial compounds, carvacrol and eugenol, into WPI edible films by an ultrasound emulsification process and to evaluate their effectiveness against 2 gram-positive bacteria of interest in food processing, *Listeria innocua* and *Staphylococcus aureus*. The effect of both the type and concentration (1 and 2% w/w) of antimicrobial incorporated into the WPI edible films was determined by using the agar disk diffusion method.

Developed antimicrobial WPI films were stables, transparent, uniform with homogeneous smooth surfaces without pores or cracks. All WPI films incorporating carvacrol or eugenol, at 1 or 2% (w/w), were active against *L. innocua* and *S. aureus* presenting different halo sizes. Films without carvacrol or eugenol, served as control to determine possible intrinsic antimicrobial effect. As expected, no inhibition area against the 2 bacteria tested was observed for WPI control films. Thus the differences in the inhibition area observed for the films that did incorporate the antimicrobials can be attributed only to the effect that each component has on every strain when diffused in the inoculated media.

Films containing carvacrol produced the largest surfaces of inhibition against the growth of the 2 bacteria tested. WPI-carvacrol films at 2% exhibited the extensive area inhibition growth with 392. 4 mm^2 for *L. innocua* and 156 mm^2 for *S. aureus*. The increase of antimicrobial concentrations in films formulations leads to an increase on inhibition area. The effect of concentration was more observed for treatments conducted against *L. innocua*. The differences in the inhibitory effects of films can be attributed to the susceptibility of each bacterial strain to the antimicrobial, to its concentration and to their interactions with the WPI matrix affecting the diffusivity of the active compounds and therefore on the final antimicrobial activity.

Keywords: Antimicrobial; Edible film; Carvacrol; Eugenol

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Antimicrobial effects of silver nanoparticles on planktonic and sessile communities of pathogenic bacteria

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In recent years, the resistance of bacteria to antimicrobials has increased due to the slapdash use of antibiotics in animal feed as growth promoters as well as their abusive utilization in medicine and veterinary. The bactericidal effects of ionic silver are known and applied since antiquity. Silver is used in several medical devices and surgical equipments such as burn dressings, scaffolds, water purification systems and medical devices. In particular, silver nanoparticles (SNPs) may damage the activity of bacterial enzymes and cell structures, which cause bacterial cells to die. Besides, the photo-induced water splitting on TiO_2 electrodes when is exposed to UV light (λ=400 nm) generate excited electrons (ecb^-) that are trapped by water (H_2O) or hydroxyl groups (OH^-) adsorbed on the surface to generate hydroxyl radicals (OH•). OH• is a powerful and indiscriminate oxidizing agent with antibacterial properties. In this work, we study the antimicrobial activity of SNPs in combination with coated TiO_2 against multiresistant strains of the human-pathogenic bacteria *Escherichia coli (EHEC), Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa,* and spores *of Clostridium perfringens and Bacillus anthracis.* Our results show the efficacy of SNPs coated (meso and dense) with TiO_2 to kill highly-grew cultures of planktonic pathogenic bacteria. Thus, the combination of noble metals (e.g., silver) and TiO_2 may enhance the photo-catalytic efficiency and so degradation of pathogen microorganisms. In addition, these SNPs also affect the viability of stability of the bacterial biofilm. Biofilms are surface-associated bacterial communities, in which bacteria are enveloped by polymeric substances known as the biofilm matrix. In the model organism *Bacillus subtilis,* biofilms display persistent resistance to liquid wetting and gas penetration which probably explains the broad-spectrum of resistance and tolerance of bacterial biofilms to antimicrobial agents. We analyze the ability of *B. subtilis*, and isogenic mutants affected in the synthesis of the extracellular matrix (ECM), to form biofilms in the presence of AgNPs. Our results show that silver nanoparticles have a greater inhibitory effect on biofilm development that the inhibition of biofilm formation produced by the germicide compound silver nitrate. In addition, it was observed that strains defective in the formation of particular ECM components (lipids, exopolysaccharide, etc.) differentially responded to the presence of AgNPs, suggesting a selective and exclusive effect of this novel nanomaterial on biofilm architecture.

Antimicrobial properties of copper in polyvinyl acetate and silicone nasal packs. An in vitro model of bacterial adhesion and survival

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Copper is the principal natural resource in Chile with several uses in the field of energy, construction and medicine, just to give some examples. This mineral has proven antimicrobial properties in biomedical materials.

For other hand, the use of Nasal packs as a therapeutic technique in otolaryngology is widespread and account for various indications. Among them, highlights their use in the management of episodes of epistaxis, by mechanical compression of the vessels supplying the nasal mucosa, and in the postoperative surgery sinuses or a rhinoseptoplasty, aiming to promote healing nasal mucosa.

Nasal packs are mostly made of polyvinyl acetate and silicone, producing complications associated to their use such as cacosmia and toxic shock syndrome, produced by *Staphylococcus aureus*, with high mortality rates.

Thereby the purpose of this study is to evaluate the antimicrobial properties of copper in a modified prototype of nasal packs with cooper, against bacteria of the nasal mucosa. An *in vitro* model was used with polyvinyl acetate and silicone nasal packs with and without copper, that were grown in a medium with strains of *methicillin-sensitive Staphylococcus aureus* (ATCC 29213) and *methicillin-resistant Staphylococcus aureus* (ATCC 43300).

There was a significant reduction of bacterial survival percentages in all modified nasal packs with copper, for both the culture medium inoculated with MSSA and MRSA, which ranged between 61% and 66% for the modified polyvinyl acetate nasal packs and between 78% and 86% for the modified silicone nasal packs, respectively. In conclusion, we suggest the incorporation of copper in the use of nasal packing, generating a wide investigating field about the use of copper in nasal packs and the decrease of infectious complications.

Key words: nasal packing, copper, bacteria, antimicrobial.

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Application of *oleuropein* for antimicrobial textile materials

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The olive plant has been an important source of nutrition and medicine of the Mediterranean. The first report for the medicinal use of olive leaf extract about its effective in treating fever and malaria was made in 1854 by Hanbury [1]. Olive leaf is one of the potent plant polyphenols having antioxidative, antimicrobial, antiinflammatory and antiviral properties due to its phenolic compounds, for example, oleuropein, hydroxytyrosol and rutin. The mostly abundant and bioactive components of olive leaf extract oleuropein (Figure 1), has been intensively studied for its promising effects on human health and its medical potential as antimicrobial [2]. In order to use this compound effectively in the industry, it should be extract from the olive leaf.

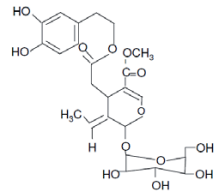

Figure 1. Oleuropein chemical structure

In this study it is aimed to design a textile product conserve oleuropein compound as a medical textile. Oleuropein was obtained from Medical Farm Ind. and used in different concentrations. With different cross-linker oleuropein was applied to cotton fabric by pad-dry-cure method. FTIR-ATR and SEM were used to examine the presence of the oleuropein on the cotton fabrics. The oleuropein applied cotton fabrics antimicrobial activity test was performed against both gram-positive and gram-negative bacteria. The durability of the antimicrobial activity of cotton fabrics was tested after 5 washing cycles at 40 °C for 30 min. Tensile strength tests of the fabrics were performed to evaluate the effect of oleuropein solution pH value changes.

Key Words: antimicrobial textiles, olive leaf extract, oleuropein.

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Bactericidal effect of encapsulated caprylic acid on *Listeria monocytogenes*

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Mesoporous silica particles (MSPs) are considered as promising vehicles for the controlled delivery of bioactive molecules from food systems [1]. Natural antimicrobial compounds such as free fatty acid could be encapsulated in this type of support in order to protect their antimicrobial activity against degradation processes. This encapsulation system has been recently reported, showing promising results as antimicrobial delivery supports [2, 3]. In the present study we proposed two different MSP (MCM-41 nano- and microparticle) for the encapsulation of caprylic acid (CA) to establish the minimum bactericidal concentration of the entrapped fatty acid against *Listeria monocytogenes*. Results show that entrapped fatty acid produced a reduction of microbial population in both encapsulation systems. The most effective encapsulated condition was found for nanoparticles being the minimum bactericidal concentration of CA encapsulated in this support in the range of 18.5 – 20 mM. Otherwise, CA encapsulated in microparticles produced a reduction of microbial growth, but this encapsulation system was not bactericidal for any of the studied concentrations. Furthermore, bacterial viability assay were done with unloaded MSPs to test the possible toxicity of the MSPs. Results show that unloaded MSPs did not affect the population counts of the microorganism. These results suggest that MSP supports are not toxic to bacteria and the effect of loaded solids is due to the fatty acid delivery. Therefore, the encapsulation process allows to maintain the antimicrobial activity of CA and could provide a new system to guarantee food safety in food industry using a more stable form of administration.

Keywords: caprylic acid; minimum bactericidal concentration; encapsulation; mesoporous silica particles

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Bactericidal efficiency of UV-active TiO_2 thin films on adhesion and viability of *Listeria monocytogenes* and *Pseudomonas fragi*

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Pathogenic bacteria present in biofilms are a major concern in food industries due to their high resistance to cleaning and sanitizing procedures [1]. The development of photoactive bactericidal surfaces, effective defense against the biofilm growth on cutting tools, unit fronts or worktops, could be an alternative. One solution is to deposit a well-known photocatalyst (TiO_2) top-layer on the conventional materials used in food plants. Our aim is to study the photocatalytic activity of such layers on the adhesion and the viability of two species encountered during food processes: *Listeria monocytogenes* and *Pseudomonas fragi.*

Glass substrates were coated with TiO_2 thin layers by radio-frequency magnetron sputtering under various deposition conditions (deposition temperature, oxygen partial pressure). Thin films were observed using scanning electron microscopy and analyzed by X-Ray Diffraction. Photocatalytic activity was evaluated by measuring the discoloration of methylene blue under UV-A light (365 nm). By controlling the deposition parameters, different TiO_2 thin films, amorphous and crystallized were elaborated. Only the anatase-based layers presented an efficient photocatalytic activity under UV-A and UV-C radiations. The TiO_2 thin layer with the most significant photocatalytic activity was selected for bactericidal activity tests. This activity was observed both on the reference and on the environment strains of *L. monocytogenes* and *P. fragi*. After a contact of three hours and a subsequent UV exposure, adherent bacteria were enumerated and *in-situ* fluorescent labeling was detected. Either the adherent *P. fragi* population or the adherent *L. monocytogenes* population decreased by 1 log on the TiO_2 thin layer illuminated by UV-A. This bactericidal activity was confirmed by *in-situ* labeling that showed dead cells with damaged walls.

A surface functionalization by a TiO_2 photoactive thin film helps to reduce the adhesion and the viability of food born bacteria. Nevertheless an optimization is required to shift this photo-assisted antibacterial activity towards visible light. A modification in the anionic composition would modify the thin film optical properties to this end [2]. On the other hand, to study the physiological response of bacteria in contact with these functionalized materials, the involvement of stress-related genes is under investigation.

Keywords: Photocatalysis ; TiO_2 ; *Listeria monocytogenes* ; *Pseudomonas fragi* ; oxidative stress.

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Biobased antibacterial finishing for textiles

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There is a constant need for the improvement of materials applied in textile industries. Technical and interior textiles, which are used under conditions of high humidity, are susceptible to bacteria or fungi. This applies to outdoor textiles, like tents, rucksacks, outdoor furniture, sunshades etc., as well as interior textiles used in a moist environment, such as kitchen and bath wear. Coating or impregnation of such textiles with biocides, which effectively prevent the growth of microorganisms on the textile surface, is therefore a common procedure. Conventional bactericides and fungicides both show disadvantages. On one hand microorganisms often develop resistance against biocides, especially when the mode of antimicrobial action is rather specific. On the other hand effective bactericides and fungicides, like chlorophenols or organo-tin compounds, are often not harmless to humans or the environment and therefore these substances are not suitable for textiles. Next to this there is a tendency for “bio, eco, natural and environmental friendly” consciousness of the consumer. As a consequence there is an urgent need for environmentally friendly antimicrobial substitutes.

The background of the research is to get an idea about the availability of biobased biocides for textile coating purposes, their applicability (with the existing technical equipment) and the properties obtained (compared with standard coatings and finishes). It is crucial for textile materials not to lose their mechanical properties and texture. Often alternative biocidal substances exhibit an excellent antimicrobial efficiency, but once applied the substrates become hard and lose their mechanical properties. Regarding the production of the finishing formulation the main efforts are focussed on waterbased solutions and dispersions. Different substances are considered as suitable candidates for antibacterial textile protection such as antibacterial substances derived from marine organisms (chitosan), coconut oil (monolaurin), essential oils, plant and herbal extracts (tannic acid and derivatives, carvacrol and thymol). The antibacterial efficiency of the finished textiles towards *Staphyllococcus aureus* and *Klebsiella pneumonia* was evaluated.

Keywords: biobased, antibacterial, textile, finishing, coating

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Characterization and mitigation of fungal growth on polymer coated building materials

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Pre-painted steel sheet, produced by coil coating, is a popular roofing material for home-owners in Australia. In most Australian environments, however, small melanised colonies (<100 μm^2) of microorganisms can be found on these roofs after less than a year in service. The dark colonies absorb incident infrared radiation, dramatically impacting on the thermal efficiency of the roof by reducing total solar reflectance (TSR). In addition, in areas where growth is stimulated, unsightly black staining, typical of sooty moulds, can be observed. In this paper, we describe the nature of the infestation and preliminary efforts to characterise the biological diversity and its spatial progression. Secondly, we discuss the requirements of the preventative or remediative strategies that may be employed to mitigate the growth.

Over 250 white, coil coated, steel samples (90 x 235 mm^2) were mounted at an outdoor exposure testing site in Burrawang (NSW, Australia), forming the basis of this study. The Burrawang site has historically shown incidence of microbial infestation on test samples. Exposure commenced in November 2012 with six standard gloss and three high gloss panels collected every 4 weeks for laboratory study using a combination of classical microbiological and analytical chemistry techniques.

The species involved and their temporal and spatial growth patterns were studied, with optical microscopy and image analysis employed for quantification and growth rate estimation. These measurements were correlated with TSR data obtained from UV-visible spectrometry. DNA sequencing and modern mass spectrometry techniques (employing novel extraction and ionisation methods to study lipid profiles) were applied to both individual colonies and biological material removed from whole panels. These techniques provided complementary information to identify the organisms present and elucidate the sequential colonisation of surfaces.

Results of the exposure experiment suggest a strong inverse correlation between TSR and microbial area coverage, which is also seasonally dependent with the most rapid growth rate observed in warmer months. Two distinct morpholgies, prevalent on panels exposed in Burrawang, are also seen elsewhere in Australia and South East Asia, and provide primary target organisms for study. DNA analysis of individual colonies with these morphologies has produced several plausible identities. Efforts are currently underway to correlate the lipid profile of these organisms to that of colonies removed from Burrawang panels.

Approaches to remediation must be compatible with coil coating; be retained; have long term activity; avoid toxic additives, due to the domestic application of collected run-off water; and finally, must not compromise aesthetic durability. To date, several (>25) commercial biocidal additives have been tested as additives or surface treatments but have been rejected because most are toxic and *none* have exhibited inhibition against these hardy organisms.

Keywords: microbial darkening, aesthetic deterioration of building products, airborne microflora, sooty moulds, total solar reflectance, pre-painted steel, lipid profile, DNA sequencing.

Covalent grafting of hyaluronic acid onto PMMA for antifouling applications

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The prevention of adhesion of bacteria on surfaces of materials is of crucial importance in diverse fields such as medical devices, health care, hospital and dental surgery equipment, textiles, ship hull fouling and water purification systems.1 Once adhered on a solid surface, bacteria form colonies and subsequently biofilms that can develop into pathogenic infections.1 Generally the polymers used in the medical device industry are hydrophobic and can result in the fouling or uptake of biological components that can then mediate a host of negative biological reactions. One of the most promising ways of preventing non-specific bioadhesion is by tethering superhydrophilic macromonomers onto solid surfaces. Hyaluronic acid (HA) is a naturally occurring glycosaminoglycan that is present ubiquitously in the extracellular matrix, vitreous humour, synovial fluid and cartilage and has the potential to provide non-fouling coatings on biological substrates if grafted in the correct conformation.3 In this study, HA was immobilised onto poly(methyl methacrylate) (PMMA) surfaces for the purposes of repelling protein, cellular and bacterial adhesion. Grafting was achieved by the following steps: treatment the surfaces with atmospheric pressure plasma processing, amination by self assembly of a 3-aminopropyltrimethoxysilane linker molecule and reaction of the surface bound amine with the carboxylic acid on HA using carbodiimide chemistry. The HA grafted PMMA surfaces showed a decrease in protein and cellular adhesion when tested with bovine serum albumin and human corneal epithelial cells, respectively. The ability of these coatings to resist bacterial adhesion was established using Staphylococcus aureus NTC8325. In this case the coatings did not repel bacterial adhesion, showing that the mechanism of adhesion of bacterial cells is different to that of mammalian cells. This indicates that conformation of the microstructure/architecture of the HA coatings is an important factor in fabricating surfaces intended to repel proteins, mammalian and bacterial cells.

Keywords: Polymer brushes; antifouling coatings; implant associated infection; hyaluronic acid

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Development of durable antimicrobial textiles for health care and sports applications using *N*-halamine Chemistry

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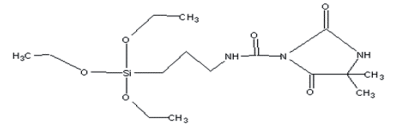
Textile fabrics, particularly those made from natural fibers, are susceptible to microorganism growth because of their hydrophilic nature. The use of antimicrobial agents in textiles has become an indispensable way to avoid cross-infection by pathogenic microorganisms. These agents are used on fabric, to control the infestation of microbes and arrest metabolism, in order to reduce odor and health hazards. Several antimicrobial agents have been used in the past. These include metal salt solutions such as $CuSO_4$ or $ZnSO_4$, and such organic compounds as zinc pyrithione (Zn (1-hydroxy-2-pyridinethione)$_2$).[1] Silver nanoparticles have been used on fabrics for bacteriostasis in many studies[2]. It has been found that an effective antimicrobial agent binds with protein molecules and inhibits cellular metabolism, consequently destroying microorganisms. In this process silver ions induce either contraction of the cytoplasm membrane or its detachment from the cell wall. As a consequence, DNA molecules become condensed and hence lose their ability to replicate upon the infiltration of silver ions. In a previous study[3], Amine end groups in PAMAM-G 3 Dendrimers were converted into quaternary ammonium salts using a synthesis procedure. Upon application on textiles, the modified Dendrimers exhibited strong biocidal activities. The cost of Dendrimers is prohibitive to the application on large surface areas of textiles. The current study explores *N*-halamine chemistry to create an effective antimicrobial textile. A heterocyclic amine such as 5, 5-Dimethylhydantoin (DMH) was used to create an N-halamine group using Sodium Hypochlorite (NaOCl). We used sol-gel chemistry to attach *DMH* to the fabric. *DMH* and γ-Isocynatopropyltriethoxysailane (CPTES) were used to create an adduct (Figure 1) and applied onto the fabric. Treated fabric was chlorinated using NaOCl to produce N-halamine groups. The treated fabric was tested against a number of Gram positive and Gram negative Bacteria using AATCC 100 procedure that exhibited strong biocidal activities even after 50 washing cycles.



γ –Isocyanatopropyltriethoxysilane - Dimethyl hydantoin adduct

Figure 1.

Keywords: Antimicrobial; textile fabric; microorganism

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Enzyme multilayer coatings inhibit quorum sensing-regulated *Pseudomonas aeruginosa* biofilm formation on silicone urinary catheters

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Bacteria use a signaling mechanism called quorum sensing (QS) to form complex communities of surface-attached cells known as biofilms. This protective mode of growth allows bacteria to resist antibiotic treatment and originates the majority of hospital-acquired infections. Emerging alternatives to control biofilm-associated infections and multidrug resistance development interfere with bacterial QS pathways, exerting less selective pressure on bacterial population. In this study, biologically stable coatings comprising the QS disrupting enzyme acylase were built on silicone urinary catheters using a layer-by-layer technique. This was achieved by the alternate deposition of negatively charged enzyme and positively charged polyethylenimine. The acylase-coated catheters efficiently quenched the QS in the biosensor strain *Chromobacterium violaceum* CV026, demonstrated by approximately 50 % inhibition of violacein production. These enzyme multilayer coatings significantly reduced the *Pseudomonas aeruginosa* biofilm formation under static and dynamic conditions in an *in vitro* catheterized bladder model. The quorum quenching enzyme coatings did not affect the viability of the human fibroblasts (BJ-5ta) over seven days, corresponding to the extended useful life of urinary catheters. Such enzyme-based approach could be an alternative to the conventional antibiotic treatment for prevention of biofilm-associated urinary tract infections.

Keywords: quorum sensing; quorum quenching enzymes; biofilms inhibition; urinary catheters

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Evaluation of the antimicrobial activity of Whey Protein Isolate emulsions and films against the autochthonous microbiota isolated from hake fresh fillets

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Currently, active edible films and coatings can be considered an innovative preservation technology to extend the shelf life of fresh products. Edible films and coatings incorporated with natural antimicrobial agents, such as plant essential oils, have been developed for reducing, inhibiting or stopping the growth of microorganisms in food surface.

In this study whey protein isolate (WPI), which forms flexible, odourless, tasteless and transparent films, was used as carrier of natural antimicrobials.

The antimicrobial activity of WPI emulsions and films incorporating Oregano (*Oreganum* vulgaris) and Thyme (*Thymus zygis*) essential oils (EOs) at three different concentrations (1, 3 and 5%) was evaluated against the autochthonous microbiota isolated from hake fresh fillets. Besides, O/W emulsions prepared with the same concentrations of EOs were evaluated and compared with the activity of WPI emulsions.

Hake microbiota was isolated from fillets packed under air and MAP conditions and stored ad 4°C for 8 and 15 days respectively. Isolation was done during days 0, 4, 8 and 15 of the storage period.

As it was expected, emulsions containing 3 and 5 % of EOs showed the higher percentage of inhibition regardless type of EO. WPI emulsions containing 5% of EOs inhibited almost the total growth of the isolated microbiota. On the other hand, WPI emulsions containing 1% of EO were capable of inhibit about 35 to 50% of bacterial growth. It was also observed that the WPI emulsions were more effective than the O/W ones, showing differences in inhibition around 20 to 50%, demonstrating the usefulness and effectiveness of WPI as carrier of natural preservatives.

Enterobacteriaceae, Mesophilic Aerobic and Lactic Acid Bacteria were the most sensitive bacteria to the action of WPI films, showing higher inhibitions zones. On the other hand, *Pseudomonas* spp. were the less sensitive. Same as before, the formulation containing 5% of EO was the most effective. In the case of the tested films, Thyme EO showed higher antibacterial activity than Oregano EO.

The obtained results supported previous researches which shown that WPI emulsions and films are a promising alternative for fresh fish preservation.

Key words: edible films; fish preservation

Fabrication of SELP/Ag nanocomposite materials with antimicrobial properties by electrospinning and solvent casting

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Natural structural proteins evolved in nature to fulfil structural and mechanical roles. Conservative blocks of amino acid sequences propagate through the natural protein to create elastic, rigid or tough materials such as elastin or silk. Advances in protein engineering combined with the use of recombinant DNA technology allow the design and production of recombinant Protein-Based Polymers (rPBPs) with an absolute control over its composition, sequence and length. This new class of protein-based materials, inspired in nature and with precisely controlled amino acid sequences, mimic the properties of their natural counterparts but can also display in the same polypeptide chain the properties of two or more different proteins. Silk-elastin-like proteins (SELPs) are a class of rPBPs which composition is based on silk and elastin repeating units [1]. Silver (Ag) is a metal with well-known antimicrobial activity against a broad spectrum of microorganisms [2].

In the present work, we report the fabrication of SELP/Ag antimicrobial materials by solvent casting and electrospinning techniques. For the production of films and fibers, pure lyophilized SELP-59-A (composition S5E9, where S represents the silk block and E the elastin block) was dissolved in water or formic acid with $AgNO_3$ at different concentrations (1, 3, 5 wt%). The produced materials were characterized by UV-Visible spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction and scanning electron microscopy analysis. The antimicrobial performance of the SELP/Ag materials was evaluated by disk diffusion assays against Gram+ and Gram– bacteria namely *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Both SELP/Ag fibre mats and films were effective against all the bacteria tested, independently of the solvent used; however, in comparison to the other microorganisms tested, the Gram-negative *Bacillus subtitlis* showed to be more resistant to the silver impregnated materials. Viability of normal human skin fibroblasts (BJ-5ta telomerase immortalized cell line) in the SELP/Ag materials was evaluated *in vitro* demonstrating that these materials do not show significant cytotoxicity.

These results thus suggest that SELP/Ag nanocomposite materials can be used as effective inhibitors of microorganisms growth, making them promising materials for medical applications.

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Keywords: silver nitrate; antimicrobial activity; silk-elastin-like proteins

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Facile immobilization of enzymes on electrospun nanofibrous membranes

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The worldwide water crisis can be attributed to a scarce supply of fresh water and increased pollution of the little there is available. One class of pollutant receiving much attention is endocrine disrupting compounds (*EDCs)*. Increased abundance of *EDCs* in fresh water systems is a cause for major concern. The wide spread use of pharmaceuticals and the extensive use of chemicals that mimic hormones and steroids leads to a buildup of these species in wastewater. This in turn has adverse effects on the well-being of marine life and ecosystems. Treatment of water contaminated with *EDCs* is difficult due to a lack of safe chemical remediations and conventional filtration processes are ineffective in removing such small compounds. Advances in the production of filtration membranes, made up of electrospun nanofibers, is set to address such difficulties in water purification. Electrospinning is a technique that allows for the manufacturing of nanofibers from polymer solutions or melts. This leads to non-woven fiber mats/membranes with a wide variety of surface functionalities and a very high surface to volume ratio. Not only can elecrospun nanofibrous membranes (*ENMs*) be used for filtration and the removal of very small pollutants in air and water, but tunable surface functionalities allow for site-specific surface modification. We would like to present work done in our group on the immobilization of enzymes on sub-micron electrospun nanofibers[1]. In this paper we describe the use of an electrospun functional copolymer *poly(styrene-alt-maleic anhydride)* for the enzyme immobilization of horseradish peroxidase (*HRP*) and glucose oxidase (*GOX*). The immobilized enzymes retained their activity and were used to perform a cascade reaction oxidizing *D-glucose* by *GOX* to yield H_2O_2 and employing *HRP* to catalyze the reaction between the generated H_2O_2 and *Ampliflu* Red to yield *Resorufin*. *ENMs* as an alternative for conventional filters is becoming increasingly more viable and in an extension of this investigation, preliminary results for the immobilization of commercial biocides, amylase and protease on *ENMs* has indicated the potential for producing fibers with inherently antimicrobial and anti-biofouling properties.

Keywords: enzymes; nanofibers; anti-biofouling; antimicrobial

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Fluorine activity of antibacterial ammonium hexafluorosilicate solution for the prevention of dental caries

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Ammonium hexafluorosilicate [**SiF**: $(NH_4)_2SiF_6$] was prepared in order to overcome the tooth discoloration caused by diamine silver fluoride [**AgF**: $(NH_3)_2AgF$] application. However, the antibacterial activity of SiF seems to be weaker than that of AgF due to silver having a high antibacterial activity. To increase the antibacterial activity of SiF for the prevention of dental caries, various antibacterial agents were added to SiF solution. The aim of this study was to evaluate the fluoride activity of the several antibacterial SiF solutions.

Four kinds of antibacterial SiF solutions were prepared with the addition to chlorhexidine (CHX), cetylpyridinium chloride (CPC), isopropyl methylphenol (IPMP), or epigallocatechin gallate (EGCG), respectively. Hydroxyapatite powder and pellets were treated with SiF solution with or without antibacterial agents for 3 min. And then, the pellets were immersed in demineralized solution for 24 hours. Demineralized depth of hydroxyapatite pellets after several SiF treatments were measured by surface roughness analyzer. Also, crystallinity of hydroxyapatite powder before and after several SiF treatment was measured with powder X-ray diffraction (XRD) analysis.

XRD analysis was shown that formation of calcium fluoride on hydroxyapatite surface was decreased with the addition of antibacterial agents to SiF solution. SiF+CPC solution showed equivalent acid resistance (demineralized depth) compared to SiF and AgF treatment. In contrast, the original acid resistance activity of SiF solution was diminished by the addition of other antibacterial agents (CHX, IPMP and EGCG). SiF with the addition of CPC was the most effective for reducing the demineralized depth, showing the same level as SiF and AgF, in contrast, the addition of other antibacterial agents to SiF reduced the original acid resistance activity of SiF solution.

It was concluded that the the addition of CPC to SiF solution was not reduced the fluorine activity of SiF solution, indicating that it may be useful for the prevention of dental caries.

Keywords: ammonium hexafluorosilicate;dental caries

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From mono-functional enzymatic coatings to bi-functional coatings to impair Staphylococci adhesion

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Despite the remarkable advances in modern healthcare, there are some drawbacks associated to the extended use of medical devices and biomaterial implants when microorganisms are able to reach their surface, forming biofilms and becoming the focus of biomaterial-associated infections (BAI) which are hardly to treat. The growing number of BAI has led to the need of developing novel antibacterial coatings for medical devices. The use of enzymes able to degrade biofilm matrix components, such as proteins and extracellular DNA, represents a promising approach to fight these infections.

The first aim of this study was to apply a biologically inspired strategy for covalent immobilization of different enzymes (lysozyme, proteinase K and DNAse I) on clinically relevant substrata (silicone) to obtain mono-functional coatings able to prevent staphylococci adhesion or to kill the adhered bacteria, depending on the enzyme used. The coating developed with the best anti-adhesive properties was afterwards combined with an antimicrobial peptide (colistin), generating a bi-functional coating.

Compounds immobilization was mediated by a polydopamine (pDA) coating and the anti-adhesive and antimicrobial performances of the generated surfaces were investigated for a clinical isolate of *Staphylococcus aureus* using fluorescence microscopy.

Results showed that unmodified silicone allowed the adhesion of bacteria without compromising their viability. Silicone modified with polydopamine coating had no significant effect on bacterial attachment and viability. Lysozyme immobilization was not able to reduce bacterial attachment or compromise their viability. On the other hand, proteinase K was able to reduce the percentage of bacterial attachment and a significant fraction of these adhered bacteria was found dead. Regarding the functionalization with DNAse I, these coatings presented the best anti-adhesive properties and since it is know that this enzyme is not cytotoxic, it was further combined with colistin and the bi-functional coating obtained proved to be more effective on reducing the fraction of bacterial attachment.

The overall results suggest that the use of coatings functionalized with enzymes is able to degrade biofilm matrix components and their conjugation with antimicrobial peptides presents a promising strategy for creating antibacterial surfaces to be applied in biomaterials for medical devices and implants.

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Keywords biofilm-dispersive enzymes; antibacterial coatings; biomaterial-associated infections; polydopamine

Human clinical testing of antimicrobial contact lenses

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Contact lenses offer an excellent alternative to spectacles to correct refractive errors. Indeed, they are superior to spectacles in many ways offering better peripheral vision and also the possibility of preventing the progression of refractive errors such as myopia (short-sightedness). However, wearing contact lenses can cause ocular inflammation and infection. If not treated quickly and with appropriate antibiotics, the infections can progress rapidly and lead to loss of vision, even the whole eye. Contact lens-induced inflammation and infection are most commonly caused by bacteria, initially adhering to contact lens surfaces. Thus, creating antimicrobial contact lenses has the potential to reduce these adverse responses and make contact lens wear safer.

Contact lenses were produced that were coated with two different forms of antimicrobials. In one case, the contact lenses were coated with an inhibitor of the quorum-sensing system of bacteria, a fimbrolide. In another case, lenses were coated with a chimeric cationic peptide, melimine. To demonstrate the broad spectrum antimicrobial properties of the coated lenses, they were incubated for 24 h with various microbes (*Pseudomonas aeruginosa (*incl. multi-resistant strains), *Staphylococcus aureus* (incl. MRSA), *Serratia marcescens*, *Candida albicans,* or *Acanthamoeba* sp. After incubation lenses were washed, and either stained and examined with microscopy or macerated and the numbers of colony forming units of microbes from antimicrobial-coated or control uncoated lenses were enumerated. After safety testing using standard *in vitro* and *in vivo* (animal) tests, the lenses were tested in 1-day trials using human subjects. Clinical and symptomatic responses were evaluated during and after wear.

The fimbrolide-coated lenses significantly reduced the adhesion of all microbial strains tested (between 1-4 log reduction depending on the microbial type). Fimbrolide-coated lenses were not toxic in *in vitro* or *in vivo* tests. Similarly, melimine-coated lenses gave significant reductions in microbial adhesion (2-4 log reduction). These lenses were also not toxic in *in vitro* and *in vivo* tests. In the 1 day human clinical trials, the antimicrobial-coated lenses showed similar responses in terms of ocular redness and conjunctival staining, as the control lenses. In the trial with the melimine-coated lenses only, there was significantly more corneal staining in 25% of subjects, but this was not associated with changes in symptoms during wear. However, both types of antimicrobial-coated lenses were associated with slightly, but significantly, worse symptoms during wear.

Short-term wear of antimicrobial-coated contact lenses was safe. The slightly worse comfort during wear is likely to be due to the processing of the lenses rather than the antimicrobials themselves. Further clinical trials are planned with subjects wearing lenses for 6 months on a 14-day lens replacement schedule.

Keywords: clinical trial; antimicrobial contact lenses

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Inhibition of *in vivo* microbial colonisation of biomaterials based on cationic peptide Melimine

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Biomaterials are used in a variety of medical devices and implants, such as catheters, prosthetic implants and contact lenses. The use of biomaterial implants and medical devices is an increasingly common and often life-saving procedure. However bacterial infections on biomaterials have emerged as a major problem. Implanted devices account for approximately 45% of all hospital-acquired infections and consequently represent a public health issue of major concern. Existing approaches for management of infection involving antibiotic treatment are ineffective for use with implanted devices. Often, the only treatment is surgical intervention by removing the infected device, which leads to high morbidity and mortality. The rapid emergence of bacterial resistance to current antibiotics adds further incentive to the need for development of alternative anti-infective strategies for implanted devices.

We have developed a cationic peptide “melimine”, with excellent broad-spectrum antimicrobial activity, that is not cytotoxic at active concentrations and is readily sterilisable. Furthermore, melimine is unusual compared to other AMPs in its ability to retain its activity when covalently attached to surfaces, making it ideal for development as potent antimicrobial coatings and therapies.

In this study we explored the ability of melimine to prevent *in vivo* bacterial adhesion and colonisation in rodent subcutaneous models when covalently tethered on titanium and polymer disks. The *in vitro* antimicrobial efficacy of the melimine-modified surfaces against model pathogens (*P. aeruginosa* and *S. aureus)* was first determined by fluorescence confocal microscopy using live/dead staining. For the mice model of biomaterial infection, the disks were implanted subcutaneously in the flank of mice and 10^5 to 10^7 colony forming units of *Staphylococcus aureus* were injected into the pocket. Clinical responses including wound area, swelling, and redness were recorded each day for 5 days. On the 5th day, the total viable bacteria on the disks *ex vivo* were enumerated.

Up to 6-fold reductions in in vitro bacterial adhesion were observed. *In vivo* efficacy of melimine-modified surfaces was demonstrated by up to 1 log reduction in viable bacteria compared with the controls. The starting concentration of melimine correlated positively with reduction in bacteiral numbers, with the highest reduction seen in animals with the highest concentration of inoculum at 10^7. Further evaluations with animal models using *Pseudomonas* are being examined and melimine-coated prototype devices will be tested at longer endpoints at 7 and 21 days.

Keywords cationic peptides; antimicrobial biomaterials, surface coatings

Multigradient porous surfaces for bacterial removal: role of the pore size and pore chemistry

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Gradient surfaces (GS) consist of interfaces in which a particular characteristic gradually varies as a function of their position between two extremes. Surface gradients are characterized by a different number of attributes that indicate: directionality (orthogonal, radial, triangular, etc), type (chemical, mechanical, topographical), dimensionality (1D, 2D, 3D), gradient length scale (narrow or broad) and time dependency (responsive, dynamic, etc.). Among the characteristics varied it is worth to mention, among others, the chemical composition, the topography or even the mechanical properties.

The interest in the preparation of gradient surfaces, which constitutes a particular case of surface patterning, relies on their potential uses. Gradient surfaces have been employed in combinatorial chemistry, in biomedical applications including the discovery of drugs, to analyze cell-substrate interaction phenomena, cell culture systems or to prepare biological devices by using microfluidic systems.

Typically, the methodologies explored to prepare gradient surfaces allow us to control either surface pattern or surface composition. Herein, we describe a straightforward and cheap alternative in order to control simultaneously chemical composition, size and shape of the porous surface. For this purpose we will employ the breath figures (BF) approach to create porous interfaces with variable average pore size and chemical composition. As will be depicted, the choice of the preparation conditions and, among others, the solvent employed are crucial to produce broad or narrow gradients. In addition, the use of blends of a polymer matrix (polystyrene in this particular case) and a block copolymer allow us to simultaneously vary the surface chemical composition.

Moreover, we will illustrate how the porous interfaces with variable pore size and chemical composition can direct the microorganism immobilization. In this study we will use a particular bacterial strain, Staphylococcus aureus, which is one of the most common pathogenic bacteria that cause disease in humans. In addition, S. aureus bacteria are spherical in shape with about 1 μm in diameter size, thus in the range of the pore dimension of surface and adequate to evaluate their surface immobilization as a function of the pore diameter. Therefore, we will evidence the role of the surface features on the retention of bacteria.

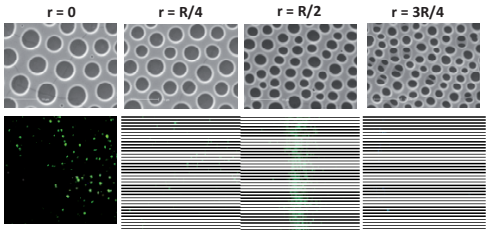

Figure 1. Interaction of the porous surface with fluorescent S. Aureus as a function of the pore size

Keywords: Polymer surfaces, honeycomb structures, Breath figures, biomolecular patterning, molecular recognition, bacterial adhesion.

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Photoinactivation with fullerenes

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Photoinactivation has been a useful platform for dealing with deleterious microbial populations and generally involves the use of photocatalysts to harness the energy from photons of light to produce reactive oxygen species (ROS) that can attack and inactivate microbes. Much research has gone into using a wide array of photocatalysts for these means, including conventional TiO_2 and nitrogen or noble metal doped TiO_2 photocatalysts, among other novel photocatalyst systems such as Cd-based quantum dots (QDs), platinized WO_3, and Bi_2WO_6. However, one material that has not been extensively studied for this use is fullerene. Upon excitation with light, fullerene readily photosensitizes $^1O_2$ when molecularly dispersed with quantum yields near 1.0 due to its highly efficient intersystem crossing and long excited triplet state lifetime when molecularly dispersed, as is the case in organic solvents (*ca*. 100 μs). Fullerene presents numerous advantages over other photocatalysts used for photoinactivation, including: 1) the production of mainly $^1O_2$ instead of hydroxyl radical species as observed in other photocatalysts, where $^1O_2$ is more selective and can be used in complex water matrixes to target select microbial populations; 2) fullerene and fullerene derivatives can be excited via visible light, which has plagued TiO_2 based photocatalyst systems; and 3) fullerene is composed of carbon and poses minimal risk to environmental exposure, compared to photocatalysts composed of toxic elements such as Cd-based QDs. However, obstacles arise when fullerene is placed in water, where fullerene molecules aggregate to form colloidal suspensions and lose much of their intrinsic photoactivity. In fact, this loss of much of fullerene's photoactivity in the aqueous phase is well documented and has been postulated to be due to increased fullerene-fullerene quenching leading to short triplet excited state lifetimes (picosecond range) and surface area limitations brought about by aggregation.

Herein, we present two cases where efficient $^1O_2$ photosensitization and photoinactivation are achieved using fullerene. In the first, a positively-charged fullerene derivative is used in aggregate form to inactivate MS2 bacteriophage and *E.coli* populations using visible light and sunlight excitation. The positively-charged fullerene colloids maintain long-lived triplet excited states in the aqueous phase and efficiently photosensitize $^1O_2$ in water, likely due to changes in aggregate characteristics as a result of inclusion of a positive charge onto fullerene's cage. MS2 inactivation was found to be the most rapid and efficient inactivation for any previously reported fullerene derivative and achieved close to six log inactivation in less than 2 min upon sunlight exposure. *E.coli* inactivation was considerably slower, likely a result of the susceptibility of the viral capsid to $^1O_2$ damage compared to the relatively robust cell membrane of *E.coli*. In the second case, $^1O_2$ photosensitization by fullerene is achieved via covalent immobilization of pristine C_{60} or C_{70} onto silica supports to prevent gross aggregation of fullerene molecules. Supported fullerene materials were found to efficiently inactivate MS2 bacteriophage under visible light illumination. C_{70} based materials exhibited enhanced photoinactivation compared to C_{60} materials, likely a result of C_{70}'s significantly larger visible light absorption and hence greater $^1O_2$ production. Our results presented herein indicate that fullerene holds great potential for photoinactivation processes.

Keywords: Fullerene; singlet oxygen; photoinactivation; visible light; photosensitize

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Preventing bacteria spread using the photodynamic effect in polymer-coated antimicrobial surfaces

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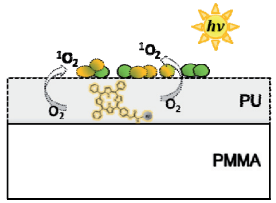
Rising resistance of bacteria against antibiotics requires new disinfection strategies. Especially in hospitals spread of bacteria via contaminated surfaces are to be controlled. A promising technique might be self-disinfecting coatings that are able to generate bactericidal reactive oxygen species (ROS) such as gaseous singlet oxygen ($^1O_2$) upon irradiation with light. Using this photodynamic approach we present coatings for many kinds of surfaces using a thin polymeric layer with embedded photosensitizer. The photodynamic procedure starts whenever ambient or artificial light is shining on such primed surfaces. As a result, singlet oxygen is generated, escapes from the surface by diffusion and may induce non-specific oxidative damage of attached bacteria (see figure).



In this work a plate of poly-methyl-methacrylate (PMMA) was coated with a 30 µm layer of polyurethane (PU) that contained an irreversibly polymerized derivative of the photosensitizer *meso*-Tetraphenylporphyrin (TPP). We investigated the photophysical and the photodynamical properties of that surface. The long-term photostability of the photosensitizer and its potential leakage from PU was analyzed with absorption spectroscopy. Singlet oxygen was generated by irradiating the probes with an OPO tunable laser at λ = 420 nm and was detected directly by spectral and time resolved single photon counting in the near-IR (1270 nm) using an infrared-sensitive photomultiplier. In order to investigate singlet oxygen escaping the PU-coating, a triiodide-assay was performed based on the reaction of potassium iodide with singlet oxygen. The photodynamic inactivation of bacteria on the coated surface was exemplarily shown using gram-positive *Staphylococcus aureus*. Therefore, a 50 µL water drop containing *S. aureus* (OD = 0.6, ~ 10^8 CFU per mL) was dried on the surface; this highly and artificially contaminated surface was then irradiated with incoherent light source (wavelength range 400 – 800 nm) and irradiation time and light dose were varied.

No leakage of the photosensitizer into water surrounding was detected and a loss of photosensitizer in EtOH was minimal, confirming the irreversibility of photosensitizer polymerization into PU. PU is gas permeable and a sufficient amount of oxygen reaches the photosensitizer. This enabled singlet oxygen generation that was directly proven by the detection of its luminescence at 1270 nm. The rising and decaying part of the luminescence signal were evaluated in order to estimate the diffusion properties of singlet oxygen in the coating material. The obviously low quenching rate constants of PU polymer yielded a lifetime of singlet oxygen that was longer as compared to pure water. Further, singlet oxygen diffusing from the surface into the surrounding was detected by the formation of triiodide in aqueous suspension. The escaping singlet oxygen was sufficient to enable an inactivation of bacteria of > 99.9 % (3 $\log_{10}$ steps) every 2 hours. This was achieved by an applied light dose of 3.6 J cm^{-2} (2 h irradiation time with 5 mW cm^{-2}) using a photosensitizer concentration of $2 \cdot 10^{-4}$ M in the material. Similar phototoxic results were gained using ambient light over a period of few days.

Using the photodynamic effect against bacteria with ambient light and the herein presented photodynamic coatings may help to reduce constantly the number of bacteria on surfaces. This is a preventive technique which may help to reduce the spread of diseases.

Keywords: photodynamic inactivation of bacteria; singlet oxygen; antimicrobial surface

Producing of electrospun nanofibers containing the antimicrobial peptide Cm-p1 as drug delivery system

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Candida albicans is a common human-pathogenic fungal species with ability to cause several diseases including surface infections [1]. Despite the clear difficulties of Candida control, antimicrobial peptides (AMPs) have emerged as alternative strategy for fungal control. In this study, different concentrations of Cm-p1 were electrospun in nanofibers for drug delivery. Nanofibers were characterized by mass spectrometry confirming the peptide presence on scaffold. Atomic force microscopy and scanning electronic microscopy were used for diameter measures and showed that the Cm-p1 affects fiber morphology and diameter and scaffold thickness. The release behavior Cm-p1 from nanofibers was evaluated and the nanofibers released peptide up to three days. Cm-p1 scaffolds presented less active than free peptide and also when compared with previous studies. Biocompatibility of scaffolds containing peptide was assessed though MTS assay and ROS generation by HUVEC cells showing that scaffolds do not affect cell viability in none concentrations evaluated; only scaffold containing 10 % Cm-p1 was able in significant ROS generation. In addition, the secretion of pro-inflammatory cytokines IL6 and TNF by HUVEC cells also was evaluated. As in ROS generation, only 10 % Cm-p1 nanofiber induced IL6 and TNF releasing. In conclusion, the electrospinning technique can be used as a tool in developing new systems for drug delivery incorporating drugs, proteins, peptides and growth factories.

Keywords: Antimicrobial peptides; nanofibers.

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Recognition and selective bacterial adhesion on porous polymer films

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The development of scaffolds with controlled surface properties and topography and the ability of patterning biomolecules on the surfaces have a great interest in fields such as protein adhesion, biosensors, cell attachment diagnostic tools development or tissue engineering. If we can combine a controlled topography and chemistry with specific biomolecules to provide functionality to the material surface we will have tools to generate sophisticated biomaterials for specific applications.

The breath figures (BF) approach has been proposed as a very simple and versatile method to fabricate micropatterned substrates. The depth and the pore size of the microporous films are factors which influence among others the attachment of cells to solid substrates, and these parameters can be modulated. Additionally, a wide variety of polymers can be used as precursors, including non-cytotoxic and biodegradable polymers. Herein, we present the development biopatterned surfaces by combining the controlled synthesis of honeycomb structures and precise biofunctionalization. We will describe the formation of honeycomb structures with pores enriched in carboxylic functional groups that can be functionalized in a further step. We present the application of a universal amine coupling method that allows us the functionalization of the surface pores with any peptidic molecule. Peptide sequences can mediate specific recognition processes that can be used in detection and diagnosis and additionally to pattern proteins and cells. Compared to previous reported systems we employed not only protein recognition sequences but also antimicrobial peptide sequences that allowed us to immobilize single bacteria, which have a similar size to the pores. As a consequence, this work supposes a significant improvement in the creation versatile patterned platforms that can be used for the display of active peptidic molecules and proteins, for sensing as reporters of different recognition processes and for cell array applications such as single cell experiments.

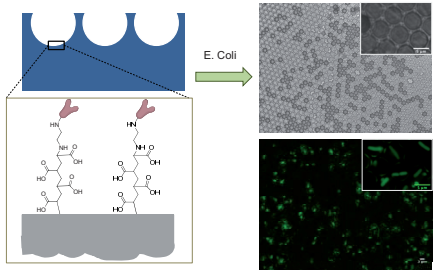

Figure 1. Interaction of the porous surface with fluorescent bacteria.

Keywords: Polymer surfaces, honeycomb structures, Breath figures, biomolecular patterning, molecular recognition, bacterial adhesion.

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Studies on biocidal properties of textile materials modified by silane compounds

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Textile materials containing natural fibres exposed to harmful external factors, e.g. high humidity, temperature and insufficient air circulation, require protection against microbiological deterioration caused by bacteria and especially by mould fungi, what leads to degradation of cellulose i.e. the main component of natural fibres. Endowing textile materials with biocidal properties is an element of their multifunctional character. The antimicrobial agents used for finishing of textile materials include for example quaternary ammonium compounds, silver and gold nanoparticles, triclosan and silane compounds [1], [2], [3].

The paper presents the research conducted to increase the resistance of cotton fabric modified by silanes compounds to action of mould fungi. Tetraethoxysilane with different quaternary ammonium salts modifiers, silver ions and triclosan were used for modification of cotton fabric. The modified fabrics were tested for action of a mixture of 5 mould strains, which most often cause decomposition of cellulose (*Chaetomium sp., Aureobasidium sp., Paecilomyces sp., Aspergillus sp., Penicillium sp.*). Evaluation of antifungal effect was done by determining the degree of mould fungi growth on the surface of tested fabric samples, the growth inhibition zone around the samples and the change of breaking force. Microscopic evaluation of the tested fabrics was also made with the use of Scanning Electron Microscope. Promising results were obtained for cotton fabric modified by tetraethoxysilane with quaternary ammonium salts modifier labelled as M8, and tetraethoxysilane with triclosan and silver ions, where no mould fungi growth was observed on the tested fabric surface, and the growth on agar medium around the sample was inhibited. The values of tensile strength for cotton fabric modified by silanes used in the studies were also very good.

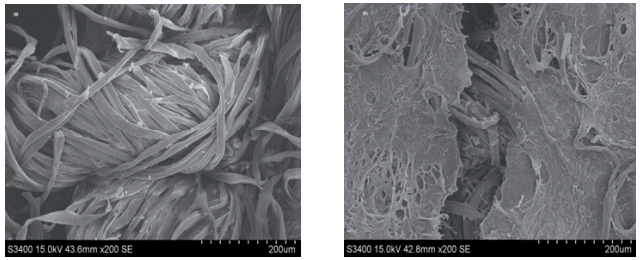

Figure 1: SEM images - Cotton fabric modified by tetraethoxysilane with silver ions (a) and reference sample (b) after mould fungi action

The achieved results will enable to continue the studies further in order to determine the washing durability of cotton fabric modified by selected silane compounds and to confirm their biocidal properties.

Keywords: biocidal properties; silane compounds; textile materials

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Study of molecular parameters of polysaccharide layer on surface antiadhesive properties

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It is now accepted that microbial populations use cell attachment to solid supports to colonize and survive forming structured communities called biofilms [1]. Recently, we elaborated antiadhesive surfaces based on a natural polysaccharides layer [2] allowing prevention of the initial bacterial adhesion on surfaces. Even if some key parameters important for chemical surface modification are well known, e.g., hydrophilicity, roughness, layer thickness or molecular weight and charge of the immobilized molecule [3-5], those governing the antiadhesive properties of covalent polysaccharide layer are yet not well defined.

In the present work, we studied the relationship between molecular parameters of the polysaccharide layer and its ability to inhibit the bacterial adhesion. To this aim, polysaccharides with various chemical characteristics were covalently grafted on silicon surfaces, previously modified by a self assembled monolayer (SAM) of aminoundecytrimethoxysilane (AUTMS).

The parameters controlling the bacterial repulsion, i.e., surface wettability, roughness and chemical composition, were characterized by contact angle, atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS), respectively. The antiadhesive properties of the modified surfaces were then evaluated against *Staphylococcus aureus*, one of the major pathogen involved in biofilm contaminations.

Keywords: Biofilms; Adhesion; Polysaccharides; *Staphylococcus aureus*

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Synthesis and antibacterial properties of some new fluorine containing nitrofurans

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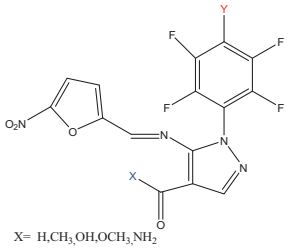
Nitrofurans (***NF***) are synthetic chemotherapeutic agents with a broad antimicrobial spectrum[1]; they are active against both Gram-positive and Gram-negative bacteria, including *Salmonella* and *Giardia spp*, trichomonads, amebae, and some coccidial species. The nitrofurans appear to inhibit a number of microbial enzyme systems, including those involved in carbohydrate metabolism, and they also block the initiation of translation. However, their basic mechanism of action has not yet been clarified. Their primary action is bacteriostatic, but at high doses they are also bactericidal. They are much more active in acidic environments (pH 5.5 is optimal for ***NF*** activity).

Enenitriles such as enolethers derived from malononitrile, cyanoacetone or cyanoacetates, respectively are attractive building blocks for the synthesis of biologically relevant heterocyclic compounds[2]. In recent years, fluorinated heterocycles find an important place in the manufacture of drugs. Perfluorophenyl pyrazoles can be efficiently prepared by reaction of enenitriles with perfluorophenylhydrazine. We report here a simple method for preparing a set of new 5-nitro-furfurylidene derivatives (***5NFPFPP***) by reacting a series of 1-(5-amino-1-pentafluorophenyl-1H-pyrazol-4-yl)-carbonyl compound with 5-nitro-2-furaldehyde derivatives. Incorporation of fluorine can alter the couse of the reaction as well as biological activities.



X= H,CH_3,OH,OCH_3,NH_2
Y= H, N_3, NH_2, NHR,N=PPh_3,N=nitrofurfurylidene

5NFPFPP

Nucleophilic displacement of a single fluorine atom in pentafluorophenyl group by azide anion affords new possibilities for cross linking, colour incorporating, efficient photosensitive dyes and photolabeling. The procedure represents a novel, as yet unpublished synthesis of ***5NFPFPP*** derivatives. The target compounds can be used as building blocks for novel materials with optoelectronic properties, OLED materials, fotolabeling reagents or as photosensitizers and excellent antibacterials.

This work was financially supported by grants from the Ministry of Education of the Slovak Republic No. 1/0829/14, and APVV-0038-11

Keywords: Nitrofurans; Pyrazoles; Antibacterial agents; Biological activity;

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The influence of nanosilver incorporated into the surface of packaging on the quality and storage of cut gerberas (cultivar 'Kimsey')

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During the time of transport and storage different factors influence on the quality of flowers. Several aspects such as environmental conditions (such as temperature, humidity), pathogens presence (fungi and bacteria), mechanical damages have an impact on the shelf life of flowers. All of them are correlated, and for example high humidity can promote the increase of bacteria growth. To reduce the influence of external factors and to protect flowers against loosing of quality (what causes huge losses, even 15-20% before flowers are put on the market) some solutions have been developed. The most important is packaging, that can protect flowers and also extend the shelf life. The nanosilver is well-known because of its antimicrobial activity, but in the case of plant application it is mostly used as additive to vase water to extend the shelf life of flowers [1].

This study focuses on the evaluation of the influence of nanosilver ions, that have been incorporated in the active coating applied on corrugated boxboard surface (EkoPak Plus, Poland), on the shelf life of gerbera - cultivar 'Kimsey'. This research work includes storage tests, the evaluation of quality of gerberas after taking off from the packaging, and the assessment of the vase life of flowers after contact with nanosilver ions during storage. To evaluate the quality of gerberas two–steps assessments has been performed:

1) Visual evaluation (including microscopic analysis), control of weight and microbiological tests to control the nanosilver effect;

2) Vase life assessment, visual evaluation (including microscopic analysis), control of the weight and the usage of the water (important parameter to test the conductivity of vessels, that can be chopped by bacteria), and microbiological analysis (total number of bacteria, and identification of pathogens, if there were any).

After 1 week of storage at cool conditions and into the packaging with nanosilver coating the reduction of bacteria amount has been noticed (approx. 70% in the comparison to the references). The same effect has been noticed during the assessment of the vase life of cut flowers.

Keywords: nanosilver, packaging, cut flowers

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The toxicity of the fluorides in oral hygiene products

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Many studies have shown antimicrobial and caries preventive effects of fluorides applied directly to the tooth surface. Fluorides are commonly added in oral hygiene products. According to the World Health Organization, today fluoride toothpastes are used by over 500 million people in the world. The most common caries preventive compound of toothpaste are fluorides.

Although, acute or chronic poisoning from fluorides in oral hygiene products is unlikely, it is necessary to draw attention to the existing threat. Toothpastes designed for use in adults, usually contain 1450ppm fluoride or 0.32% of sodium fluoride. Toothpastes designed for use in children of 6 to 12 years, usually contain 1000ppm fluoride, or 0.2% of sodium fluoride. Toothpastes designed for use in children younger than 6 years, usually contain 500ppm fluoride, or 0.1% of sodium fluoride. Fluoride is often added to the mouthwash usually containing sodium fluoride at concentration of 0.02 to 0.05%. A mouthwash is intended for use in adults only. The lethal dose for man is from 32 to 64 milligrams of fluorine per kilogram of body weight. A lethal dose of sodium fluoride is 5 to 10 grams for men weighting 70kg. In children even a half of a gram of sodium fluoride can be fatal. In 100 grams of toothpaste for adults is 0.32 grams of sodium fluoride. So, for a man who weights 70kg lethal dose of sodium fluoride is contained in about 1500 grams of toothpaste for adults. For a child who weights 20kg lethal dose of sodium fluoride is contained in about 440 grams of toothpaste for adults, ie from 700 to 1400 grams of toothpaste for children. Mouthwashes are usually packaged in vials of 200-500ml size and containing from 0.1 to 0.25 grams of sodium fluoride in the packaging. Thus, for a man who weights 70kg lethal dose of sodium fluoride is contained in 20 vials per 500ml of mouthwash. For a child who weights 20kg lethal dose of sodium fluoride is contained in about 5.5 bottles per 500ml of mouthwash.

The victims of fluoride poisoning from oral hygiene products are, mostly, children. It is necessary to keep the oral hygiene products out of the reach of children. When your child is brushing teeth, it is necessary to adult to put a proper amount of toothpaste on the toothbrush, and the tube with the rest of the paste immediately return to the place beyond the child's reach. It is not recommended that a household have stock (more tubes or bottles) of oral hygiene products. The same measures are also needed for people with developmental disabilities and in some psychiatric patients. For the prevention of chronic poisoning, it is important that among the available modes of fluoridation (endogenous, professional topical, fluoridated toothpaste) select and implement only one.

Keywords: fluoride; toothpaste; toxicity; prevention

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Zinc oxide as an alternative to conventional preservatives: antimicrobial properties and use in cosmetic products

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Introduction: Cosmetic products must be kept free of microorganisms to ensure the safety of their users. Several authorized preservatives taken in the Annex V of the European regulation 1223/2009 are usually added to the products so as to keep their microbiological quality during their shelf life. Nevertheless, the consumers but also the scientific community worry about the use of antimicrobial preservatives in cosmetics since few years because they are afraid of possible side effects. According to this context, alternatives to conventional preservatives are searched for. Inorganic antimicrobial agents are such promising alternatives and zinc oxide (ZnO) appears of major importance owing to its high antimicrobial activity. Besides, ZnO is a safe material that is already used in sunscreens as UV filter. So as to improve the prospect of using ZnO as a preservative in cosmetics, the antimicrobial efficacy as well as the antimicrobial mechanisms of ZnO were studied, and the antimicrobial potential of ZnO in various cosmetics was evaluated. All these studies were performed on the five germs of the Challenge Tests (Ph.Eur). This article is focused on a single bacterial strain: *Staphylococcus aureus*.

Methods: Four ZnO grades were selected according to their physicochemical characteristics. Microbiological tests were carried out in liquid media (MH broth, AES Chemunex) and MIC and MBC of each ZnO grade were determined against *S. aureus* CIP 4.83. Specific studies were carried out in order to investigate the contribution of each antimicrobial mechanism of ZnO particles on their global antibacterial efficiency:
- Direct contact of ZnO to bacterial cells: the interaction of ZnO particles to cell walls was evaluated by zeta potential measurements on bacterial cell suspensions;
- Generation of zinc ions: the release of zinc ions by dissolution of ZnO particles was measured by HPLC coupled to UV detection and the antimicrobial efficiency of Zn^{2+} was evaluated;
- Generation of reactive oxygen species (ROS): the generated H_2O_2 was quantified with an OxiSelect^{TM} *In Vitro* ROS/RNS assay kit (CellBiolabs) depending on the chemical and physical environment. The influences of the light conditions as well as the nature of the aqueous media were evaluated.
Lastly, the antibacterial effectiveness of ZnO against *S. aureus* in three types of cosmetic formulations (oil-in-water emulsion, colored water-in-oil emulsion, and compact powder) was assessed according to Challenge Tests of the standard ISO 11930.

Results and discussion: Microbiological tests revealed that the tested ZnO grades exhibited a bactericidal activity against *S. aureus*. The MIC was 0.25% and the MBC was 1.72% (w/w) for the most efficient ZnO grade [1]. Antimicrobial mechanisms were studied so as to improve the understanding of each mechanism and get an overview of their respective contribution to the global efficiency of ZnO particles. Such fundamental data help for the optimization of the efficacy of ZnO in complex formulations. *S. aureus* was mostly sensitive to the direct contact of ZnO particles to the cells walls. Zeta potential measurements showed that it was governed by an electrostatic attraction phenomenon. Moreover, the irradiation of ZnO suspensions by UV light enhanced the antibacterial efficacy of the particles *via* the production of ROS. Zinc ions also contributed to the global antibacterial efficacy of the particles; the dissolution of ZnO appeared dependent to the environmental conditions. Finally, the influence of ZnO on the microbiological quality of the three cosmetic products was definitely demonstrated since the A criteria was met on *S. aureus* in the presence of 1% of ZnO while ZnO-free products did not comply with A or B criteria. As conclusion, *S. aureus* was highly sensitive to the antibacterial activity of ZnO particles in complex formulations, which opens the way to the application of ZnO as an antimicrobial agent into such products.

Keywords: Zinc Oxide; Antimicrobial activity; Antimicrobial mechanisms; Cosmetic formulations

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Antimicrobial chemistry

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A facile one-pot green synthesis and antibacterial activity of some new polyfunctionalized 2-amino-4*H*-pyrans

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The reaction of cyclic CH-acids such as 4-hydroxy-6-methyl-2*H*-pyran-2-one, 4-hydroxycumarine, 1,3-cyclohexanedione, or 5,5-dimethyl-1,3-cyclohexanedione, with dialkyl acetylenedicarboxylates in the presence of tosylmethylisocyanide (TosMIC) in polyethylene glycol (PEG-300) lead to corresponding novel desired highly functionalized 2-amino-4*H*-pyrans at room temperature in good yields within 1 hour. All compounds are tested against Gram positive and Gram negative bacteria and results showed that compounds inhibited bacterial growth even resistance isolates.

Keywords CH-acid, TosMIC, polyethylene glycol, antibacterial activity, trimetoprimsulfametoxazole.

Antimicrobial activity and cytotoxicity of novel eugenol derivatives

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Eugenol (4-allyl-2-metoxy-phenol), the major phenolic component of clove essencial oil, has been largely used in medical and dental practice for its analgesic, local anesthetic, anti-inflammatory, anti-oxidant, antibactericidal and antifungicidal properties. It is well known that eugenol can denature proteins and react with cell membrane phospholipids changing their permeability and inhibiting a great number of Gram-negative and Gram-positive bacteria as well as different types of yeast. This compound has ever proven antimicrobial properties; thus, the search of eugenol derivatives for the optimization of its property through structural changes appears to be interesting for the development of new antimicrobials. This study aimed to evaluate the antimicrobial activity and cytotoxic characteristics of eugenol analogues. From natural eugenol, fourteen derivatives were obtained by typical acylation and alkylation. Their antimicrobial activity was evaluated by the broth microdilution method. The compounds were assessed against *Staphylococcus aureus* ATCC 19095, *Enterococcus faecalis* ATCC 4083, *Escherichia coli* ATCC29214, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 62342 and the following clinical isolates from the human oral cavity: *C. albicans* (3), *C. parapsilosis, C. glabrata, C. lipolytica* and *C. famata*. Cytotoxicity against mouse embryonic fibroblast (NIH/3T3) cell line was evaluated with the MTT colorimetric assay. The antimicrobial effect was characterized by the IC_{50}, which is the concentration that induces 50% inhibition of bacterial/fungal growth relative to the growth control, and the MIC, which is the lowest concentration of the substance required for complete inhibition of the bacterial and fungal growth after incubation time. IC_{50} values were determined from logarithmic graphs of growth inhibition versus concentration. The compounds showed IC_{50} values ranging from 30 to >500 μg mL^{-1} and MIC and MMC values ranging from 62.5 to >500 μg mL^{-1} against fungal strains and IC_{50} values ranging from 1.92 to >500 μg mL^{-1} and MIC and MMC values ranging from 125 to >500 μg mL^{-1} against bacterial strains. In general, the derivative compounds presented very low or no cytotoxicity ($p>0.05$), with absorbance values similar to those presented by the control group. These results demonstrate the potential of eugenol derivatives to selectively induce antimicrobial activity in the strains tested without promoting cytotoxicity in normal cells.

Keywords: antimicrobial activity; cytotoxicity; eugenol; *in vitro* assays

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Antimicrobial activity of newly synthesized indolizidines

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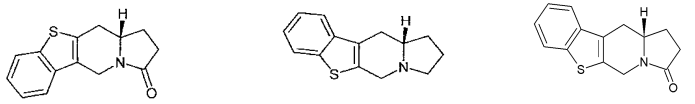
The treatment of microbial diseases is an important and challenging worldwide problem. In spite of a large number of available antibiotics and chemotherapeutics for medical use, at the same time the emergence of microbial resistance revealed a substantial medical need for the development of new classes of antimicrobial agents. Indolizines, the nitrogen containing heterocyclic systems, are widely distributed in the nature. The synthesis of biologically active indolizine derivatives continues to attract the attention of organic chemists, because of their importance as potent pharmaceutical drugs due their wide spectra of biological activities. Owing to the increasing importance of indolizine heterocycles in the field of biology and pharmacology, 21 novel indolizine derivatives was synthesized and subjected to antibacterial and antifungal screening studies against *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Salmonella* Typhimurium and *Escherichia coli*, *Candida parapsilosis, Botrytis cinerea, Alternaria alternata* and *Microsporum gypseum.* Antimicrobial activity *in vitro* was assayed by dilution method. The assessments of antibacterial and antifungal activities were expressed as the concentration of the derivative that inhibits the growth of bacteria on 50% (IC_{50}) and MIC values that are representing the minimal concentration, that fully inhibits the microbial growth (on 100%). The IC_{50} and MIC values were evaluated from toxicity curves. Chromatographically pure compounds were dissolved in dimethylsulfoxide (DMSO); its final concentration never exceeded 1.0% vol. either in control or treated samples. Assessment of mutagenicity and antimutagenicity was performed using classical plate incorporation method (Ames test without metabolic activation) using *Salmonella* Typhimurium TA 98 and TA 100 (antimutagenicity was assayed only on *Salmonella* Typhimurium TA 98). As positive mutagen 3-(5-nitro-2-furyl) acrylic acid (NFAA) was used. Positive response was defined as a reproducible two fold increase of revertans with dose response relationship and statistical evaluation using the *t*-test. The best growth inhibition of model bacteria was observed in the presence of derivative **20** and **21** (Fig.1). The growth of *S. aureus* and *M. smegmatis* was fully inhibited at the concentration of 25 μg.mL^{-1} with bacteristatic effect on the cells. The IC_{50} values were noticed at the concentration of 12 μg.mL^{-1}. These compounds also inhibited the growth of the gamma proteobacteria *E. coli* (MIC_{20}= 400 μg . mL^{-1} MIC_{21}= 100 μg. mL^{-1}; bacterisatic effect on cells). Concerning the antifungal activity of our studied set of derivatives, only the derivative **13** has shown an antifungal effect on model yeast *C. parapsilosis* (MIC = 200 μg . mL^{-1}). The growth of model filamentous fungi was inhibited by several derivatives with comparable effect on the growth, but the best activity was obsereved in the presence of derivative **13.** Significant morphological changes of *B. cinerea* accompanied with partial growth inhibition were also observed. The effect of increased ramification of the hyphal tips of *B. cinerea* was occurred in the presence of the compound **13.** Finally, derivatives were tested for the potential mutagenic activity. Based on our obtained data we can conclude that the newly synthesized derivatives have not increased the number of revertants of *S.*Typhimurium TA 98 nor TA 100. It means that they do not induce point and frameshift mutations at any tested concentrations and are considered to be non-mutagenic. Some of newly synthesized derivatives had shown antimutagenic activity, that means the decrease of induced revertants by NFAA was observed.



derivative 20 derivative 21 derivative13

Fig. 1 Structure of derivatives with the best antimicrobial activity

Keywords: indolizine derivatives, antimicrobial activity, antimutagenic activity

Antituberculotic and cytotoxic properties of new hydrazone derivatives

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Mycobacterium tuberculosis is the persistent bacterium that causes one of the major public health problem, tuberculosis (TB) [1]. The pathogen primarily affects lungs (as pulmonary TB), subsequently the brain and meninges, lymph nodes, bone and joints, the genitourinary system and the intestine and liver [2]. Due to its special structural feature which is including thick, lipid-rich cell wall, it is hardly permeable for chemotherapeutic compounds [3]. In addition to strong characteristic profile of the microorganism, occurrence of resistance to currently available drugs and laborious, lengthy treatment were prompted medicinal chemists to design and synthesize new compounds with potential antituberculer activity [4]. Accordingly, we synthesized and investigated a series of *N'*-(arylidene)-4-[(pyrimidine-2-yl)thio]butanohydrazide derivatives (**3a-3m**) considering the reported data about the antimycobacterial activity of pyrimidine ring [5] and hydrazone moiety [6]. Thereafter we investigate their potential antituberculotic and cytotoxic activity. The antitubercular activities of the newly synthesized compounds were tested against *Mycobacterium tuberculosis* H37Rv strain by MABA (Microplate Alamar Blue Assay) method and cytotoxic properties were tested against NIH/3T3 and A549 cell lines by MTT method. The title compounds have exhibited moderate antitubercular activity. Compound **3m** including *para* nitro substituted phenyl moiety showed promising cytotoxic activity when compared with standard drug.

Keywords: pyrimidine; hydrazone; alkylsulfanyl, antituberculotic, cytotoxic

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Application of photochromism to the molecular design of antimicrobial agents: synthesis of phenolic derivatives and their bactericidal activity based on a photo-reaction with ultraviolet-A light

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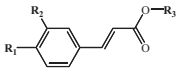
Photo-isomerization of cinnamic acid, a phenolic acid compound, arises from photochromism following irradiation with ultraviolet (UV) light [1]. The *trans*-form is predominant in plants due to its stability, but the *cis*-isomer exhibits higher physiological activity. The *cis*-isomer provided a growth inhibitory effect against a multidrug resistant mycobacterium at 1/200th the concentration required of the *trans*-isomer [1]. In addition, anti-invasive activity of the *cis*-isomer was demonstrated against human lung adenocarcinoma cells [2]. Recently, the expected high biofunctionality of the *cis*-form of phenolic acids has prompted efforts to directly synthesize diastereomerically-pure *cis*-phenolic acids, followed by modification of the substituent groups [3].

We explored the design of antimicrobial agents incorporating a photo-isomerizable moiety. Specifically, our intent was to generate improved bactericidal compounds by a UV-A-triggered photo-reaction. *p*-Coumaric acid (CA) [4] and its analogue, ferulic acid (FA), were used as UV-A-isomerizable moieties. The carboxyl group was modified with methyl, *n*-butyl or phenyl groups to increase the hydrophobicity, and the hydroxyl group was reacted with *L*-serine trifluoroacetic acid (TFA) salt to accelerate adsorption onto the bacterial membrane. The effect of the modification of *L*-serine TFA was compared to CA and FA derivatives protected with an acetyl group at the same position. The bactericidal activities of phenolic derivatives derived from *trans*-CA and *trans*-FA, shown in Table 1, were evaluated by triggering their isomerization during the assay using UV-A light.

CA and FA were esterified with a methyl or *n*-butyl group by dissolving in methanol or *n*-butanol containing a catalytic amount of sulfuric acid. A phenyl group was installed at the carboxylic acid *via* the acid chloride intermediate. The ester was conjugated with *L*-Ser using a carbodiimide reagent. The synthesized derivatives were consistent with the target structures and masses, as determined by NMR and LC/MS. Eight derivatives (Table 1) were tested in bactericidal assays using *Escherichia coli* NBRC12713. The samples, containing 100 μM derivative, were illuminated with 8.6 J/ cm^{-2} UV-A from a UV-A light-emitting diode (350 to 385 nm, λ_{max} = 365 nm), or were kept in the dark, then the number of surviving bacteria was determined using a colony-forming assay. Compounds **4c**, **10** and **12** reduced survival by approximately 3.5-logs, whereas the reduction following exposure to UV-A in the absence of any derivative was approximately 1.8-logs. Compound **12**, binding *L*-Ser to a FA derivative, resulted in a remarkable increase in activity compared with the corresponding derivative protected with an acetyl group (compound **9**). In the absence of UV-A irradiation, none of the compounds exhibited significant bactericidal activity, indicating that an increase in bactericidal activity was dependent on both an oxidative reaction [5] and isomerization by UV-A irradiation. *Trans*-**4c** and **-10** were isomerized to the *cis*-form in 35% and 50% yield, respectively, using a UV-A dose of 8.6 J cm^{-2}. Isomerization increased the bactericidal activity of each derivative. However, **12** did not isomerize, suggesting that another photo-reaction was responsible for its high antimicrobial activity, such as the generation of reactive oxygen species by energy transfer or by electoron transfer reaction initiated by UV-A light.

Keywords: antimicrobial agents; phenolic acid derivatives; photo-isomerization; ultraviolet-A

Table 1. Structures of the synthesized phenolic acid derivatives



	Phenolic acid derivatives							
	3	**4c**	**6a**	**6b**	**6c**	**9**	**10**	**12**
R_1	AcO	HO	NH_2(TFA)SerCOO			AcO	HO	NH_2(TFA)SerCOO
R_2	H	H	H	H	H	CH_3O	CH_3O	CH_3O
R_3	Ph	Ph	Me	*n*-Bu	Ph	Ph	Ph	Ph

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Changes in tularemia progression due to melatonin in a BALB/c mouse model

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Melatonin is a hormone with antioxidant properties. In the body, melatonin is involved in regulation of circadian biological rhythm. However, receptors for melatonin are expressed on disparate organs and they can be found on immune cells as well. The present experiment is focused on research whether melatonin would regulate pathogenesis caused by a model intracellular pathogen, *Francisella tularensis*. For the reason, laboratory mice BALB/c were infected with *F. tularensis*. Melatonin was given in two doses: 10 and 100 μg/kg. Animals were sacrificed after either three or five days. Spleen and liver were sampled for bacterial burden. Interferon gamma (IFN-γ), interleukin 2 (IL-2) and total immunoglobulins were assayed from plasma samples. We proved that melatonin is able to reduce bacterial burden in the organs in a dose response manner. Surprisingly, IFN-γ and IL-6 levels were reduced as well. Immunoglobulins remained unchanged. We conclude our experiment that melatonin is potent to reduce tularemia progression. We infer that the effect of melatonin lay in regulation of cell cycle rather than immunity regulation.

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Design and synthesis of antimicrobial cyclic lipopeptides

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The vulnerability of modern agriculture to diseases and pests has resulted in an intense research effort to develop new antimicrobial agents that are safe for the host organism and the environment, and that are unlikely to cause the emergence of resistant strains. Due to their broad spectrum of activity, low intrinsic cytotoxicity, and unique mechanism of action, antimicrobial peptides are considered to be suitable candidates for use in plant protection [1]. A subfamily of antimicrobial peptides that has emerged in recent years includes lipopeptides [2]. They consist of a linear or cyclic peptide sequence, either cationic or anionic to which a fatty acid moiety is covalently attached. Lipopeptides have been shown to exhibit significant antibacterial and antifungal activity [3]. Moreover, acylation of synthetic or natural antimicrobial peptides with fatty acids has been demonstrated to be a useful strategy to improve their antimicrobial activity. In fact, it has been reported that the presence of an acyl chain enhaces peptide-membrane interactions and it has been attributed to the changes in peptide overall hydrophobicity and organization in membranes [4]. In addition, lipopeptides have also been described to be active against plant pathogens. For instance, natural cyclic lipopeptides isolated from *Bacillus* strains have well-recognized antimicrobial activity against phytopathogenic microorganisms [5].

Within our efforts of finding new agents to control plant pathogens, in a previous study we designed and synthesized a library of cyclic decapeptides with general structure c(X_5-Phe-X_3-Gln) where X is Lys or Leu [6]. From this library we identified c(Lys-Lys-Leu-Lys-Lys-Phe-Lys-Lys-Leu-Gln) (**BPC194**) with high activity against the plant pathogenic bacteria *Erwinia amylovora*, *Xanthomonas vesicatoria* and *Pseudomonas syringae*. This peptide also exhibited low hemolysis. The antimicrobial activity of **BPC194** and the excellent properties described for lipopeptides prompted us to design and synthesize lipopeptides derived from this cyclic decapeptide. We devised a concise strategy for the solid-phase synthesis of these cyclic lipopeptides that included as key steps: (i) synthesis of the cyclic peptidyl resin incorporating the Lys residue to be acylated protected at the N^{ε}-amino group with an ivDde group, (ii) selective removal of the ivDde group, and (iii) acylation [7]. Using this protocol, we prepared a family of cyclolipopeptides by acylating **BPC194** at Lys^5 with a range of fatty acids. We also analyzed if the position of the hydrophobic chain influenced the biological activity. Futhermore, taking into account that we have previously observed that the presence of a D-amino acid or of a His residue generally renders less hemolytic peptides, we decided to include in this study cyclolipopeptides incorporating a D-Lys, a D-Phe or a His [8]. All cyclolipopeptides were screened against the above phytopathogenic bacteria, and the hemolytic activity against red blood cells and the phytotoxicity in tobacco leaves were also determined. Results showed that best derivatives incorporated an acyl substituent of 4 to 6 carbons. In general, acylation of Lys^1, Lys^2 or Lys^5 rendered the sequences with the highest activity. Incorporation of a D-amino acid maintained the antimicrobial activity while significantly reduced the hemolysis. Replacement of Phe with a His also yielded cyclolipopeptides with low hemolytic activity. Furthermore, the best derivatives exhibited low phytotoxicity in tobacco leaves.

Keywords: acylation; plant pathogens

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Design and synthesis of antimicrobial peptidotriazoles

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Plant pathogenic bacteria and fungi are responsible for many diseases in plants affecting the quality of crops and producing significant economic losses. Their control is mainly based on copper compounds and antibiotics, but they are regarded as serious environmental contaminants and are currently subjected to strong restrictions and regulatory requirements [1]. Moreover, although antibiotics are highly efficient, their use is hampered by the rapid emergence of resistance developed in plant pathogens [2]. In recent decades, the search for new antimicrobial agents as an alternative to antibiotics has acquired great interest. Considering their structural and biological properties, antimicrobial peptides offer promising perspectives to become a new class of pesticides for plant disease control [3].

Our current research is focused on finding antimicrobial peptides able to control the economically important plant pathogenic bacteria *Erwinia amylovora*, *Pseudomonas syringae* and *Xanthomonas vesicatoria*, and the fungi *Fusarium oxysporum* and *Penicillium expansum*. The main features expected for these peptides are a high antimicrobial activity, a low eukaryotic cytotoxicity and a high stability to protease digestion. Up to now, we have identified linear and cyclic peptides which inhibited the in vitro growth of these plant pathogens. Two of the best peptides, KKLFKKILKYL-NH_2 (**BP100**) and c(KKLKKFKKLQ) (**BPC194**), also showed minimized cytotoxicity and low susceptibility to protease degradation [4,5].

In our efforts to further improve the biological profile of **BP100** and **BPC194**, we decided to explore the effect of incorporating a 1,2,3-triazole ring onto the side chain of a selected residue of these sequences. The conjugation of **BPC194** to linear sequences derived from **BP100** through a 1,2,3-triazole ring was also studied. The synthesis of these peptidotriazoles was accomplished on solid-phase and involved as key step a copper-catalyzed cycloaddition reaction between an alkyne and an azide. The peptidotriazoles were screened for in vitro growth inhibition of the above phytopathogens and for their cytotoxic effects on eukaryotic cells. Sequences with high antimicrobial activity and low hemolysis were identified [6-8].

Keywords: phytopathogenic bacteria; click chemistry

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Design, Synthesis and Anti-HIV Evaluation of Novel DAPY Derivates Targeting an Additional Tolerant Region Ⅱ in The NNRTI Binding Pocket

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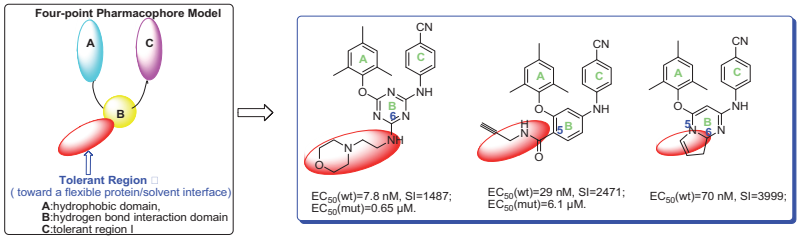
The "Four-point Pharmacophore Model" for next generaction HIV-1 NNRTIs has been proposed recently, which is refered to the spatial arrangement of three common chemical features (hydrophobic domain, hydrogen bond interaction domain, tolerant region I), togerther with a newly identified tolerant region Ⅱ toward a flexible protein/solvent interface. Inspired by this concept, three series of novel DAPY derivates that targeting an additional tolerant region Ⅱ in NNRTI binding pocket were designed, synthesized, and evaluated for their anti-HIV activity, in which diverse long-chain substitutions were incorported to the 5/6 position on the cetral ring of DAPYs, or a five-numbered heterocycle ring was fused to the 5/6 position of pyrimidine. Most of the tested compounds were proved to be effective in inhibiting HIV-1, particularly, some promising compounds were identified with single or double-digit nanomolar activity against wild-type HIV-1 and moderate activity against the double mutant strain. These gratifying results indicate that targeting an additional tolerant region Ⅱ is a valuable clue for design of novel NNRTIs. Moreover, the preliminary structure-activity relationship (SAR) and molecular modeling results were also briefly discussed in all these series, which provided some useful information for further modification.

Keywords: HIV, NNRTIs, DAPY Derivates , Tolerant Region Ⅱ



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Diphenyl diselenide $(PhSe)_2$ inhibits biofilm formation by *Candida albicans* by a mechanism evolving ROS production

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The opportunistic fungal *Candida albicans* can produce superficial and systemic infections in immunocompromised patients. An essential stage to both colonization and virulence by *C. albicans* is the transition from budding yeast form to filamentous form, producing biofilms. In this work, we studied the effect of the organochalcogenide compound $(PhSe)_2$ on both cell growth and biofilm formation by *C. albicans*. $(PhSe)_2$ inhibited both growth and biofilm formation by *C. albicans*. The inhibitory effects of $(PhSe)_2$ depended on the cell density and $(PhSe)_2$ concentration. We have also observed that $(PhSe)_2$ stimulated ROS production (67%) and increased cell membrane permeability (2.94-fold) in *C. albicans*. In addition, $(PhSe)_2$ caused a marked decrease in proteinase activity (6.8 fold) in relation to non-treated group. $(PhSe)_2$ decreased both cell growth and biofilm development. The toxicity of $(PhSe)_2$ towards *C. albicans* can be associated with an increase in ROS production, which can increase cell permeability. The permanent damage to the cell membranes can culminate in cell death.

Keywords: *Candida albicans*; $(PhSe)_2$; biofilm formation

Discovery of Novel Arylazinylthioacetanilides as Potent HIV-1 NNRTIs Using "Follow-on"-based Lead Optimization Strategy

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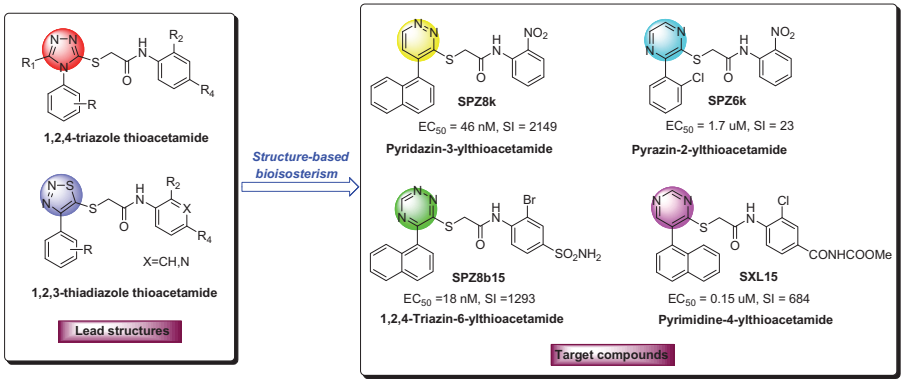
Prompted by the inspiring findings of existing arylazolylthioacetanilides and with aim to further explore the chemically diversed space, activity landscapes and cliffs, we employed the structure-guided bioisosterism, an common and efficient "follow-on"-based lead optimization strategy to obtain novel arylazinylthioacetanilides with desired potency, selectivity. The surprising initial findings coming out of our ongoing investigations was that replacement of the five-membered azoles moiety by an array of six-membered heteroaromatic bioisosteres (such as pyrazine, pyridazine, 1,2,4-triazine and pyrimidine), led to the identification of additional series of arylazinylthioacetanilides that possessed potent anti-HIV activities in cell culture assay. In particular, 1,2,4-triazin-6-yl thioacetamide **SPZ8b15** was identified as the most promising compound with double-digit nanomolar activity against wild-type HIV-1 (EC_{50} = 0.018μM) and moderate activity against the double mutant strain RES056 (EC_{50} = 3.3μM). These results indicated that six-membered heterocycles can be used as novel bioisosteres to replace the five-membered azoles. Very recently, in the newly designed pyrimidine series (exemplified by **SXL15**), diverse substituents, varying in size and electronic nature, were introduced in the phenyl ring of the anilide moiety which is located at the protein-solvent interface region of RT, to further investigate potential interaction. The preliminary structure-activity relationship (SAR) and molecular modeling results were also briefly discussed in all these series, which provided some useful information for the further design of novel NNRTIs.



Keywords: Arylazinylthioacetanilides; heterocycle; drug design; SAR; molecular modeling

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DNA aptamers blocking activity botulinum toxin type A

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Botulism is dangerous bacterial disease of humans and other mammalian species caused by pathogenic clostridia. Botulism is often fatal due to paralytic action of botulinum neurotoxins exerted by blocking neurotransmission. There are seven types of botulinum toxin, at least four of them, A, B, E, and F are dangerous to humans. Although *Clostridium botulinum* is primary toxin vehicle, other Clostridia can also produce certain toxin types. Recently, eights type botulinum toxin has been found and considered as the deadliest one, also hinting that more toxin species can be discovered in the future.

Botulinum toxin (BoNT) is the most toxic substance in the Earth, yet specific therapy represented by homologous or heterologous polyclonal antibodies is of limited supply and moderate treatment effectiveness. As the result, botulism outbreaks still have significant mortality rate. Botulinum toxin is also potential agent of biological terrorism

Limited number of therapeutic options is being developed against botulism. Meanwhile, not only there is a need for control of natural outbreaks, but also the need of management of potential complications during treatment with medical BoNT preparations, as the spectrum of neural and muscular disorders treated by therapeutic botulinum toxin has broadened significantly.

For now, a combination of three monoclonal antibodies (3AB mAb) developed by XOMA is the sole new-generation botulism therapeutic entered clinical trials. Notably, only a combination of three mAb was effective in BoNT neutralization. In addition, antibodies cannot neutralize toxin already entered the cell.

One possibility to neutralize BoNT inside the cell is development of the target-specific nucleic acid aptamers capable to enter mammalian cells [1]. Aptamers represent new class of affinity molecular tools capable of efficient binding to the target. Diagnostic and therapeutic potential of DNA aptamers is actively explored in different fields of modern molecular medicine, including infectious pathogen detection and neutralization. We identified DNA aptamers binding to BoNT type A employing new technique tailored to avoid carryover of target-specific DNAs with aptamers binding to the solid phase [2]. For this purpose recombinant C-terminal heavy chain fragment (HC50) of BoNTA was produced as fusion protein with His6 affinity tag and SUMO peptide. Aptamers bound to HC50 were then specifically eluted from the solid phase by cleavage of the fusion protein with SUMO protease.

Random oligonucleotide library (10^{14}) was subjected to 6 rounds of panning and proteolytic elution against the LF fusion immobilized on IMAC magnetic beads. Selected aptamers analyzed for capability to block *in vitro* proteolysis of internally quenched proteinaceous fluorescent BoNTA substrate. Positively acting aptamers were fused to DNAs selected for the ability to internalize in target neuroblastoma cell line and tested for blocking cleavage of internally quenched fluorescent protein substrate in cell-based assay. Some of the aptamer constructs displayed inhibition of BoNTA substrate cleavage inside the cell. The data obtained outlines DNA aptamers as prospective tools for the development of the new generation of therapeutic agents against botulism.

Keywords: DNA aptamer; botulinum toxin type A, botulism toxin; toxin neutralization

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Dual-acting hybrid antibiotics on the basis of azithromycin and glycopeptides – synthesis and antibacterial activity

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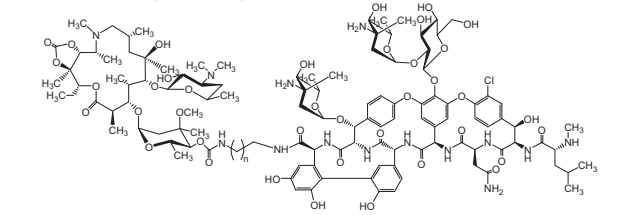
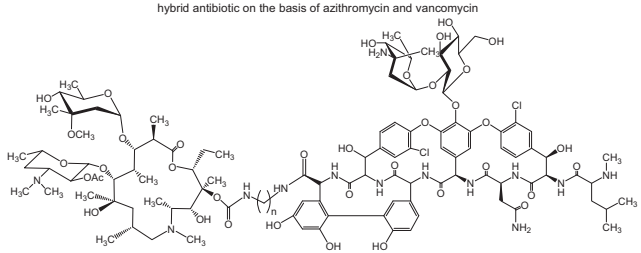
The growing resistance of microorganisms to currently available antibiotics calls for the development of new strategies that can solve the problem of antibacterial resistance. One of such strategies is the development of dual-acting hybrid antibiotics – structures that contain two covalently linked antibacterial drugs that interact with different targets in a bacterial cell.

We developed a method of synthesis of novel dual-acting hybrid antibiotics which contain a glycopeptide antibiotic (vancomycin or eremomycin or teicoplanin aglycon) covalently linked *via* spacer to a 4”- or 11-position of azithromycin (Figure 1). The developed method allows to vary the position of the attachment of glycopeptide antibiotic to azithromycin as well as the length of the spacer. The structures of the obtained hybrid antibiotics were confirmed using NMR spectroscopy and HR mass spectrometry methods, including MS/MS data.

Antibacterial activity of the obtained series of hybrid antibiotics was tested on a panel of gram-positive bacterial strains. It has been demonstrated that all novel dual-acting antibiotics are as active as azithromycin and vancomycin against different *Staphylococcus aureus* strains and have superior activity than azithromycin and vancomycin against *Streptococcus pneumoniae* strains. Hybrid antibiotics on the basis of azithromycin and eremomycin or teicoplanin aglycon were active against *Enterococcus faecium* and *Enterococcus faecalis* strains resistant both to azithromycin and vancomycin. Thus, synthesis of the dual-acting antibiotics on the basis of azithromycin and glycopeptides lead to highly-active compounds that in some cases overcome bacterial resistance. Further investigations are needed to expand the spectrum of antibacterial activity and establish some structure-activity relationships such as the influence of the structure of glycopeptide antibiotic and length of the spacer on the antibacterial activity of such hybrid antibiotics.



hybrid antibiotic on the basis of azithromycin and vancomycin



hybrid antibiotic on the basis of azithromycin and eremomycin

Figure 1. Examples of structures of hybrid antibiotics on the basis of azithromycin and glycopeptides.

Keywords: azithromycin; vancomycin; eremomycin; teicoplanin aglycon; dual-acting antibiotics; synthesis; antibacterial activity.

Employing molecular 3D fitness evolutionary algorithm to introduce novel anti-TB property for approved drugs

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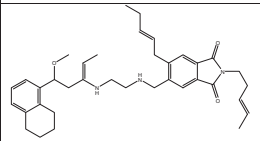
Tuberculosis (TB) with 8.6 million new cases in 2012 which it is claiming approximately 1.3 million lives, could be defined as the worldwide health problem. Furthermore, it is estimated that one-third of the human population is infected with the *Mycobacterium tuberculosis*. In addition to HIV, a wide range of the aspects continue to undermine TB control measures in the world, involving the enhancing multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains that are resistant to the common anti-tubercular drugs. In addition, the increasing of prevalence of chronic diseases is known as the undesirable side effects of frontline anti-TB drugs. On the subject of mentioned serious challenges in drug treatment of TB based on current standard regimen of Directly Observed Treatment Short-*course* (DOTS), it would be critical to discover and design new anti-TB drugs with maximum effect in addition to minimum side effects. In this regard, based on using *de novo* drug design approach of 3D fitness evolutionary algorithm as SSDD software (Ver.1.0, 2013), the current research has been finalized. Initially, chemical structures of one of the anti-TB drug, Rifampicin; selected as input pattern for *de novo* designing of novel anti-TB compound by SSDD. The matching process between input drug and *de novo* designed structures built based on the fragmented chemical library of SSDD were completed. The resulted structures were filtered by Lipinski's rule of five. Finally, competed 5-((2-((Z)-5-methoxy-5-(5,6,7,8-tetrahydronaphthalen-1-yl)pent-2-en-3-ylamino)ethylamino)methyl)-6-((E)-pent-2-enyl)-2-((E)-pent-3-enyl)isoindoline-1,3-dione with fitness degree 998.1, was reported as output SSDD and candidate for similarity search in second round of the current investigation. The similarity search procedure via available approved drugs was carryied out via drugbank.ca website. Findings of the similarity search as three approved drugs: Palonosetron, Quinapril and Valrubicin (table 1.); could be presented as novel anti-TB drugs with decreased side effects to overcome MDR and XDR strains of *M. tuberculosis*.

Keywords: anti-TB; cheminformatics; SSDD software

Table1. *de novo* drug design procedure of novel anti-TB drug based on SSDD software

Input pattern anti-TB drug	Resulted structure from SSDD software	Resulted approved drug from similarity search via drugbank.ca
Rifampicin		Palonosetron
		Quinapril
		Valrubicin

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Evaluation of antimicrobial efficiency of new polymers

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During the last two decades, the field of macromolecules with antimicrobial properties, including the synthesis of novel structures and modifications of known polymers, as well as biological, physicochemical, and biochemical research and engineering design, made a great advance due to the rather complex epidemiological situation, nosocomial infections, microbial contamination, and infection risks in hospital and dental equipment, in water purification system, food, and general consumer markets that has led to an ever-growing need for prevention of microbial infection in these various areas [1, 2, 3]. The scope of our research was to evaluate the antibacterial efficiency of eleven copolymers bearing quaternized ammonium or phosphonium groups bounded either electrostatically or covalently to the polymeric backbone [$PSSAmC_{16}$, PVBCHAM, P($SSAmC_{16}$-co-VBCHAM65), P($SSAmC_{16}$-co-VBCHAM25), $PSSPhC_{16}$, P(MMA-co-VBCHAM47), P(SSNa-co-VBCHAM20), P(SSNa-co-VBCHAM85), P(AA-co-VBCHAM88), P(AA-co-VBCHAM20), P(GMA-co-VBCHAM70)]. Certified reference bacterial strains used were *E. coli* NCTC 9001, *S. aureus* NCTC 6571, *P. aeruginosa* NCTC 10662 and *E. faecalis* NCTC 775. Under aseptic techniques a film of each polymer sample was placed on a coupon cover glass 18 x 18 mm and was then transferred to a sterile petri dish, inoculated with 20μl of the overnight culture of one of the test organisms. Coupons were incubated at 22° C and 4° C for 3 and 24 hours. Each inoculated coupon was then diluted in a tube containing 10 ml sterile phosphate-buffered saline (PBS) and the survived bacteria were enumerated after spreading onto nutrient agar plates at 37±1°C for 18-24 h. A diffusion test of the polymers was performed by evaluating the inhibition zone created when the tested polymers were in contact with the inoculated nutrient agar plates. The antibacterial efficiency of these polymers was differential to different polymers and varied from less than1 log to more than 5 log reduction, depending on microorganism, polymer type, time, as well as temperature. The most effective polymers were $PSSAmC_{16}$, PVBCHAM, P($SSAmC_{16}$-co-VBCHAM65), P($SSAmC_{16}$-co-VBCHAM25), $PSSPhC_{16}$ and P(AA-co-VBCHAM88), which reduced significantly bacterial numbers of mainly gram positive bacteria ($p<0.05$). These results show clearly the antibacterial effects of these polymers and their potential use for selected medical, industrial and environmental applications, such as surgical equipment, food industry systems or anti-fouling paints, respectively. These novel materials would promote the protection of public health, as well as saving money.

Keywords: polymers; antibacterial effect

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Fluoroquinolone-metal complexes: a route to counteract bacterial resistance?

Maria J. Feio & Paula Gameiro

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Microbial resistance to antibiotics is one of the biggest public health threats of the modern world. Various strategies have been suggested to control the growing problem of microbial resistance to available antibiotics however resistance to antimicrobials is a result of an intrinsic property of the pathogens, their astonishing adaptability. Thus, resistance is currently and will continue to be a problem, and safe and effective new antimicrobials are needed now and will continue to be needed in the future.

There has been an increasing threat of bacterial resistance to quinolones and the concept that metal complexes as novel derivatives of fluoroquinolones (FQs) could be an alternative to conventional drugs has been pushed forward. Numerous studies regarding the interaction between quinolones and metal cations have been reported and reviewed in the literature. In particular, the study of quinolone-copper and quinolone-copper-1,10-phenanthroline (phen) complexes has become an increasingly important field since they seem to exhibit high affinity towards DNA binding as well as nuclease activity towards plasmid, genomic and internucleosomal DNA [1, 2].

In this work, the solution behaviour of FQ-complexes with several transition metal ions in the presence and absence of phen in aqueous solution is presented providing a consistent view of divalent metal ion complex behaviour across the FQ family. In all cases, the results obtained show, that under physiological conditions (micromolar concentration range and pH 7.4), only copper(II):FQ:phen ternary complexes are stable. Hence, the synthesis, characterization and single-crystal X-ray diffraction (XRD) structure of several copper(II) complexes of FQs and the N-donor heterocyclic ligand were undertaken [3].

Overall data on the antibacterial activity of these compounds and intake route are also reported and discussed. Minimum inhibitory concentration (MIC) determinations of the complexes and comparison with free FQ in various *E. coli* strains indicate that the Cu-complexes are as efficient antimicrobials as the free antibiotic. Moreover, results strongly suggest that the cell intake route of both species is different supporting, therefore, the complexes' suitability as candidates for further biological testing in FQ-resistant microorganisms. Moreover, discovery and development of antibiotics has become scientifically more complex, more expensive, and more time consuming over time; the pathways to antibiotic approval through the U.S. FDA and European Medicines Agency have become confusing, generally inapplicable and of questionable relevance to patients and clinicians. The data presented in this work, suggesting the potential of FQ metal complexes as antimicrobial agents proposes the use of new formulations, and not necessarily new compounds which could be a means to introduce faster-track therapies with reduced trial periods and fewer regulatory barriers.

As a final remark, [Cu(FQ)(phen)] complexes appear as suitable candidates for more advanced metalloantibiotic testing, potentially exhibiting efficacy, stability and toxicity advantages over their respective free FQs.

Keywords: Fluoroquinolones, Metalloantibiotics, Bacterial resistance, Solution equilibria

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From azoloquinolones to azoloquinolones through selenadiazoloquinolonoes

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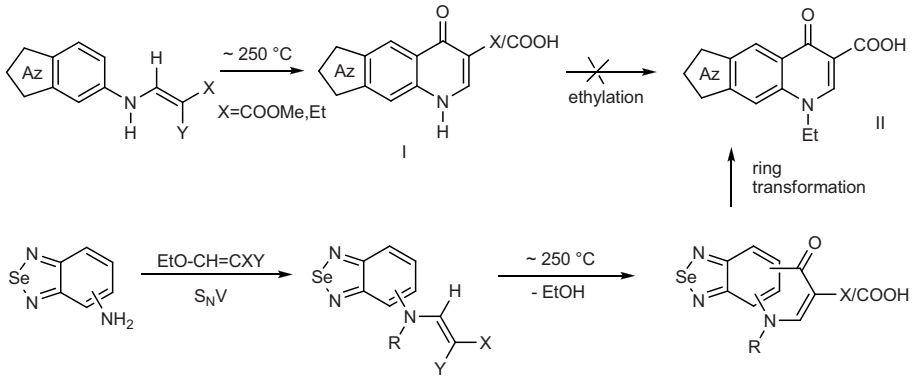
Angularly annelated azoloquinolones I (azole = imidazole, triazole, pyrazine) were prepared as bioisosters of oxolinic acid – a nalidixic acid type drug using a modified Gould-Jacobs reaction [1]. The fixed structure of fused 1-alkyl-4-oxodihydropyridine-3-carboxylic acid II is required to achieve bioactivity, due to interaction with topoisomerase II / IV (gyrase). But introduction of the alkyl group into position 1 using classical alkylations or alkylated precursors failed due to polyalkylation of tautomeric nitrogen atoms. We decided also to prepare selediazoloquinolones to enhance biological activity using the same protocol:



Az = in text; X, Y = COOEt, COOMe, COMe, CN; R = H, Et

By this way we prepared also exclusively the regioselectively angularly annelated selenadiazoloquinolines. Starting from 4- or 5-aminoselenadiazole, we utilized the reaction with activated enolethers under conditions of nucleophilic vinylic substitution with inversion of configuration, followed by thermal cyclocondesation to arrive at the desired skeleton [2]. Following ethylation offered us the ring opening of selenadiazole offered us a chance to prepare fused ethylated azoloquinolone acids - analogues of nalidixic acid type antibacterial drugs. We discuss antibacterial and photochemotherapeutic potential of the prepared compounds as well as their physico-chemical and spectral data.

Keywords: azoloquinolinones, selenadiazoloquinolinones, Gould-Jacobs reaction, nucleophilic vinylic substitution, inversion of configuration

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[2] 15 References to selenadiazoloquinolones will be given during lecture

From nitrogenous cationic surfactant as disinfectant to o-substituted pyrazines as the antituberculotics– synthesis and evaluation

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The first part of work deals with the preparation and testing of compounds of type cationic surfactants such as disinfection agents. Since the quaternary cationic surfactants are substances widely used in many of applications (pharmaceuticals, chemical industry, food industry etc.) are still of great interest. It was designed and prepared more than 40 surfactants based on quaternary nitrogen. Substances derived from structures commonly used (benzalkonium, cetylpyridinium or cetyltrimethylammonium). We have prepared several sets of surfactants (with different hydrophilic part). Each set contain seven homologues differing in two methylene units (C10-C18). Prepared structures were confirmed with analyzes of NMR, MS and EA. Furthermore HPLC method was developed to distinguish the individual homologues in the mixture.

For most compounds was measured the critical micelle concentration as a fundamental characteristic of surfactants. It was confirmed the structure relationship between the value of CMC and lipophilic chain length in the molecule. The compounds were evaluated for antimicrobial activity expected. Some compounds significantly influenced the growth of several strains of bacteria or fungi.

The second part of the work deals with the preparation of derivatives of pyrazinamide (PZA). PZA is one the most useful antituberculotic drug in a clinical practice. We have prepared several O-substituted derivatives as a new potential antituberculotics. The structures were confirmed by NMR and elementary analysis. The compounds were tested against several strains of Mycobacteria. The values of IC_{50} were determined.

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Initial experience with procalcitonin (PCT) determination and its use for guidance in treatment of critically ill patients with severe pneumonia

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Introduction and Objectives: The use of biomarkers, such as procalcitonin (PCT), to guide the initiation and termination of antibiotic therapy may reduce antibiotic exposure and treatment duration, without increasing risk in patients. In clinical practice, it may improve the management of severe pneumonia (SP), and may also help by reducing treatment duration. The purpose of this paper is to present the preliminary results of the utility of PCT levels in managing critically ill patients admitted to our ICU with the diagnosis of SP.

Methods: Retrospective observational study based on patients admitted to our ICU on the last six months with diagnosis of SP. All patients received antibiotic and/or antiviral therapy according to protocol. PCT levels, leukocytes, PaO2/FiO2, need for vasoactive drugs and microbiological data were obtained on admission. Radiological control, PaO2/FiO2, PCT, leukocytes and vasopressor withdrawal were obtained at 48-72 hours as follow-up. Finally it was checked whether PCT levels significantly decreased at day 5-7 and if they helped to the end of treatment and clinical outcome.

Results: 27 patients, male 81% (22/27), 17 were Community-Acquired Pneumonia (CAP), and 10 were Hospital-Acquired Pneumonia (HAP). The mean age was 54 years (SD± 13), 11 patients with chronic disease including 2 patients with COPD and 6 DM. All patients required mechanical ventilation. The mean SOFA was 6 (SD ± 2) and APACHE II of 17 (SD± 4). The mean of PCT at ICU admission was 3.7 (SD ± 4), Leukocytes 12,830 (SD± 4250), PaO2/FiO2 163 (SD± 62). 56% had purulent secretions by orotracheal tube. Positive bacterial cultures were positive only in 12 patients (44%), in 5 (19%) were PCR positive for H1N1. At 48-72 hours there was improvement in PaO2/FiO2 in 25 patients (93%), decreased PCT levels average of 1.99 (SD ± 2.1) and leukocytes decrease in 17 patients (63%). Vasopressors were removed after 48-72 h in 20 patients (76%). The PCT helped in the ending of antibiotic therapy in 10 cases (37%). 7 patients died before the end of treatment (26%), from which 3 (43%) showed a rise of PCT at 48-72h have started, being a total of 4 patients who presented a PCT level rise at this time.

Conclusions: Procalcitonin was a support for both entities CAP and HAP. It helped to reduce the duration of treatment in some cases. Although clinical worsening and other work up were more important for the adjustment or change of antibiotic therapy. 75% of patients with an increase of PCT at 48-72h died, however it represents 43% of all deaths. Even when PCT predicts the severity and course of severe pneumonia, it has always to be correlated with clinical course and other complementary tests.

Keywords: Pneumonia1, Antibiotic Therapy2; Procalcitonin (PCT)3

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Investigating transformation and degradation of scaffold compounds in the rumen to advance the development of methanogen-specific inhibitors

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Methane emissions from ruminants are increasingly being recognised as a significant factor in contributing to climate change. One strategy for mitigating these emissions is to develop effective inhibitory compounds that specifically target rumen methanogens. However, little is known about the chemical and biological processes that could affect the stability of compounds introduced into the rumen. Recent reviews of chemical diversity, and of current drugs and antimicrobial compounds, have highlighted the fact that the large majority of marketed drugs and veterinary compounds can be classified into a limited number of chemical scaffolds types (~30-40), that typically contain at least one ring system (Welsch *et al.* 2010, Curr. Opin. Chem. Biol. 14: 347-361). As a result, large compound collections can be quickly classified into much fewer ring systems, with various linkers and side-chains. In addition, scaffolds are representative of a far larger chemical diversity (10,000-fold) which allows for vastly improved access to the available chemical diversity in a cost-effective manner (Hubbard and Murray 2011; Meth. Enzymol. 493:509-531). For these reasons, scaffold compounds and select derivatives are an excellent choice for conducting studies into the stability and transformation processes that occur in the rumen. One approach for monitoring scaffold stability is NMR spectroscopy which has been used in some metabolic studies for simultaneously assessing numerous compounds in a sample. Significantly, there are relatively few aromatic compounds at significant levels in rumen fluid (e.g. aromatic amino acids and their breakdown products, and lignin constituents). However, specific NMR spectral peaks can be affected by the presence of other components (e.g. plant fibre, proteins, microbes). These changes could affect the ability to quantify the compounds in question. We have undertaken initial studies into the stability of eight scaffolds using NMR in rumen fluid incubations. The scaffolds include: quinazoline, 2-aminopyridine, 1-phenylpiperazine, benzimidazole, 4-benzylpiperidine, 4-aminoquinoline, 4-benzoylpiperidine hydrochloride, and phenyl-piperidin-4-yl-methanol. The above-mentioned primary scaffolds have been examined by ^{1}H NMR spectroscopy in a standard buffer that is used for rumen *in vitro* analyses and which has a pH value close to that typically found in the rumen (pH 6.8). In almost all cases that we have examined thus far, the peaks for the scaffolds in the presence of centrifuged and filtered (0.45 µM) rumen fluid were highly attenuated (indicating an interaction with high molecular weight species), and shifted to slightly lower chemical shifts. Further investigations with three scaffolds (4-aminoquinoline, quinazoline and 4-benzoylpiperidine), that were spiked into additionally clarified rumen fluid aliquots (using 0.45 µm- and 0.22 µm-pore size filters and a 3 kDa MW cut off spin-filter), showed that these compounds were interacting with macromolecules greater than 3 kDa, which attenuated signal strength and hindered quantification. This sample processing markedly reduced the interference in the spectra suggesting that the stability of these three scaffolds can likely be assessed using NMR. Future analyses may include an extraction technique and incubation step prior to the filtration steps and could also utilise LC-MS techniques as an additional analytical method. These new approaches should enable us to identify the type of scaffolds that are stable in the rumen leading to an improved understanding of how compounds are degraded and transformed in the complex rumen environment. This knowledge could be critical for optimising the development of effective small molecule methanogen-specific inhibitors for use in ruminants.

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Naturally occurring and synthetic derived catechins induce membrane alterations and reduce MRSA phenotype

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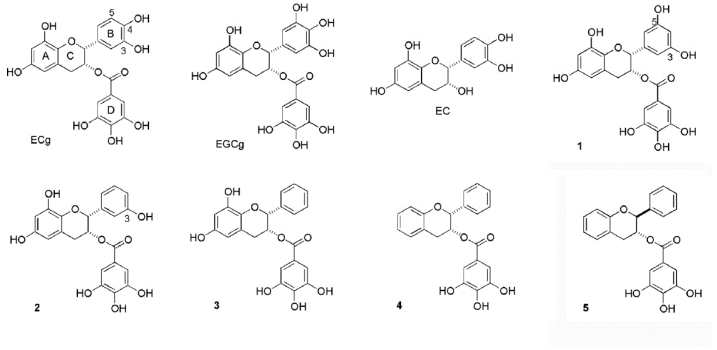
Galloylated catechins, in particular (-)-epicatechin gallate (ECg), have the capacity to abrogate β-lactam resistance in methicillin-resistant strains of *Staphylococcus aureus* (MRSA). Current evidence suggests that these reversible phenotypic traits result from their intercalation into the bacterial cytoplasmic membrane. We have endeavoured to potentiate the capacity of ECg to modify the MRSA phenotype by stepwise removal of hydroxyl groups from the B-ring pharmacophore and the A:C fused ring system of the naturally occurring molecule. Some of these catechin analoges are even more effective than ECg by reducing β-lactam resistance in MRSA. Studies with artificial membranes modelled on the lipid composition of the staphylococcal bilayer indicated that ECg adopts a position deep within the lipid palisade, eliciting major alterations in the thermotropic behaviour of the bilayer. The non-galloylated homolog (-)-epicatechin enhanced ECg-mediated effects by facilitating entry of ECg molecules into the membrane. ECg analogs with unnatural B-ring hydroxylation patterns induced higher levels of gene expression and more profound changes to MRSA membrane fluidity than ECg but adopted a more superficial location within the bilayer. ECg possessed a high affinity for the positively charged staphylococcal membrane and induced changes to the biophysical properties of the bilayer that are likely to account for its capacity to disperse the cell wall biosynthetic machinery responsible for β-lactam resistance. The ability to enhance these properties by chemical modification of ECg raises the possibility that more potent analogs could be developed for clinical evaluation.

Keywords: MRSA; catechin; membrane.



Structures of (-)-epicatechin gallate (ECg), (-)-epigallocatechin gallate (EGCg), (-)-epicatechin (EC), (-)-3,5-dihydroxy B-ring modified (-)-ECg (1), (-)-3-hydroxy B-ring modified (-)-ECg (2), (-) B-ring modified (-)-ECg (3), (-)- A,B-ring modified (-)-ECg (4) and A,B-ring modified (-)-Cg (5).

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Optimization of a 1-H-benzimidazole fragment hit yields biologically active, high-efficiency inhibitors for glutamate racemase (RacE)

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Glutamate racemase (GR), the enzyme responsible for the stereo-interconversion between D- and L-glutamate, plays a critical role in the biosynthesis of the peptidoglycan layer of bacterial cell walls, for both Gram-positive and Gram-negative species, and has been validated as a drug target for the development of novel antimicrobial agents. Although there are a number of high resolution crystal structures of GRs that have been solved in recent years, the inherent flexibility of the enzyme and the nature of the active site pocket have proved challenging for the development of drug leads. Our group has designed a novel family of lead compounds against GR, using the enzyme from *B. subtilis* (RacE) as a model system. Our approach to the receptor flexibility problem has been tailored around the needs of dealing with GR specifically, and is a hybrid of steered molecular dynamics (SMD), classical docking and Poisson-Boltzmann solvation energy calculations, called FERM-SMD (Flexible Enzyme Receptor Method for Steered-MD Docking)[1]. Steered MD is used to provide an enhanced force applied to the natural substrate in the GR-D-glu complex, in order to accelerate the unbinding in the MD simulations, while maintaining atomistic detail in the simulation. We used this method to synthetically optimize a 1-H-benzimidazole scaffold [2], which was previously identified as a GR competitive inhibitor. The FERM-based optimization scheme was successful in distinguishing between high- and low-affinity binders with minimal experimental structural information, saving time and resources in the process [2]. In the case of RacE, *in vitro* potency was increased approximately fourfold. The outcome of this study is a novel small-molecule inhibitor of RacE with the following characteristics: Ki =2.5 μM, LE=0.45 kcal mol^{-1} $atom^{-1}$, LiPE=6.0. Ongoing additional *in silico* screening focused on *S. aureus* and *E. coli* GRs will be discussed.

Keywords: glutamate racemase; structure-based drug discovery

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Plant systemic acquired resistance inducers – salt derivatives of benzo[1,2,3]thiadiazole-7-carbothioic acid, *S*-methyl ester (BTH) as bifunctional ionic liquids

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Plant protection against different pathogens such as viruses, bacteria and fungi is one of the most challenging problem in modern agriculture, due to the fact that those pathogens cause high losses in crop yield. To prevent against bacteria and fungi pesticides are used. Unfortunately it is not as easy to fight against viruses. It is mostly due to the fact that they are nonliving matter very closely associated with the host and thus it is not possible to combat them with common plant protection compounds. Systemic Acquired Resistance (SAR) is a phenomenon that allows the plant to induce self-resistance against many microorganisms, especially viruses.[2] It is, besides crossing more resistant types of plants together or genetic modification, the only way to act against viruses. Commercially available compound which is used as SAR inducer is benzo[1,2,3]thiadiazole-7-carbothioic acid, *S*-methyl ester (BTH, Bion®) (Figure 1) [2]. Unfortunately its usage is limited because of its very low solubility in water that resulted in past in inaccurate dosing. Due to this fact possibility to create bifunctional salts forms of BTH derivatives with counterion which acts against bacteria, increases solubility in water and allows to preserve SAR induction properties would be very promising.

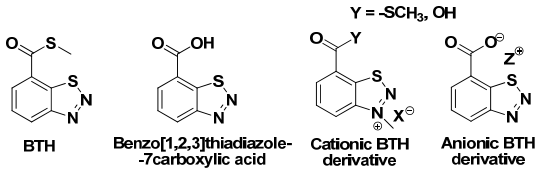

Figure 1. BTH and its derivatives

Presented research concerns test on *Nicotiana tabacum* var. *Xanthi* and viruses: *tobacco mosaic* (TMV) and *latent olives* (OLV-1) to check whether salt derivatives of BTH are still an effective inducer of plant resistance.[3,4] To assess the induction resistance in plants, the hypersensitivity phenomenon occurring between virus and host plant was used (manifested by the presence of local necrotic spots on the leaves after virus infection). Tobacco plants were sprayed or watered once with compound solutions at the concentrations between 20 and 100 mg/L and 7 days after treatment were mechanically infected with purified Tobacco mosaic virus at the concentration of 3-4 μg/ml. After 4 days the number of local necrotic spots on the leaves treated with salt was calculated and compared to control.

Direct influence of ionic liquids on the growth of bacteria was also examined for the determination of MIC (Minimal Inhibitory Concentration) on *Pectobacterium carotovorum* subsp. *carotovorum* ATCC 15713 (Pcc), *Erwinia amylovora* K3 (Eam), *Pseudomonas syringae* pv. *syringae* 840 (Pss), *Clavibacter michiganensis* subsp. *michiganensis* 153 (Cmm) to confirm that obtained compounds are dual-functional ionic liquids with preservation of SAR induction and antibacterial properties.

Research is sponsored by HOMING PLUS (HOMING PLUS/2012-5/13) programme of Foundation for Polish Science, co-financed from European Union, Regional Development Fund.

Keywords: SAR; Systemic Acquired Resistance, ionic liquids, resistance induction, viruses

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Probing the mechanisms of pyoverdine recognition by the FpvA receptor using molecular simulations

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Pyoverdine is a fluorescent siderophore molecule synthetized by the Gram-negative bacterium *Pseudomonas Aeruginosa*, to scavenge the iron indispensable to bacterial growth from the outside medium. Its very strong affinity for Fe^{3+} (10^{32} M^{-1}) effectively enables this molecule to 'rob' other binders of this ion by displacing the binding equilibrium in its favor. The pyoverdine-Fe^{3+} complexes thus created are then internalized *via* specific TonB-dependent FpvA receptors on the outside membrane and eventually release their payload into the cytoplasm, marking the start of a new cycle.

To date, the mechanisms of this complex harvesting process are not fully understood. In particular, the recognition between the pyoverdine-Fe^{3+} complex and the FpvA receptor appears to be only loosely specific: noncognate pyoverdine-Fe^{3+} complexes have been shown to bind to FpvA with varying affinities depending on the first few N-terminal aminoacids of their peptidic part. This finding provides incentive for the design of pyoverdine analogs capable of either blocking the Fe^{3+} harvesting cycle, or acting as a Trojan horse to deliver antibiotic compounds into the cell. However, this can only be effectively achieved if the codes of the underlying recognition mechanisms are well understood.

Molecular dynamics simulations, which transcribe the detailed movements of all the constituent atoms of these complex systems and their environment (lipopolysaccharidic outer membrane, water, ions…), can provide valuable insights into such mechanisms. We have simulated the binding and unbinding processes of cognate and noncognate pyoverdines to FpvA and obtained the associated mechanisms and free energy profiles. We discuss the implications of these results on the rules of pyoverdine-FpvA recognition: in particular, the effects of pyoverdine structure on the collective motion of FpvA which favor or impede communication with the TonB motif, and the necessity to consider kinetic effects in addition to the more usually considered thermodynamical binding free energies when discriminating between pyoverdine analogs.

Keywords: pyoverdine; FpvA; Pseudomonas; recognition; free energy; simulations; molecular dynamics.

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Pyoverdine analogues: design, chelating properties and molecular recognition

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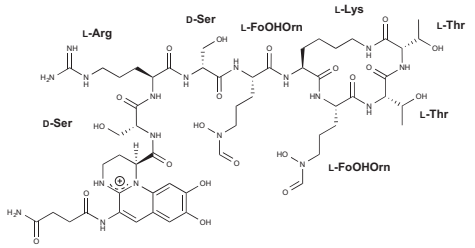
Pyoverdines are sophisticated siderophores synthesized by *Pseudomonas* bacteria under iron deficiency conditions in a strategy to capture Fe^{3+} and cause acute infections. Three parts constitute pyoverdines: (i) the chromophore, (ii) a carboxylic acid side-chain and (iii) an either linear or cyclic peptidic part. Once loaded with Fe^{3+}, the complexes are internalized *via* specific TonB-dependent FpvA outer membrane receptors. The specificity of the receptor is quite relative and the bacteria are able to import exo-siderophores as well. In this study, we propose to design simplified pyoverdines, yet possessing all the structural requirements to bind FpvA, and featuring additional moieties such as antibiotics.



In a first step, we will study the iron chelating properties of a series of analogues by means of molecular dynamics simulations and compare them to data obtained on reference pyoverdines. Then, in a second step, and starting from available crystallographic structures, we will study the conformational differences between the apo-FpvA, Pvd-FpvA and Pvd(Fe)-FpvA systems and check very carefully the different interactions taking place and the nature of the different pockets present in the receptor. Eventually, we will use docking methods to dock the analogs onto the receptor so to check for their compatibility and potential siderophore behavior.

Keywords: pyoverdine ; siderophore ; Pseudomonas

Pyoverdine analogues: Trojan horse strategy against *Pseudomonas aeruginosa*

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Because of its resistance to classical antibiotics, *Pseudomonas aeruginosa* has become an important public health problem. To ensure its development, *P.aeruginosa* needs iron present in low quantity in biological media. To obtain it, *P. aeruginosa* produces the siderophore pyoverdine, which is secreted into the extra-cellular environment where it binds Fe^{3+} ions. Subsequently, the newly formed pyoverdine-iron complexes are transported back into the cell *via* specific receptor proteins named FpvA. Our objective is to synthesize analogues of pyoverdines, simultaneously featuring a significant siderophore activity and able to antagonize the FpvA receptor or/and carry antibiotics. As a first step toward this goal, we present the synthesis of promising analogues.

Keywords: Pseudomonas ; FpvA ; siderophore

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Rational design, synthesis and bioactivity of DAPY derivatives as potent HIV-1 NNRTIs based on the NNRTI/RT binding model

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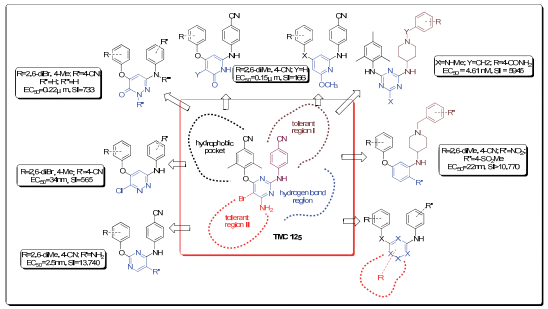
Diarylpyrimidine (DAPY) derivatives, represented by the clinically approved drugs etravirine (TMC125) and rilpivirine (TMC278), have developed a potential chemical skeleton with excellent activity against a large panel of HIV-1 mutant strains. However, a defect in bioavailability of most DAPY derivatives resulted from their low water solubility has significantly limited their clinical application. Numerous NNRTI/RT binding models and pharmacophoric analyses showed that, an excellent HIV-1 non-nucleoside reverse transcriptase inhibitor (NNRTI) should occupy at least three of the following RT regions in an NNRTI/RT binding model: a) the hydrophobic pocket, b) the hydrophilic tolerant region I, c) the hydrogen bond region and d) the tolerant region II. Guided by the binding model, a great deal of compounds which can be classified according to the modification region were designed and synthesized based on the lead compound TMC 125: modification aimed at the tolerant region I to improve the water solubility of the compounds; modification at the core ring with the purpose of generating multiple hydrogen bonds with RT which was important in improving the resistance profiles; and the modification specific to the tolerant region II to enhance the multiple affinity between the inhibitor and RT (**Fig.**). Many target compounds were verified to be potential lead compounds with potent anti-HIV-1 potency against both wild strains and mutant strains. The anti-activity results were rationalized by molecular simulation and the preliminary SAR was also studied which provided helpful guidance for further rational design of novel DAPY HIV-1 NNRTIs[1-5].



Keywords: HIV-1; NNRTI; DAPY; Binding model; Anti-HIV-1 activity

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Stilbene inclusion complexes as a natural-based strategy with improved anti-*Campylobacter* activity

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Foodborne illness still is a serious public health threat. Recent reports show that *Campylobacter* continues to be the leading cause of foodborne infections both in the EU and USA with *C. jejuni* and *C. coli* being the two most prevalent species both in human campylobacteriosis and chicken colonization. Due to the highly epidemic character of *Campylobacter* infections and also to the increased antibiotic resistance of these microorganisms [3], new approaches to their control are in demand. In this work, we investigated both the anti-*Campylobacter* activity and mode of action of stilbenes encapsulated in modified cyclodextrins [1] to circumvent the poor water solubility of these compounds and possibly potentiate stilbenes' antimicrobial activity. The results showed that pterostilbene and pinosylvin and their inclusion complexes (ICs) had antimicrobial activity against *C. jejuni* and *C. coli*. Furthermore, pinosylvin inclusion complexes had a bactericidal activity against both *Campylobacter* species at 37 ºC and this activity remained even at lower temperatures of 4 or 20 ºC. This bactericidal action is due to the membrane damage caused by these compounds, resulting in the impairment of several cellular functions such as membrane polarization, permeability and efflux activity [2]. This is the first study describing the antimicrobial activity or stilbene phytoallexins and cyclodextrins inclusion complexes against *Campylobacter* spp. and the results described here deeply encourage the use of pinosylvin as a preservative for the effective control of *Campylobacter* in foods.

Keywords: stilbenes; inclusion complexes; anti-*Campylobacter* activity; mechanism of action

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Structural and functional studies of novel Antimicrobial peptides from Chinese odorous frogs

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Antimicrobial peptides (AMPs) are small peptides with microbicidal properties. AMPs length varies from 6 to 50 amino acids. These peptides selectively interact with bacterial membranes and eventually kill the bacteria[1]. The growing problem of resistance to conventional antibiotics and the need for new antibiotics has stimulated interest in the development of antimicrobial peptides as human therapeutics. The skin of Chinese odorous frogs secretes an extreme diversity of natural antimicrobial peptides, which may serve as classic antibiotics' counterpart in solving life-threatening resistance of pathogenic microorganisms[2]. Peptidomic analysis of purified AMPs reveals that the post-translational modification rarely happens in odorous frogs and thus helps to characterize AMPs by cloning techniques. While a number of peptides have been isolated till date, their mechanistic aspects remain unclear. High-resolution three-dimensional structures of these potent antimicrobial peptides provide insights into the mechanism of action. This project is focused at cloning a few selected genes of frog peptides in pET32a+ expression vector, *E.coli* BL21 (DE3) expression and purification, chemical synthesis of short length peptides, antimicrobial activity assays and mechanistic studies. The isotopically enriched peptides have been expressed and purified by Ni-NTA chromatography for further studies by solution NMR.

Keywords: Antimicrobial peptides; NMR spectroscopy

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Study of antibacterial activity and toxicity of functional analogues of ubiquinone

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The design of new antimicrobials must include biological targets that are evolutionarily conserved to avoid the generation of resistance, be present in strains of clinical relevance, and to be part of critical mechanisms in bacterial metabolism. One such target is ubiquinone, an important component in the transfer of electrons in the electron transport chain, the regulation of reactive oxygen species generation and virulence factors and resistance. This multifunctionality makes this biological target attractive because its intervention ubiquinone analogues alter multiple metabolic processes, triggering irreversible events that will lead to bacterial death. In this work we designed, synthesized and evaluated functional analogs of ubiquinone. The novel compounds were synthesized from commercially available precursors, using one-pot synthesis and reductive Michael additions. Its purification was performed by column chromatography. Lipophilicity was calculated with ChemDraw software and on-line predictor ALOGPS 2.1. The half wave potential ($E_{1/2}$) was measured by the technique of cyclic voltammetry in a potential range of 0 to 2 volts in acetonitrile at room temperature. The minimum inhibitory concentration (MIC) by broth microdilution was determined in prototype strains *E. coli* methicillin-sensitive (ATCC 25922), *S. aureus* methicillin-resistant (ATCC 29213), *S. aureus* (ATCC 43300), *E. faecalis* (ATCC 29212), *P. aeruginosa* (ATCC 27853) and clinical strains *S. aureus* methicillin-resistant, *E. faecalis* and *E. faescium* according to CLSI protocol. Cytotoxicity against SHSY-5Y cells at 24 hours of acute exposure model was evaluated with the MTS colorimetric assay. The compounds with electron-withdrawing groups had MICs of 1 to 8 μg/mL in Gram positive strains. No activity was observed for Gram negative strains. Cell viability was 80% at the concentrations used as antibacterial($p>0.05$). 3D QSAR model was developed by using CoMFA technique in SYBYL X 1.2 program. The results of this study indicated that bulky groups adjacent to the electron-arylmercapto ring substituent may enhance the antibacterial activity. Based on this information was synthesized and evaluated a new dihaloderivative showed moderate activity. This result suggests that the obtained 3D QSAR model could be useful in directing the rational synthesis of a new series of compounds. Finally, all synthesized compounds showed antibacterial activity with low toxicity for normal cells. Acknowledgements: [1]FONDECYT 11110516, [2]FONDECYT 1130425; [3]FONDECYT 11130701; [6]FONDAP 15130011.

Keywords: ubiquinone, mercaptoquinone, antibacterial resistance, antimicrobials.

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Synthesis and Biological Activity Observation of Some New Thiazole Derivatives

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Thiazole derivatives are interesting compounds in medicinal chemistry field due to their diverse pharmacological activity profile [1]. Antifungal drug abafungin [2], acetylcholine esterase inhibitor acotiamide are clinically important thiazole containing agents. Thiazole residue is also sited in chemical structures of penicilins. Thus, in this study our research group synthesized some new thiazole derivatives so as to perform antimicrobial and anticholinesterase activity investigations. Structures of the synthesized compounds were confirmed by spectral analyses. MIC values of the tested compounds were recorded by microbroth dilution method [3] and enzyme inhibitions were measured by a colorimetric assay. Compounds **3c** and **3j** displayed remarkable acetylcholine esterase inhibitory activity. Most of the compounds displayed anticandidal effects that can be comparable with reference drug ketoconazole.

Keywords: Thiazole, anticandidal, microbroth dilution method

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Synthesis and anticandidal activity of some 2-mercaptobenzothiazole derivatives

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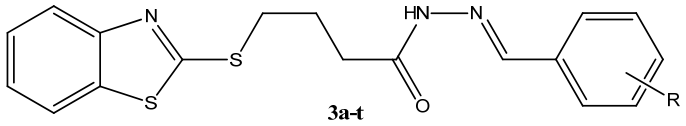
Infectious diseases constitute an important part of human diseases. In recent years, all over the world fungal infections, especially *Candida* infections have increased seriously. The main reasons for this situation are weakening of immune system because of a variety of diseases (AIDS), drugs (anticancer agents) and organ transplantation and also enhancement of microorganisms resistance to existing drugs. There is different approaches in treatment but, chemotherapeutic agents are still unavoidable [1,2].

Fungi, differing from bacteria are eucaryotic structure which are similar to human cell. This situation makes antifungal theraphy difficult. Polyene amphotericin B, lipopeptide caspofungin, and terbinafine and azole compounds are most commonly used drugs in the treatment of fungal infections by acting with different mechanisms of action [3].

In this study, we have performed the synthesis of new *N'*-(arylidene)-4-[(benzothiazol-2-yl)thio]butanohydrazide derivatives (**3a-t**) including 2-mercaptobenzothiazole structure which have been reported with high antifungal activity in many studies [4-6] besides lipophilic alkyl structure. Target compounds have obtained with a three step reaction starting from 2-mercaptobenzothiazole and the structure of the final compounds have been elucidated with IR, NMR, Mass spectroscopy data and elemental analysis results. Antifungal activity of the target compounds have been determined against different *Candida spp.* by using microdilution method. At the end of the study, the synthesis of new, pure substances have provided, thereby new compounds have gained to the literature. The anticandidal activity of the compounds (**3a-t**) were found in different ratios.

Keywords: 2-mercaptobenzothiazole; hydrazone; synthesis; antifungal; anticandidal



3a-t

R: H, CH_3, OCH_3, Br, Cl, F, NO_2

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Synthesis and Biological Activity of Some Novel Thiadiazole Derivatives

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Today, there is a necessary to design a new and different kind of antimicrobial agents to solve the problem of bacterial resistance to most of the known antibiotics [1]. Thiadiazole is a heterocyclic nucleus and it has occupied a basic position in medical chemistry because its derivatives have a wide spectrum of pharmacological activities especially potent antimicrobial activity against a wide variety of microbes like bacteria and fungi [2]. Therefore, in this study we synthesized a new series of 1,3,4-thiadiazole derivatives, and investigated their antimicrobial [3] and anticholinesterase activities so as to obtain new biologically active compounds[4]. Structures of the final compounds were confirmed by spectral data and elemental analyses. Final compounds, showed antimicrobial activities and anticholinesterase activities to different extents.

Keywords: Thiadiazole, antimicrobial activity, anticholinesterase activity

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Synthesis and in-vitro antimicrobial activity of novel succinimides derivatives

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A diversity of biological activities and pharmaceutical uses have been attributed to cyclic imides such as succinimides, maleimides, glutarimides, and their derivatives, such as antibacterial, antifungal, anticonvulsant, antitumor.

A series of cyclic succinimides derivatives were synthesized and their structure confirmed by FT-IR, ^{1}HNMR, ^{13}CNMR, elemental analysis.

In vitro antimicrobial activity of compounds was determined against *Enterobacter* ATCC 13048, *Klebsiella pneumonia* ATCC 700603, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 43071 as (Gram-negative) bacteria and *Enterococus faecalis* ATCC 29212, *Staphylococus* ATCC 25952 as (Gram-positive) bacteria was done by use of the paper disc diffusion method on Mueller Hinton agar (Merck). Chloramiphenicol and ciprofloxacin were standard reference antibiotics. The zone of inhibition against bacteria was measured after 24 hours at 37°C. Compounds 1, 3, 5were the main antibacterial compounds against Gram-negative, but not Gram-positive bacteria.

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Synthesis and very potent antistaphylococcal activity of polyhalogenated 2-phenylbenzimidazoles

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Since methicillin-resistant *Staphylococcus aureus* (MRSA) was firstly identified in 1961, it has been the leading cause of morbidity and mortality in various nosocomial and community-acquired infections [1]. With the increasing use of Vancomycin, as the last line of defense against MRSA in recent decades, vancomycin-resistant *Enterococccus faecium* (VRE) has emerged and can lead to bacteremia and death. Therefore, a tremendous need exists to discover novel anti-MRSA and anti-VRE agents for overcoming these emerging bacterial resistance problems [1]. On the other hand, halogenated compounds have become particularly important in higher value added pharmaceutical and agrochemical products [2]. One method to combat drug and antimicrobial resistance is to introduce small modifications (such as halogenation) to existing drugs, which can restore potency. Nearly 20% of drugs on the market and a quarter of new drugs in the developement pipline are halogented, clearly showing that halogenation can be a major advantage in new drug development [3].

Halogenated benzimidazoles and their derivatives have raised special interest because of their diversified biological activity [4]. For example, tetrabromobenzimidazoles and tetraiodo-benzimidazoles are known as very strong inhibitors of antiapoptotic protein kinase CK2. Among antiviral agents, benzimidazole nucleosides and acyclonucleosides have also received much attention. The 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB) and its derivatives (TCRB and BDCRB) were found to show activity against RNA and DNA viruses. Also some benzimidazole L-ribonucleosides, particularly 5,6-dichloro-2-isopropylamino-1-(β-L-ribofuranosyl)-benzimidazole (Maribavir) inhibit replication of human cytomegalovirus and have favorable safety profiles in animal species. Benzimidazole system is present in numerous antiparasitic, fungicidal, anthelmintic, and anti-inflammatory drugs. Substituted 2-trifluorobenzimidazoles are potent decouplers of oxidative phosphorylation in mitochondria. These compounds also inhibit photosynthesis and therefore exhibit appreciable herbicidal activity. Their antibacterial, antifungal, and antiprotozoal activity has been reported [4].

These finding have inspired us to widen the list of halogenated benzimidazoles and to test the new derivatives against selected Gram-positive *Staphylococcus aureus* (ATCC 25923) and methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300). We have prepared a series of 36 new halogenated benzimidazoles. Some of them exhibited very important activity against *Staphylococcus aureus* and MRSA. Among them, compound **11** (having tetrachloro atom) exhibited lowest MIC values with the 0.19 µg/mL.

In conclusion, our results suggest that new derivatives of polyhalogenated benzimidazoles are promising group of antistaphylococcal agents.

Key words: Antistaphylococcal activity, MRSA, polyhalogenated benzimidazoles.

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Synthesis Antifungal and Anticholinesterase Activity Evaluation of Some Substitituted Carbodithioic Acid (3,4-Disubstituted-Phenylcarbamoyl)-Methyl Esters

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It is well known that, dithiocarbamate derivatives have various pharmacological activities such as anticholinergic [1], antibacterial, antifungal [2] and herbicidal [3]. Hence, in the present study, we aimed to synthesis some Substitituted carbodithioic acid (3,4-disubstituted-phenylcarbamoyl)-methyl ester derivatives and investigate their antifungal activity. The structures of all the newly synthesized compounds were characterized by spectral data and elemental analysis. Target compounds were tested against Candida albicans, Candida glabrata, Candida parapsilopsis and Candida krusei using a microbroth dilution technique. Anticholinesterase activity evaluation by using Ellman's Assay [4] revealed that title compounds possess low enzyme inhibitory potency. The results showed that the final compounds displayed antifungal activity to different extends.

Keywords: dithiocarbamate, antifungal activity, Ellman's Assay

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Synthesis of New Substituted Hydroxy Heterocyclic Nitrogen Systems Derived from α, β-Unsaturated Ketones as Antimicrobial Agents

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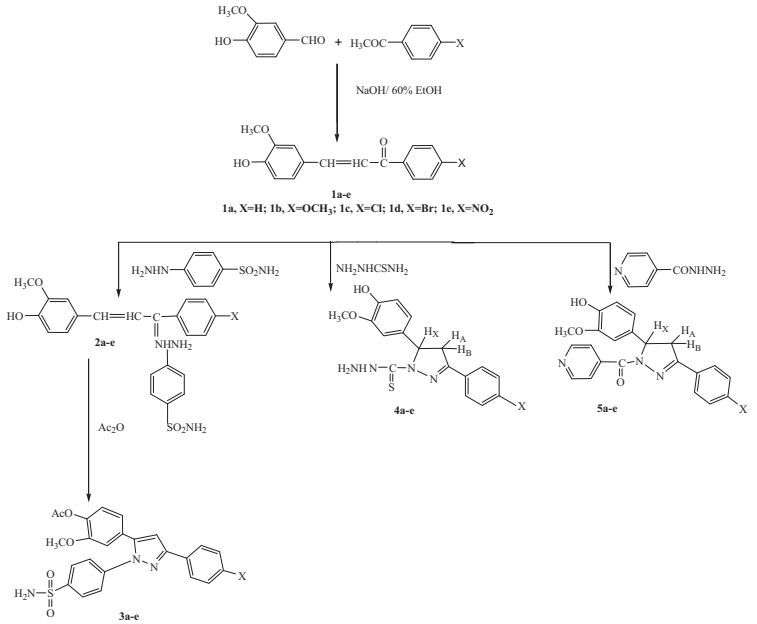
Number of α, β-unsaturated ketones were prepared by condensing 4-hydroxy-3-methoxybenzaldehyde (vanillin) with *p*-substituted acetophenones in 60% ethanolic NaOH solutions. The characterized chalcones were reacted with *p*-sulfamyl phenyl hydrazine in glacial acetic acid to give the corresponding hydrazones**,** which on treatment with acetic anhydride gave the corresponding acetoxy sulfonamide pyrazole derivatives. Refluxing of chalcones with either thiosemicarbazide or isonicotinic acid hydrazide in ethanol containing few drops of acetic acid gave pyrazoline-1-thiocarboxamides and isonicotinoyl pyrazolines, respectively. The synthesized compounds were fully characterized on the basis of their elemental analyses and spectroscopic data. All of the newly isolated compounds were tested for their different biological activity.



Synthesis of some novel Antimicrobial Sulfonamid-arylazo H-Acid

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Several new of sulfonamide based reactive dyes (D1-D4) has been synthesized by coupling reaction of sulfonamide diazonium salt with sulfonamido-cyanurated H-acid. The chemical structure of the synthesized dyes was secured by their spectral data e.g. Elemental analysis, IR, ^{1}HNMR and MS spectroscopy. The principle advantage here for using sulfonamide based moiety is that the activity of antimicrobial is high, short reaction time and reaction procedure is done in few steps, the work up is convenient and thus the starting material can be easily found.

Keywords: Sulfonamide; H-acid; antimicrobial; reactive dyes.

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Synthesis, Anticandidal and Anticholinesterase Activity of Some Benzothiazole Derivatives

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Azoles constitute a class of drugs that are the first choice in antifungal medication. Benzothiazol is a nitrogen and sulphur containing ring system reported with varying pharmacological activity profile as antifungal, antibacterial [1], anticancer [2] and anticholinesterase [3]. Hence, in the present study we designed and synthesized some novel benzothiazole compounds to investigate their antifungal activity and inhibitory potency against acetylcholine esterase enzyme. Structure elucidations were performed by spectroscopic methods. Compounds did not exhibit notable enzyme inhibitory activity. However, moderate anticandidal effects were observed with some of the compounds in the series.

Keywords: Benzothiazol, anticholinesterase, anticandidal

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The design and functional characterization of the antimicrobial and antibiofilm activities of MelitAP-27, a rationally designed hybrid peptide

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Background: Many pathogenic and free living and biofilm forming bacterial organisms are resistant to almost all known conventional antibiotics. Global research is currently focused on finding novel therapies to counteract the threat of bacterial and biofilm infections rather than using conventional antibiotics. Antimicrobial peptides (AMPs) represent an attractive group of molecules for development as novel therapeutics for the purpose of combating microbial infections and specifically the ones that are caused by resistant forms of bacteria.

Methods: In this project, we have rationally designed a hybrid antimicrobial peptide by using two antimicrobial peptides named Melittin and BMAP-27 as platforms for structural analysis of the amino acids participating in the formation of the helical fragments of the peptides and the generation of a novel hybrid peptide named MelitAP-27. MelitAP-27 was assesed for its antimicrobial and antibiofilm activity against representative strains of planktonic and biofilm forming Gram-positive and Gram-negative bacteria. The time needed to kill the bacterial strains in logarithmic and stationary growth phases was also evaluated after peptide treatment. Adiditionally, the cytotoxic activity of the peptide was assesed against human mammalian cells and eryhtrocytes using cell culture techniques and hemolytic assays. The effect of the hybrid peptide and its mechanism of action against bacterial membranes was evaluated using the β-galactosidase assay.

Results: Our results show that MelitAP-27 displays potent antimicrobial activites against a range of Gram-positive and Gram-negative planktonic bacteria with MIC values in the range of 1 to 5 μM. MelitAP-27 was also able to eradicate *Pseudomonas aeruginosa* biofilm formation while showing weak hemolytic activity towards human erythrocytes and mammalian cells. Studies on the mechanism of action MelitAP-27 revealed that the peptide is probably inducing bacterial cell death through membrane permeabilization determined by the release of β-galactosidase enzyme from peptide treated *E. Coli* cells.

Conclusion: The results of our studies idicate that MelitAP-27 has a considerable potential for therapeutic application as a novel drug candidate for eradicationg bacterial infections

Keywords: Antimicrobial peptides; Hybrid peptide; Antibiofilm

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The evaluation of sonication influence on antimicrobial efficacy of ZnO nanoparticles

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The nanoparticles of ZnO are well – known, because of their antimicrobial activity [1]. The purpose of this research work was to optimize the sonication parameters and autoclaving conditions to obtain the stable ZnO nanoparticles. The aim was also to evaluate the influence of sonication on the antimicrobial properties of ZnO nanoparticles. The 5 different types of commercial nano-ZnO samples were examined during the experiments:

1)Alta / Aesar, Nanotek C2, 44901, organosilanes coated hydrofobic;

2)AMCN 123, ~ 20 nm, spherical;

3)AMCN 122, 90-210 nm, mixed shapes;

4)AMCN 122 , 90-120 nm, mixed shapes (referred to as 122a);

5) And AA 44899, ~ 70 nm, mixed shapes.

The *S. aureus* DSMZ 346 and *Escherichia coli* DSMZ 498 were used in this study to test antimicrobial properties of nanoparticles. The 0.1% aqueous solutions of ZnO were prepared using all of commercial samples. The solutions were sonicated. The different parameters of sonication were compared during the tests. As references 0.1% ZnO samples which were magnetically stirred have been used. These samples were not sonicated. After sonication and stirring the particle size and distribution of ZnO nanoparticles were measured.

The results of the experiments showed that AMCN 123 ZnO sedimented very fast. The sample of C2, 44901 had hydrophobic character. It was not possible to sonicate it in water. Because of the best particle size distribution and the highest stability after sonication, AA 44899, ~ 70 nm (mixed shapes) has been chosen for the further tests. The most effective parameters of sonication (used to prepare the samples for microbiological tests) were: cycle – 0.5; amplitude – 50%; and time – 30 minutes.

The antimicrobial properties of these nanoparticles were checked as well. Further attempt was made to determine the effect of autoclaving on the antimicrobial activity of ZnO. Two solutions were prepared: AA 44899 sonicated and AA 44899 sonicated and autoclaved.

The results of experiments showed that 1 ml – 0.1% aqueous dispersions of ZnO had no activity against *S. aureus* strain (no difference in the bacteria growth in the comparison to the references – sample without ZnO nanoparticles). The higher volume of 0.1% aqueous dispersions of ZnO (2 and 4 ml) showed moderate activity against *S. aureus*. In addition, it has been shown that the autoclaving had no positive effect on antimicrobial properties of ZnO. As it has been demonstrated the ZnO had very low activity or none activity against *E. coli* strain. In addition, in this case the autoclaving had no positive effect on antimicrobial properties of ZnO as well.

Keywords: ZnO nanoparticles, sonication, antimicrobial activity

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Theoretical investigation on the origin of the stereoselectivity in the alkylation of 2-oxopiperazine enolates

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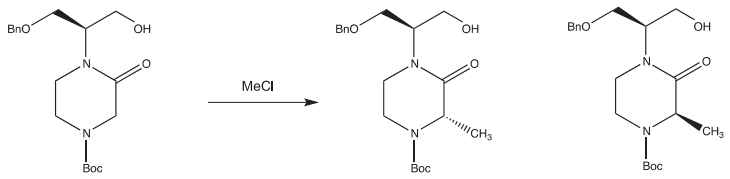
Piperazines, consisting of a six-membered ring containing two nitrogen atoms in opposite position, are moieties present in numerous drugs as they possess important pharmacological and anti-microbial properties. Substitution of a piperazine might lead to more potent drugs if the synthesis is controlled. Indeed, the piperazine core presents four asymmetrical centers.

The aim of this study is to explore the origin of the stereoselectivity upon alkylation of 2-oxopiperazines by means of quantum chemical calculations. Upon alkylation of the reagent **1**, products **2** and **3** could be obtained, theoretically, in equal amounts (see figure). Actually, it has been experimentally demonstrated that if the chirality of the reagent **1** is *R*, than the product **2** is obtained in a 97 % yield.



1 **2** **3**

To gain a better understanding of this stereoselectivity, in a first step, conformational searches will be performed on the reagents and both the products in order to determine the most stable structures of each entity in different environments (gas phase and solvent). Then in a second step, we will investigate the transition states structure so to establish the possible reaction paths leading to each product. The relative free energy differences corresponding to the reactions are evaluated as well. Moreover, structural analyses of the different paths will provide some insight on stereoselectivity. Indeed, upon complexation, phenomenons occur, such as: (i) formation of hydrogen bonds, (ii) steric hindrance at the complexation site, (iii) steric hindrance of the substituent and (iv) modification of the piperazinic cycle conformation.

Keywords: piperazine ; alkylation ; anti-microbial ; ab initio

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Time-kill curve kinetics of 4-chloro-*N*-{(2*S*)-1-[(3,4-dichlorophenyl)amino]-3-methyl-1-oxobutan-2-yl}-2-hydroxybenzamide against multidrug-resistant clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA)

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Despite antibacterial therapy, methicillin-resistant *Staphylococcus aureus* (MRSA) infections are still associated with serious clinical consequences, especially treatment failure, higher morbidity and mortality, prolonged hospitalization, increased health care costs, etc. Considering ineffectiveness of vancomycin, the first line therapy of MRSA infections, in the recent years, it will be necessary to broaden the spectrum of anti-MRSA drugs in the future. Because of the increasing numbers of immunocompromised patients with meningitis or endocarditis, bactericidal effect will probably be desired in this new generation of antibacterial drugs.

The synthesis of a novel salicylanilide derivative, 4-chloro-*N*-{(2*S*)-1-[(3,4-dichlorophenyl)amino]-3-methyl-1-oxobutan-2-yl}-2-hydroxybenzamide (**Figure 1**), with supposed antibacterial activity was described previously [1]. The aim of the current study is evaluation of new biological characteristics of this compound, the dependence of bactericidal activity on time and concentration against three clinical isolates of MRSA and *S. aureus* ATCC 29213 as a reference strain. Time-kill curve assay with a final concentration of the compound equal to 1×, 2× and 4× MIC and aliquots removed at 0, 4, 6, 8 and 24 time points was used for this evaluation.

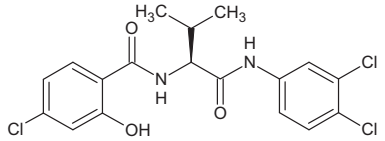

Figure 1. 4-chloro-*N*-{(2*S*)-1-[(3,4-dichlorophenyl)amino]-3-methyl-1-oxobutan-2-yl}-2-hydroxybenzamide.

No bactericidal effect was recorded for the *S. aureus* reference strain. Bactericidal effect against MRSA 63718 was maintained at 4× MIC at 6 and 8 h after incubation (with a reduction in bacterial count of 3.54 and 3.31 $\log_{10}$ CFU/mL, respectively). For MRSA SA 630 concentration-dependent killing was observed at 4× MIC at 6, 8 and 24 h after incubation with $\log_{10}$ differences in CFU/mL ranging from 3.18 to 3.39 $\log_{10}$ CFU/mL. For MRSA SA 3202 bactericidal effect was maintained only at 4× MIC at 24 h after incubation with a reduction in bacterial count of 3.02 $\log_{10}$ CFU/mL. Thus, 4-chloro-*N*-{(2*S*)-1-[(3,4-dichlorophenyl)amino]-3-methyl-1-oxobutan-2-yl}-2-hydroxybenzamide can be a suitable candidate for a novel bactericidal anti-MRSA agent presenting a promising starting point for further investigations.

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Keywords: MRSA; salicylanilides; bactericidal; time-kill assay

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Non-antibiotic biocides

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Control of bacteria isolated from frozen foods using preservatives

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In recent years, various kinds of foods (perishable foods such as fresh vegetables, fish and shellfish, and precooked foods) have been delivered at low-temperature or frozen conditions. To secure their quality and safety, the temperature must be maintained at a set level through distribution (storage and transport). When the temperature is not controlled, bacteria can increase in the partially defrosted food. The purpose of this study was to observe the bacterial growth during the transitory thaw of frozen food and determine how to control the bacteria using preservatives.

Leuconostoc mesenteroides, *Staphylococcus lentus*, *Pseudomonas fluorescens* and *Listeria grayi* were isolated from the delivered frozen foods (Japanese new year‘s dishes, fresh vegetables, and shrimp) under culture conditions at 7℃ for 10 days. Their optimum growth temperatures were 20, 35, 25 and 10℃, respectively. Frozen hamburger samples inoculated with the test bacteria (four isolates and *Yersinia enterocolitica* NBRC105693) were prepared, and defrosted at 25℃ for 6, 12, and 24 hours. The number of bacteria inoculated to the surface of the sample increased considerably. In particular, *L. mesenteroides* and *L. grayi* showed more rapid growth than other bacteria, and the number of both bacteria inoculated on the inside also increased. The results indicate that contamination with bacterial strains having a low optimum growth temperature can be a cause of food spoilage or food poisoning.

To reduce this risk, the antibacterial effects of food preservatives, including a food preservability improving agent, were investigated. Nisin A, in the form of Nisaplin (containing 2.5% nisin) obtained from San-Ei Gen F.F.I., Inc., Japan, showed high bacteriostatic and bactericidal action against *L. mesenteroides* (MIC=31.3ppm, MBC=62.5ppm) and the other gram positive bacteria tested. *n*-Butyl *p*-hydroxybenzoate exhibited a high activity against *S. lentus* (MIC=500ppm) and *L. grayi* (MIC=1,000ppm). Acetic acid and citric acid also exhibited an activity (MIC=2,000ppm) against all bacteria except for *L. mesenteroides*. However, the other preservatives used in this study, such as potassium sorbate, sodium benzoate, sodium acetate, and glycine, were not effective against all the bacteria tested. It was thought that food preservatives such as nisin A were useful in controlling the gram positive bacteria that can contaminate frozen foods.

Keywords: food preservative; antibacterial activity; delivered frozen food

Efficacy of neutral electrolysed water against *Pseudomonas* spp. in washing contaminating ready-to-eat vegetables

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In the present study, we evaluated the antimicrobial activity of neutral electrolyzed water (NEW) against 14 strains of *Pseudomonas* responsible for spoilage of the leaves of lettuce 'iceberg', 'trocadero' chicory and endive under cold storage. Active chlorine concentration was found stable for 14 minutes also when it was in contact with mixed vegetables; in addition, NEW resulted to be bactericide, within 2 min, for all concentrations of free chlorine above 100 ppm.

When catalogna chicory and lettuce leaves were dipped for 5 min in a NEW solution microbial loads of mesophilic bacteria counts and *Enterobacteriaceae* were reduced on average of 1.7 log cfu/g. This work demonstrates that in situ production of active chlorine, via electrolysis of a diluted sals solution, can be efficiently used against spoilage bacteria contaminating fresh cut vegetable.

Keywords: RTE vegetables, improvement microbial quality, shelf-life, risk management

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Influence of an acidifier on antibiotic resistant *E.coli* counts in feces of weaning pigs

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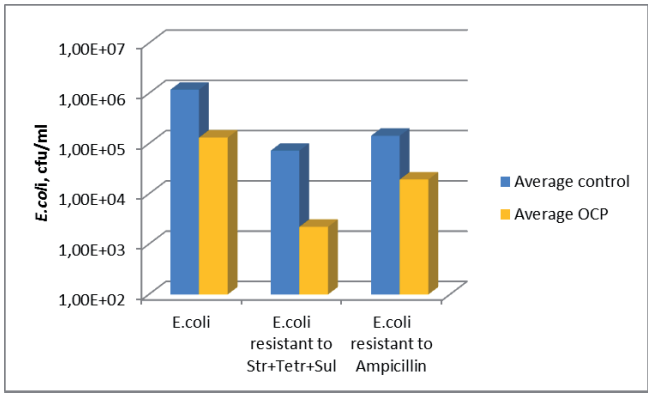
The control of antibiotic resistance has become a health priority worldwide and trials are needed to reduce the amount of resistant bacteria and find solutions of this complex issue.

The experiment was conducted to study the effects of the addition of the mixture (OCP) of organic acids, cinnamaldehyde and permeabilizer in swine diet on growth performance and fecal content of antibiotic resistant *E.coli*. Sixty weaning pigs were assigned to two treatments with three replicate pens per treatment and ten pigs per pen. The negative control group diet contained no feed additives, whereas the trial group was fed OCP. Fecal samples of four pigs in one pen were pooled and three samples per group from three pens were collected at the end of the trial on day 42. Results showed that body weight, average daily gain and feed intake were numerically higher in the trial group and feed conversion ratio was lower in the trial group compared with the control group. The number of total *E.coli* count (Figure 1) in the fecal samples of the group fed OCP was 1 log below the control group, the number of resistant *E.coli* to Ampicillin in the trial group was 1 log below the control group. The number of multi resistant *E.coli* to Tetracyline, Streptomycine and Sulfomethoxazole in the trial group was nearly 2 logs below the control group. Reduction of fecal antibiotic resistant bacteria in swine leads to the reduced resistant bacterial load in the environment, which lowers the transmission of the resistance genes to other environmental bacteria.

Figure 1: Average counts of *E.coli* in fecal samples of pigs, cfu/ml



Keywords: antibiotic resistance, acidifier, *E.coli*

Modulatory effect of LL-37 (cathelicidin) peptide in human macrophages stimulated by LPS

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The use of antimicrobial peptides (AMPs) such as LL-37 (cathelicidin), has demonstrated satisfactory effects not only as antimicrobial agents but also as modulators of the inflammatory immune response. The aim of this study was to evaluate the modulatory effect of LL-37 peptide (cathelicidin) in the quantification of tumor necrosis factor α (TNF-α) in macrophages after stimulation with *E. coli* and *P. gingivalis* lipopolysaccharide (LPS). Initially the peptide was dissolved in RPMI supplemented with 1% fetal bovine serum at concentrations of 0.2 mM, 0.5 mM, 1.0 mM, 2.5 mM, 5.0 mM and the cytotoxicity was determined using the MTS reduction assay (Methyl-tetrazolium (3 - (4,5-dimethyl-2-yl) -5 - (3-Carboxy methyl phenyl) -2 - (4-sulfophenyl)-2H-tetrazolium.The macrophages were cultured in culture medium (RPMI-1640 supplemented with 10% fetal bovine serum) at 37°C in 5% CO_2. The ability to modulate the production of TNF-α was measured after cell stimulation of macrophages with LL-37 peptide (different concentrations) for 24 hours, in the presence and absence of *E. coli* (1 μg/ml) and *P. gingivalis* (100 ng/ml) LPS. Enzyme linked immunoassay (ELISA) was performed to determine the TNF-α levels. The results were analyzed by Student's t test with $p < 0.05$ (SPSS). It could be observed by absorbance analysis that the cell viability was above 90%, showing that high concentrations of LL-37 peptide have no cytotoxic effect. Low concentrations (0.2 mM, 0.5 mM and 1 mM) of LL-37 peptide showed modulatory effect ($p < 0.05$) in reduction of TNF-α in macrophages after stimulation with both LPS. The LL-37 peptide could be considered an important regulator of immune responses and in the future could be considered as a therapeutic agent in reducing the inflammatory cytokines.

Acknowledgements: This work was supported by State of São Paulo Research Foundation (FAPESP), Grant # 2012/15705-7

Keywords: antimicrobial peptides; inflammation

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Postadaptional resistance to antibiotics of bacteria from organic foods

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The indiscriminate use of antibiotics, antiseptics and disinfectants on microorganisms has generated a survival response, which enables bacteria to efficiently prevent the bactericidal action of these agents. The widespread use of these compounds generates expectations about the possible increase in bacterial resistance caused by environmental pressure produced by these antimicrobials, and focuses the interest in the possible cross-resistance to antibiotics [1]. There is growing concern worldwide regarding the possible effect of biocides on antibiotic resistance and recent studies suggests that the selective pressure because of the use of biocides at sub-lethal concentrations could contribute to the expression and dissemination of antibiotic resistance mechanisms [2]. In recent years, there has been renewed interest in the antibacterial properties of triclosan. This stems from the finding that triclosan has a specific mechanism of action and that there might be a link between triclosan usage and antibiotic resistance [3].

The aim of this study was to evaluate the effect of a selective pressure produced by contact with growing sublethal concentrations of triclosan on the tolerance to antibiotics of 76 sensitive Gram-positive and Gram-negative bacteria isolated from organic foods. The tolerance to triclosan of these bacteria was gradually increased by serially inoculating into TSB containing growing concentrations of triclosan. Next, we studied the co-resistance of the adapted strains to clinically relevant antibiotics (ampicillin, cefotaxime, ceftazidime, ciprofloxacin, tetracycline, nalidixic acid, sulfamethoxazol and the combination sulfamethoxazol /trimethoprim). Initial and final levels of resistance were determined by minimum inhibitory concentration (MIC) tests according to CLSI [4].

21 of the 39 strains that were able to grow in increasing sub-inhibitory concentrations of triclosan, showed an increased tolerance to sulfamethoxazol, with postadaptional MICs values of twice the MIC interpretative strandard of antibiotic-resistant strains defined by CLSI standards. 13 strains acquired resistance to cefotaxime and two strains showed a postadaptional double MIC as compared to the MIC detected before the triclosan selective pressure. 18 strains increased their MICs against ampicillin or ceftazidime after triclosan exposure, 5 strains showed higher MICs against trimethoprim/sulfamethoxazol and 2 strains against nalidixic acid. Only one strain showed increased MIC against imipenem, ciprofloxacin or tetracycline after contact with triclosan. On the other hand, 10 strains showed lower MICs against cefotaxime after contact with triclosan, 6 strains showed decreased resistance against ceftazidime and 5 strains against ampicillin, so all these strains turned to be sensitive to these antibiotics, according to CLSI standards, after the selective pressure exerted by triclosan.

Since efflux pumps are not specific as to the substrates they can accommodate (eg. biocides and antibiotics), exposure to biocides and biocide tolerance could facilitate the prevalence of antibiotic resistant strains in the food industry. This study corroborates that microorganisms acquire varying levels of resistance or tolerance to environmental stresses, so this might provide protection for foodborne pathogens against antimicrobials and preservation processes. More research is needed to determine the frequency and mechanisms of resistance to food antimicrobials and process stresses in food systems and the application strategies to minimize tolerance or resistance development.

Keywords: organic foods; biocides; antibiotics.

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Postadaptional resistance to biocides of bacteria from organic foods

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Biocides are widely used in the food industry with several purposes such as decreasing the risks of transmission of human foodborne pathogens through the food chain, both from raw materials and by contamination during processing, inactivating food spoilage bacteria, and keeping food processing facilities and equipment free from microbes. In addition, they are widely used in sanitary settings where they play an important role in limiting the potential sources of infection[1]. But it has been suggested that exposure to sub-lethal concentrations of biocides could potentially have an impact on the responses of bacteria to commonly used food processes, enabling microorganisms to survive challenges such as the concentrations of biocides currently permitted for use in food environments [2].

The phenylether or chlorinated bisphenol, triclosan, is an antimicrobial agent that has been employed for a variety of purposes for more than 20 years. It is used clinically and in oral hygiene products, and is incorporated into many types of cosmetic formulations [3].

In the present study the tolerance to triclosan of a collection of 76 Gram-positive and Gram-negative sensitive bacteria isolated from organic foods was gradually increased by serially inoculating into TSB containing growing concentrations of triclosan. Next, we studied the co-resistance of the adapted strains to other biocides. Initial and final levels of resistance were determined by minimum inhibitory concentration (MIC) tests according to CLSI [4].

39 of the 76 studied strains (51.3%) were able to grow in increasing sub-inhibitory concentrations of triclosan, showing 2 to 2000-fold higher MICs than the original sensitive strains. As to other biocides tested, the highest tolerance after selective pressure caused by triclosan was obtained for chlorhexidine (87% of the triclosan-adapted strains showed 2 to 18-fold increases in their MICs against this biocide), followed by benzalkonium chloride (69% of strains increased their MICs from 2 to 300-fold) and hexachlorophene (61% of the strains showed 2 to 30-fold increases in their MICs). Lower percentages of the adapted strains showed increased MICs against cetrimide (46%), didecyldimethylammonium bromide (36%) and hexadecylpyridinium chloride (23%) after the selective pressure induced by triclosan. None of the strains tested showed increased tolerance to any of the tested biocides after growing in presence of triclosan.

Genetic determinants of efflux pumps conferring resistance had been previously described in these strains from organic foods [5,6]. Since efflux pumps are rather non-specific as to the substrates they can accommodate and they seem to be the most abundant determinants of antimicrobial resistance among biocide-tolerant bacteria, exposure to biocides could facilitate the prevalence of multi-resistant strains in the food industry. Proper cleaning and disinfection (as well as rotation of disinfectant products) might help to prevent the spread of biocide-tolerant bacteria in the food chain.

Keywords: Biocide resistance; Organic foods.

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Resistance to biocides and antibiotics following adaptation to quaternary ammonium compounds in food-associated bacteria

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Both antibiotics and biocides are chemical agents playing a crucial role in limiting the spread of infection and disease. Unlike antibiotics, biocides are widely used in different fields (household, medicine, animals, foods .....). Food industry has increased its use of biocides to satisfy consumer demands of healthy and minimally processed foods and to guarantee food safety. Concerns about the risk of selection of resistant bacteria to antibiotics with regard to the increased use of these agents have been addressed. It is well acknowledged that the main reason for antibiotic resistance increase is linked to the overuse and misuse of antibiotics in human and veterinary medicine leading to a treatment failure. The contribution of other factors such as biocides is difficult to evaluate. When they are applied during cleaning and disinfection practices at the concentrations recommended by the manufacturer, they are effective to inhibit or to destroy bacteria in the most cases. But, the biocides can be used in conditions (biofilm, insufficient rinsing...) that decrease their efficient concentrations. In consequence, this may select less susceptible bacteria to biocides coupled with the potential for the development of cross-resistance to clinically important antibiotics.

The aim of our study was to assess the effects of repeated exposure to a quaternary ammonium compound (QAC) on the susceptibility of food-associated bacteria to other biocides and antibiotics. This *in vitro* experiment was used to predict a risk associated with biocide use.

Strains (n=136) isolated from pig production chain were studied for each of the following species: *Listeria monocytogenes, Salmonella enterica, Campylobacter coli* and *Escherichia coli*. Bacteria were adapted by a daily exposure to increasing sub-inhibitory concentrations of didecyldimethylammonium chloride (DDAC) for 7 days [1]. Susceptibility tests against antibiotics and five biocides (4 active substances and one biocide formulation) were performed using a standard microdilution method.

Following adaptation, similar levels of reduction in susceptibility to DDAC were observed for *E. coli* and *L. monocytogenes*, with a 3-4-fold increase in the minimum inhibitory concentration (MIC) compared to initial MIC. For each bacterial species, no significant difference was observed in the susceptibility to hydrogen peroxide and sodium hypochlorite. Antibiotics MIC was increased in *Salmonella* but to a lesser extent in terms of antibiotics numbers and of magnitude than in *E. coli*. Moreover, most of *E. coli* strains acquired resistance to 1 or 2 new antibiotics, and two strains became resistant to 7 antibiotics. Antibiotic susceptibility was mainly reduced from 4 to 8-fold among *Salmonella* strains, but did not significantly change for *Listeria* and *Campylobacter* strains. Repeated exposure of bacteria to DDAC at subinhibitory concentrations may reduce their susceptibility to antibiotics that may be helpful for human therapy. In consequence, misuse of QAC-based disinfectants could lead to the emergence of antibiotic-resistant bacteria and may represent a public health risk. In the future, these results will be compared to epidemiological data to determine if it is a good model to predict the impact of biocide exposure on the modification of bacterial antibiotic susceptibility.

We acknowledge the financial support provided by the Contrat Plan Etat-Région administered by the Agriculture Ministry.

Keywords: Adaptation, Disinfectant, Antibiotics, Reduced susceptibility, Resistance

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Silver nanoparticles as antibacterial towards *Listeria monocytogenes*

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Bacterial resistance to antibiotics has increased worldwide, leading to treatment failure against pathogens responsible for human and animal infectious diseases (1). However, *in vitro* evidence has shown that biocides, used indiscriminately in an increasing number of applications, can also play a role in the development (or selection) and dissemination of biocides resistant pathogenic bacteria (2).

Recently, nano-technology has produced several new antibacterial agents, including metal based nanoparticles. This new generation of antibacterial substances is particularly attractive in that it might allow the development of new tools to circumvent bacterial pre-existent resistances.

Among the different metal nano-agents that can easily be purchased on the market, great interest exists toward silver nanoparticles (AgNPs) because of their strong biocidal effect against different bacteria species. Indeed silver has been used for centuries as an antimicrobial (3) to fight infections and prevent spoilage (4), and it is well known that silver ions and silver based compounds can be highly toxic to Gram-negative and Gram-positive microorganisms (5).

The purpose of the present work was to test *Listeria monocytogenes* sensitivity to AgNPs and to investigate possible resistance phenomena in a panel of wild strains isolated from different food matrices.

Listeria spp. is recognized as ubiquitous in the environment and it is well known to be persistent in food processing plants probably due to complex mechanisms of adaptation, including the growth at low temperatures, biofilm formation and resistance to antibiotics and biocides. Nevertheless, regulation EC 2073/2005 on microbiological criteria for foodstuffs compels food business operators to observe very strict criteria regarding *Listeria monocytogenes* in food intended for direct human consumption.

Thus, it is of public health interest to investigate the efficacy and applicability of new types of safe and effective biocidal compounds.

Listeria strains, obtained from the collection of pathogenic microorganisms of the Food Microbiology Laboratory (Istituto Zooprofilattico Sperimentale delle Venezie), were serotyped and tested for their antimicrobials susceptibility. AgNPs size and morphology were appropriately evaluated. Finally, the antimicrobial activity of AgNPs and Ag^+ were determined by assaying the number of culturable *Listeria* cells which formed colonies after incubation in the presence of AgNPs (300 ppm) or Ag^+ (10, 20, 30 ppm), in a time course experiment.

Results showed that both AgNPs and Ag^+ treatments reduced *Listeria* growth. However, a delayed effect caused by AgNPs if compared to $AgNO_3$, was observed.

The present study demonstrates that AgNPs can be effective as an antimicrobial even in the case of *Listeria monocytogenes*. A previous study with Salmonella (6) demonstrated an antimicrobial activity strongly strain-dependent; in contrast this experiment failed in demonstrating differences among strains.

This could be due to a lack in significant differences in antimicrobial profile among selected strains or to a low or absent selective pressure in *Listeria monocytogenes* environment.

Nevertheless, even though there is circumstantial evidence that these particles have good antimicrobial activity, the exact way in which they exert this activity is still speculative and probably strongly dependent on the broad variability of NPs biological activities that depend not only on their chemical form but also on the specific shape and dimensional range.

Keywords: *Listeria monocytogenes*; Silver nanoparticles, antimicrobials

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Study of antimicrobial compounds for the footwear sector

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Natural compounds have been applied for the treatment of various illnesses since ancient times. Among these, essential oils or zinc oxide, for instance, are being used in very different sectors, such as the food industry[1], or in finished industrial products or human hygiene products.

One of the potentially interesting applications of these compounds relates to the ability to inhibit microbial growth on feet, which is a source for problems such as foot odour or the worsening of certain conditions (athlete's foot, ulcer or wound infection in diabetics, etc.). This study aims to check whether a commercial preparation based on natural oils and a zinc oxide nanoparticle emulsion obtained by chemical synthesis can effectively serve in controlling microbial proliferation that takes place in footwear components and is caused by wear.

To this end, several testers wore shoes provided with EVA insoles for 15 days (8 hours/day). After that time, the preparations were applied onto one of the insoles of each tester. The other insole remained untreated, thus representing the control condition of the tester.

Antimicrobial growth was assessed using contact plates containing different generic microbiological culture media for bacteria and fungi, both in the metatarsal and heel areas of each insole, before and after the application of the preparations.

The results proved that, under the study conditions, both preparations reduced bacterial count and fungal coverage by up to 99% in certain cases.

In light of these results, it can be ascertained that biocides based on natural oils and those obtained by chemical synthesis are valid choices for use as reducing agents for microbial flora grown in footwear.

Keywords: antimicrobial agents; natural extracts; fungi; bacteria.

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Antimicrobial physics

Antibacterial activity of silver nanoparticles: sensitivity of different *Salmonella* serovars

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Salmonella spp. represents a major challenge in animal health and food safety; consequently great interest exists in reducing its impact in human health by lowering its prevalence in the food chain, through a farm to fork approach. Different control measures have proven to be effective against *Salmonella* at farm level, such as vaccination and proper hygiene management, whereas the use of antimicrobials is forbidden according to EU Regulation 1177/2006, due to the risk of spread of antimicrobial resistance (1). Thus, it is interesting to investigate the efficacy and applicability of new types of safe and effective biocidal compounds.

Silver has been used for centuries as an antimicrobial (2) to fight infections and prevent spoilage (3), and it is well known that silver ions and silver-based compounds are highly toxic to Gram-negative and Gram-positive microorganisms (4). Previous studies have shown that antimicrobial formulations in the form of nanoparticles (NPs) could be used as effective bactericidal materials due to their enhanced reactivity, resulting from their high surface/volume ratio (5). Particularly, silver in the form of nanoparticle (AgNPs) is known to exhibit strong biocidal effects on different bacterial species (2,6) including multidrug resistant bacteria. However, few data concerning their success against different *Salmonella* serovars are available. Aims of the present study were to test the antimicrobial effectiveness of silver in the form of nanoparticles (AgNPs) against different *Salmonella* serovars (Enteritidis, Hadar, Senftenberg) and to investigate the causes of their different survival ability, when identified, from a molecular point of view.

Salmonella strains, obtained from the collection of pathogenic microorganisms of the OIE/National Reference Laboratory for Salmonella (Istituto Zooprofilattico Sperimentale delle Venezie), were serotyped and tested for their antimicrobials susceptibility. AgNPs size and morphology were appropriately evaluated.

The effectiveness of AgNPs as an antimicrobial was determined by assaying the number of culturable bacterial cells which formed colonies after incubation in the presence of AgNPs or $AgNO_3$.

The three *Salmonella* serotypes, Senftenberg, Hadar and Enteritidis, were screened by RT-PCR for the presence of the SilB gene, which is one of the most plausible resistant determinants found to be involved in both silver and copper resistance pathway of many gram negative bacteria (7).

Results showed an immediate, time-limited and serovar-dependent reduction of bacterial viability. In the case of *S.* Senftenberg, the reduction in numbers was observed for up to 4 h of incubation in the presence of 200 mg/L of AgNPs; on the contrary, *S.* Enteritidis and *S.* Hadar resulted to be inhibited for up to 48 h.

RT-PCR experiments demonstrated the constitutive expression of the plasmidic silver resistance determinant (SilB) by *S.* Senftenberg, thus suggesting the importance of a cautious use of AgNPs.

The present study demonstrated that AgNPs can be effective as an antimicrobial even in the case of *Salmonella,* but that its success is strongly *Salmonella* strain-dependent, since great differences in terms of effective dose and time of action were observed for the three investigated serovars. This is probably due to many factors strictly related to the genetic features of each strain, including the presence of specific genetic determinants of resistance, as demonstrated in the case of *S.* Senftenberg, which specifically expressed the resistance gene SilB.

Keywords: Salmonella; Silver nanoparticles; Silver resistance

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Antibactericidal activity of blue light and hyperbaric oxygen on methicillin resistant *Staphylococcus aureus*

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Background and Objectives: It is known that certain wavelengths of blue light have antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) [1, 2], and that hyperbaric oxygen (HBO) suppresses bacterial growth [3]. HBO alone has been shown to inhibit the growth of 28% of 240±24 colony forming units (CFU) of bacteria while 470nm blue light suppresses 92% growth of a standard culture of MRSA in one shot. We studied the effects of 470nm light and HBO to determine if this combination can yield optimal bacterial activity in *in vitro* simulation of mild, moderate or severe MRSA infections. **Materials and Methods**: Different culture densities ($3x10^6$, $5x10^6$ and $\geq 7x10^6$ CFU/mL) of MRSA were treated with HBO (hyperbaric hyperoxia, 2.0 atmospheres, 100% O_2) and/or 55J/cm^2 of 470nm blue light. Irradiation protocol for all cultures involved the application of blue light and/or exposure to HBO as follows: (1) Control (no irradiation, no HBO), (2) 55J/cm^2 only, (3) 55J/cm^2 and then exposure to HBO (55J/cm^2 + HBO), (4) HBO only, and (5) HBO exposure and then 55J/cm^2 (HBO + 55J/cm^2). **Results**: The bactericidal effect of combined blue light and HBO on MRSA was commensurate with the results for blue light treatment alone, with blue light producing as high as 97.3± 0.2% inhibition in the mild infection model ($3x10^6$ CFU/mL) and the combination approximately 94.7±4% for (55J/cm^2 + HBO) and 97.5± 2.5% for (HBO + 55J/cm^2). Notably, HBO treatment at this density produced significantly ($p<0.0001$) less bactericidal activity (43.3± 0.81%) when compared to either blue light or a combination of blue light and HBO. Increasing bacteria density to moderate ($5x10^6$ CFU/mL) and severe ($\geq 7x10^6$ CFU/mL), highlighted the superiority of blue light or combined therapy over HBO. **Conclusion**: The combined bactericidal effect of blue light and HBO on MRSA was profound and similar to that observed when blue light alone was utilized.

Keywords: Antibactericidal; Blue light; Hyperbaric oxygen; Methicillin resistant *Staphylococcus aureus*

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Combined effects of temperature and electro-activated solutions on inactivation of spores of the *Clostridium sporogenes* and *Geobacillus stearothermophilus* in pea and corn purees

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A sterilization temperature (121°C) is required to destroy the heat-resistant pathogen spores of *Clostridium botulinum* in canned vegetables. However, temperature affects the quality of products and requires high energy costs.

The technology of electro-activation produces electro-activated solutions (EAS), which can be acid or alkali with oxidizing or reducing redox-potential, respectively [1, 2]. The antimicrobial properties of the EAS can potentially be applied in preserving vegetables [1, 2]. Thus, the goal of this work was to study the effects of EAS and temperatures on the survival of the spores of *Clostridium sporogenes* and *Geobacillus stearothermophilus* in both pea and corn purees.

The EAS generated near the anode layer allows the inactivation of 2 to 7 Log CFU/ml of spores at different exposition times with the combination of mild temperatures. One of the most thermo-resistant spores, *Geobacillus stearothermophilus*, can be destroyed in both pea and corn purees in $D_{125°C}$ = 7.04 min and $D_{125°C}$ = 7.42 min, respectively. However, in treatment with EAS the thermal death time decreases down to $D_{125°C}$ = 6.05 min and $D_{125°C}$ = 4.90 min for pea and corn purees, respectively. Although, this decrease might seem minor, it can considerably reduce the overall time of sterilization of peas and corn by 14% and 34%.

The obtained results showed that *Clostridium sporogenes*, which is often applied as non-pathogenic surrogate for *Clostridium botulinum*, hardly tolerated the temperatures higher than 100°C when combined with EAS. A synergetic effect between the EAS and the heat treatment was evidently revealed. While treatments of pea and corn purees with NaCl brine solution showed $D_{100°C}$=127.78 min and $D_{100°C}$=63.19 min, same treatments in combination with EAS gave $D_{100°C}$=10.11 min and $D_{100°C}$=4.78 min, respectively.

Consequently, this work showed a significant lethal effect of EAS in combination with moderate heat treatments on the heat-resistant spores of *Cl.sporogenes* and *Geo.stearothermophilus*. A synergetic effect of combined treatments will allow decreasing the time/temperature couple of sterilization and hence producing canned vegetables with higher quality. The potential application of electro-activation as a hurdle technology can be way to decrease the energy consumption and make the cannery industry more competitive.

Keywords: electro-activated solutions; thermal inactivation, hurdles, *Clostridium sporogenes, Geobacillus stearothermophilus*

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Fast and effective killing of *Bacillus atrophaeus* endospores by light-activated vitamin B2 derivatives

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Spore forming bacteria like *Bacillus* or *Clostridium* provoke massive problems in the food and packaging industries as well as in medical and biotechnological processes. Endospores exhibit a highly intrinsic resistance against a variety of stress factors including UV and gamma irradiation, wet and dry heat, oxidizing agents, desiccation and even toxic chemicals [1] Only strong chemical or physical agents like peracetic acid, hypochlorite solution, hydrogen peroxide, chlorine dioxide or formaldehyde gas show a satisfactory result in spore decontamination [2, 3] . However, these measures reveal harmful potentials to humans and are often also harmful for the environment. In addition, many chemicals are not allowed to get into contact with food.

Alternatively, the photodynamic inactivation (PDI) of microorganisms and spores presents several positive aspects regarding the killing efficacy and the environmental hazard. PDI is independent of the resistance pattern of microorganisms so far, PDI can be applied for various microorganisms, and PDI show no selection of photo-resistant cells [4]. PDI is based on positively charged dyes (photosensitizers) that attach to the negatively charged spore surface. Upon irradiation the photosensitizers generate reactive oxygen species, especially highly reactive singlet oxygen, that kill spores via oxidative damage.

In a cross-disciplinary approach of physicists, chemists and biologists, we developed new Flavin photosensitizers (FLASH-01a, FLASH-07a) that are based on naturally occurring Vitamin B2 (Riboflavin). We added one (FLASH-01a) or eight (FLASH-07a) positive charges to Riboflavin that allow the attachment of the Flavin photosensitizers to the spore surface. The new photosensitizers efficiently convert light energy into singlet oxygen with a quantum yield of 0.75 ± 0.05 (FLASH-01a) and 0.78 ± 0.05 (FLASH-07a), respectively. The absorption spectrum of 50 µM FLASH-07a matched closely to the emission spectrum of the non-coherent light source. Incubation of FLASH-01a or FLASH-07a with *Bacillus atrophaeus* endospores for 10 seconds and a following irradiation of 10 seconds with 70 J/cm² caused a biologically relevant decrease of spore survival of ≥ 3 $\log_{10}$ orders ($\geq$ 99.9%) *in vitro*. Immobilized spores on food related surfaces like polyethylene terephthalate (PET) were efficiently killed with 7.0 $\log_{10}$ orders (99.99999%) with parameters similar to those for *in vitro* experiments. Transmission electron microscopy images of endospores after PDI treatment impressively show the damage of the coat, the outer membrane, the cortex and the core. Thus, PDI with new Flavin photosensitizers offers a great potential for a safe and sustainable use in food industry and environmental technologies as well as in medical applications.

Keywords *Bacillus atrophaeus* endospores, photodynamic inactivation, vitamins, singlet oxygen.

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Inactivation of *Candida albicans* by cold atmospheric plasma jet

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Cold plasma jets are promising for biological applications because they do not cause thermal damage [1]. Their antimicrobial effects on pathogenic microorganisms and application in the disinfection of medical devices have been described [2,3]. Little is known about their effects on fungal biofilms. This study evaluated the antimicrobial effect of a cold plasma jet against planktonic cells and biofilms of *Candida albicans*.

C. albicans (reference strain ATCC 18804 and 5 clinical isolates from oral candidosis lesions) were subcultured on Sabouraud Dextrose Agar (SD) for 24 hours (37 °C) and the cells were suspended on 0.9 % NaCl solution (10^6 CFU/ml). An aliquot of 100 µl of this suspension was plated on SD agar using a swab. After 15 minutes drying, plates were exposed to the plasma jet working with helium (3 SCFH, 3 cm distance) for 30, 60, 90, 120, 150, 180 and 240 seconds. After 24 hours of incubation, inhibition zones were measured. Biofilms of reference strains were formed at the bottom of 96-well plates containing RPMI buffered with MOPS supplemented with 2% glucose. After incubation for 24 hours (37 °C) under agitation (80 r.p.m.), biofilms were rinsed once with water and exposed to plasma jet (3 SCFH, 1.5 cm distance) for 2.5, 5.0 and 7.5 minutes (n=7). Cell viability was determined with Colony Forming Unit (CFU)/ml counting.

The lowest inhibition zone on agar surface was observed at 60 s (3 mm) and the largest at 240 s (12 mm) for clinical and reference strain. Plasma jet exposition for 5 minutes significantly reduced CFU counting in biofilms ($p<0.001$). Treatment for 2.5 an 7.5 minutes promoted a 2 log10 and 3 log 10 reductions in cell viability, respectively. In conclusion, cold atmospheric plasma jet working with helium was able to reduce cell viability in biofilms and planctonic culture of *Candida albicans*.

Keywords: *Candida albicans*; plasma jet

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Membrane bound structure of SSL-25: an antibiotic peptide present in human sweat

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SSL-25 (SSLEKGLDGAKKAVGGLGKLGKDA) is one of the shortest antimicrobial peptides present in human sweat produced after the proteolytic processing of the parent antimicrobial peptide dermcidin (DCD). It has been reported that SSL-25 preserves the antimicrobial activity against both gram-positive and gram-negative bacteria [1, 2]. The structure of DCD-1L was previously reported [3] but the membrane bound structure of SSL-25 still remains unknown. To determine the structure of SSL-25 in lipid bilayers, we have exploited solid state ^{19}F-NMR and oriented circular dichroism (OCD) spectroscopy. Both techniques are well established to determine the molecular orientation and mobility of peptides in membranes [4-7]. A series of ^{19}F-labeled SSL-25 analogues were synthesized and our solution CD results show that the secondary structure of all analogues were similar to the unlabeled SSL-25, namely α-helical in the presence of small unilamellar vesicles, thus allowing the detailed ^{19}F-NMR analysis. Like several other membrane active peptides, SSL-25 remains unstructured in aqueous environment, but upon contact with SDS micelles and/or in oriented membrane bilayers it folds into an overall α-helical structure. Our OCD results suggest that SSL-25 helix resides on the membrane surface with a slight tilt. Detailed ^{19}F-NMR analysis shows that SSL-25 does not form a continuous helix. The *N*-terminal part of the peptide preserved the α-helical structure under different experimental conditions (various lipids and peptide to lipid ratios), the *C*-terminus (11 residues) appears to deviate from an ideal α-helix. Furthermore, our NMR results confirm that the *N*-terminal helical part of SSL-25 primarily resides on the membrane surface as previously observed by OCD and did not reorient into the membrane as a function of either peptide concentration or membrane composition. SSL-25 does not aggregate and remains fully mobile in the membrane bilayer as shown by ^{19}F-NMR. To support the ^{19}F-NMR results, isotope labeled SSL-25 peptides with ^{15}N-Leu at position 8 and 18 were synthesized. ^{15}N-NMR data also confirm the surface bound state of the peptide in the membrane bilayer. At high peptide concentration using ^{31}P-NMR we observed a disturbance to the membrane bilayer but our biological tests did not show any bacteriostatic or bactericidal effects as previously reported [1, 2]. We note, however, that SSL-25 shows high affinity to bind to bilayers that represent bacterial lipid composition in sharp contrast to mammalian membrane composition containing cholesterol where the peptide remains in solution and does not bind.

Keywords: Dermcidin; SSL-25; solid-state NMR; circular dichroism; oriented circular dichroism;

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Membrane dipole modifiers affect the channel forming activity of cecropins

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The dipole modifying agents, flavonoids and styryl dyes, were used for modify the membrane activity of cecropin A, B and P1. Virtually solvent-free bilayer lipid membranes were prepared using monolayer-opposition technique from equimolar mixture of phosphoethanolamine and phosphoserine in 0.1 M KCl (pH 7.4). Addition of cecropin A or B on one side of a planar lipid bilayer led to the formation of well defined and reproducible ion channels of different conductance levels. The conductance of different states of cecropin A channels varied from forty of picosiemens up to one of nanosiemens. It was found three populations of cecropin A channels: pores with the weak cationic selectivity, anionic selectivity, and non-selective. The mean conductance of cecropin A or B channels was weakly dependent on the presence of the dipole modifiers agents in the membrane bathing solution. Cecropin P1 added on one side of a planar lipid bilayer did not induce ion channel formation, it led to membrane destabilization and disintegration at the concentration of peptide less than 50 μM and full distraction of lipid bilayer at concentration above 50 μM. Herewith, the addition of phloretin to the bilayer bathing solution, which is known to decrease the membrane dipole potential, led to decrease in the steady-state cecropin A or cecropin B induced transmembrane currents. At the same time, increasing the membrane dipole potential because of the introduction of RH 421 led to a rise in the steady-state cecropin A or cecropin B induced transmembrane currents. It is concluded that the observed changes in the channel-forming activity of cecropins might be caused by an increase in the energy barrier for the interfacial accumulation of cecropin monomers due to decrease in the membrane dipole potential.

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Keywords: cecropins, dipole modifiers, planar lipid bilayers

Vitamins fight back – fast and effective killing of multiresistant bacteria by light activation of Vitamin B2 derivatives

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The increasing emergence of multiresistant bacteria like Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the most important clinical challenges today [1]. Since the early 1960s, only four new classes of antibiotics have been introduced, and none of these have made a major impact yet [2]. Besides the development of novel antibiotics, other methods for effective killing of pathogenic bacteria have been considered. One of these methods is the photodynamic inactivation of bacteria (PIB).

The mechanism of PIB is based on positively charged dyes (photosensitizers, PS) that attach well to the negatively charged cell wall of bacteria [3]. After excitation by visible light PS transfers the absorbed light energy to adjacent molecules (biomolecules or oxygen) leading to the generation of reactive oxygen species (ROS). ROS cause irreversible oxidative damage of bacterial cell wall components, lipids, proteins and DNA already during irradiation [4]. However, many PS show low efficacy when simultaneously applied for different types of bacteria or show moderate toxicity without irradiation.

Based on vitamin B2 (Riboflavin) we developed new Flavin photosensitizers (FLASH-01a; FLASH-07a) that can eagerly attach to the bacterial surface and that are effective against different types of multiresistant bacteria and show no cell toxicity without irradiation. MRSA was incubated with different concentrations of FLASH-01a or FLASH-07a for 10 seconds and was subsequently irradiated with 50 mW/cm². A FLASH-01a concentration of 10 µM and a radiant exposure of 1.5 J/cm² resulted in a bacterial killing of > 5 $\log_{10}$ orders (> 99.999% reduction of viable bacteria). Using 50 µM of FLASH-07a and a radiant exposure of 1 J/cm², the number of viable MRSA decreased up to 6 $\log_{10}$ steps (≥ 99.9999%), which is equivalent to high level disinfection. Similar results were achieved when enterohemorrhagic *Escherichia coli* (EHEC) HUSECO41 (O104:H4; ESBL producer), multiresistant *Pseudomonas aeruginosa* and multiresistant *Acinetobacter baumannii* were irradiated with our new Flavins. The killing effect was independent of the type of bacteria and its corresponding antibiotic resistance pattern.

Additionally the cell toxicity of FLASH-01a and FLASH-07a was tested against normal human epidermal keratinocytes (NHEKs). NHEK cells were incubated with FLASH-01a or FLASH-07a with concentrations up to 100 µM and irradiated with the same light parameters as used for PIB. The results clearly showed that cell viability was not affected by both photosensitizers for radiant exposures up to 9 J/cm² (FLASH-07a) or 12 J/cm² (FLASH-01a).

Multiresistant bacteria can be effectively killed by a clever combination of modified vitamin B2 molecules, visible light and oxygen. PIB with both Flavin derivatives shows a great potential of bacterial killing without harming the adjacent tissue and therefore may be a realistic prospect for a future clinical use in humans.

Keywords MRSA, EHEC, photodynamic inactivation, vitamins, singlet oxygen.

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What are we really seeing? Investigating the relevance of traditional antimicrobial assays for nanomaterials

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Metallic nanoparticles such as nano silver have found many applications as alternative antimicrobials in recent years. One such application of this technology is the use of nanoparticles in antimicrobial food packaging. In such packaging, plastic polymers are embedded with antimicrobial metallic nanoparticles. As regulation of products containing engineered nanomaterials is still an essentially untapped area, there are no defined protocols for assaying the activity of antimicrobial nanoparticles. The aim of this work was to explore the methods currently being used by researchers and investigate the relevance of the results.

The disk diffusion assay is most often used by researchers synthesising antimicrobial nanoparticles for use in food packaging. This assay was used on a number of nanoparticles including zinc oxide, and various types of silver nanoparticles. The 'Minimum Inhibitory Concentration' (MIC) and 'Minimum Bactericidal Concentration' (MBC) assays were also carried out. A new spectroscopy-based method was subsequently designed by us, and following on from this flow cytometry was employed to investigate mechanisms in more details. Results from all experiments were compared.

The disk diffusion assay was found to show varying results, often alluding to the particles having no antimicrobial activity. The MIC and MBC assays were shown to be unsuitable due to the insolubility of the nanoparticles. The spectroscopy based method coupled with flow cytometry however showed antimicrobial activity of the same particles on *Staphylococcus aureus* and *Escherichia coli,* as well as the metabolically important resident microflora *Bifidobacterium breve.*

The complex physiochemical characteristics of metallic nanoparticles, such as insolubility, density, and mechanism of antimicrobial action mean that traditional antimicrobial assays designed for antibiotics do not provide a true picture of the activity of nanoparticles. This work highlights a need for a new quantitative method, which would take into account the physiochemical characteristics governing the mechanism of action of the nanoparticles. Metallic nanoparticles often act by releasing ions from their surface, the rate of which is dependent on the amount of dissolved oxygen in the environment. Hence it was felt that a liquid based system would facilitate ion release and more closely mimic a real life *in vitro* situation. As well as this, the spectroscopic method allows the researcher to take control of the optical properties of the particles (namely surface plasmon resonance) and ensure no interfering absorbance from this. Finally, it was also found that ingesting metallic nanoparticles in food packaging may have a negative effect on the human GI tract by inhibiting the growth of gut microflora. This work has highlighted a knowledge gap in the area of food nanotechnology, and reinforced the message that the risks of nanotechnology in food need to be thoroughly assessed, and stricter regulations put in place for the use of nanotechnology in consumer products such as foods.

Clinical and medical microbiology, infectious diseases and antimicrobials Public health

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Antibacterial activity of Antarctic lichens against MDR nosocomial pathogens isolated from Chilean hospitals

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The emergence of MDR bacteria [1] is a serious problem in hospitals, leaving only 1 or 2 available antibiotics to treat infections caused by the group of bacteria called ESCAPE (*Enterococcus* spp., *Staphylococcus aureus*, *Clostridium difficile*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacteriaceae). This group is associated with high mortality and great level of resistance to antibiotics used in hospitals [2]. Additionally, a significant decreasing in the production of new antibiotics has occurred during the latest years [3]. In accordance with this scenario, there are several publications reporting investigations that are looking for new antimicrobial compounds from plants. Thus, has been reported that secondary metabolites of lichens as depsides and depsidones have antimicrobial activity againts a broad spectrum of bacteria [4,5]. In this work we assayed the total extracts and pure metabolites from antarctic lichens upon several MDR bacteria isolated in Chilean hospitals.

Antarctic lichens *Himantormia lugubris* (HL)*, Ramalina terebrata* (RT), *Stereocaulon alpinum* (SA) and *Umbilicaria antarctica* (UA) collected from King George Island, were used to prepare methanolic extracts, and from these, using chromatographic column (DIAION), methanol, acetone, ethyl ether and aqueous fractions were obtained. The antibacterial activity of was assayed using a disk diffussion method on Mueller-Hinton agar plates. Furthermore, minimal inhibitory (MIC) and minimal bactericidal (MBC) concentrations of pure compound (usnic acid, atranol and a b-orcinol depsidone) were also determined by a microdilution method [6,7]. The MDR bacteria included were methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), ESBL-producing *E. coli* (Eco), ESBL-producing *K. pneumoniae* (Kpn), *A. baumannii* (Aba) and *P. aeruginosa* (Psa).

Disk diffussion results show that total extract of RT and its methanol fraction had the best antibacterial activity upon all bacteria assayed with inhibition zone (IZ) fluctuating between 7-28 mm, followed by the total extract and methanol fraction of HL with IZ varying from 8-14 mm. In contrast to expectations, none of the extracts was active against *Psa* isolates. Acetone fraction of HL and SA had activity only upon Gram(+) bacteria. Ethyl eter and aqueous fractions had no antibacterial activity. Results of MIC and MBC show that 90% of isolates were inhibited or dead with > 100 µg/mL of atranol and the b-orcinol depsidone for MRSA, VRE, *Eco*, *Aba,* and > 250 µg/mL for *Kpn.* MIC_{90} and MBC_{90} of usnic acid was > 100 µg/mL for *Aba* and *Eco* and > 250 µg/mL for *Kpn*, but the activity was better against Gram(+) bacteria with MIC_{50}/MIC_{90} of 3,125/6,25 µg/mL for VRE and 6,25/12,5 µg/mL for MRSA. The extract of UA showed no activity against none of bacteria assayed.

We conclude that extracts of RT and HL had good activity against multiresistant isolates of Gram-negative bacteria. Since the results obtained with pure compounds, especially atranol, main compound in HL, a comprehensive study is required to determine the compounds responsible for the activity observed for the methanolic extract. Usnic acid was active exclusivelly upon Gram-positive, as has been reported previously in literature.

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Keywords: ESCAPE, multi-drug resistance, nosocomial bacteria, lichen, Antarctica

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Antibiotic Resistance of Viridans Group Streptococci Isolated from Dental Plaques

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Viridans group streptococci (VGS) are a common cause of infective endocarditis, and dental plaque is the major source of these bacteria. The present study examined the antibiotic resistance of 635 VGS isolates obtained from dental plaques. Isolates from supragingival plaques were identified using the rapid ID 32 Strep and mini API reader (bioMérieux, France), and minimal inhibitory concentrations (MICs) were determined by a broth microdilution method. High rates of resistance to ampicillin and tetracycline were detected among the isolates. The most resistant species were *Streptococcus sanguinis* and *S. salivarius*. Among the 635 isolates, 9.1% were resistant to erythromycin, and 31% to tetracycline. All isolates were sensitive to vancomycin. Resistance to amoxicillin was observed in 0.2% of all isolates. In this study, we showed the incidence of antimicrobial resistance and the susceptibility patterns among 635 VGS isolates from dental plaque.

Keywords: dental plaque; streptococci; antibiotic; resistance

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Antibiotic susceptibility of fecal *E. coli* isolates from human stool sample, Turkey

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Antibiotic resistance is a major global public health. Among normal flora, *Escherichia coli* is the main facultative anaerobic bacteria species in the bowel. However, *E. coli* is also the most common pathogen causing urinary tract and blood stream infections. Additionally, fecal *E. coli* can act as reservoir of resistance genes in the human bowel. These resistance genes might be rapidly transferred to other commensal or pathogenic organisms. The high rate of prescribing and consumption of antibiotic and usage of antibiotic without prescribing is likely to result high rates of resistance in Turkey. The main aim of this study was to describe prevalence of resistance in *E. coli* isolates from stool specimens. Stool samples negative for occult blood test were obtained from outpatient send to microbiology laboratory between March and December 2013. All stools samples were streaked on eosin methylene blue agar (EMB, Klas Medical,Turkey) for isolation of *E. coli*. Colonies morphologically resembling *E. coli* were confirmed by using BBL Crystal E/NF ID System (Becton Dickinson, USA). The antimicrobial susceptibilities of the *E. coli* isolates were determined using the standard Kirby-Bauer disk diffusion method for 23 antibiotics, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. These antimicrobials were chosen on the basis of their importance in treating human *E. coli* infections and their use in animal feed additives to promote growth. ATCC *E. coli* 25922 was used for quality control to antibiotic discs and identification tests. 80 out of 150 isolates were determined susceptibility to all antibiotic discs. Ampicillin had the rate of the lowest susceptibility (53.33%). Except for cefepime (100%), lower resistance rates were observed for the other cephalosporins. A total of 24 isolates had extended-spectrum beta-lactamase (ESBL) (16%). The susceptibility rates to fluoroquinolones were more than 80%. Higher susceptibility rates (93.33-97.33%) were also observed for aminoglycosides (gentamicin and amikacin). The susceptibility rates to trimethoprim/sulfamethoxazole were found 74.67%. The ESBL positive isolates were found higher resistance than the ESBL negative isolates. The susceptibility rates to fluoroquinolones in the ESBL positive isolates were 45.83%. The susceptibility rates to trimethoprim/sulfamethoxazole in ESBL positive isolates were found 33.33%. All isolates were susceptibility to carbapenems. In this study the low susceptibility rates were found to antibiotics in fecal *E. coli* isolates. There is a need to enhance local and national research and surveillance efforts to monitor resistance trends of fecal *E. coli* in each country.

Key words: *E.coli*; Fecal; Antibiotic resistance

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Antimicrobial Photodynamic Therapy: From Bench to Bedside and Vice Versa

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Background: Photodynamic therapy (PDT) kills microrganisms by activating a photosensitizer with selective wavelength of light. PDT targets different organelles in a cell; therefore, less likely to induce resistant strains.

Aims: We present our experience in antimicrobial PDT in Taiwan, from bench to bedside and vice versa.

Results: Antimicrobial PDT was examined *in vitro*, in mice, and in patients. More than half of the mice with *Vibrio vulnificus* septicemia survived after toluidine blue O-mediated PDT (150 J/cm^2 at 80 mW/cm^2). PDT inactivation of enteroviruses is demonstrated in animal experiments. More importantly, PDT enhanced wound healing in chronic infected ulcers in patients. Both direct bactericidal effects and altering local microenvironment by PDT may play roles in optimizing wound healing.

Conclusion: PDT has potential to become an alternative or adjuvant antimicrobial therapy.

Keywords: antimicrobial; photodynamic therapy, drug resistant, chronic ulcer

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Antimicrobial susceptibility pattern of bacterial isolates from surgical site infections from a tertiary care cancer centre

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Introduction: Surgical site infection (SSI) is the most frequent cause of nosocomial infection in surgical patients, accounting for 38% of the total. Surgical site infection doubles the risk of postoperative mortality, increases intensive care unit stay by 60%, and is associated with a 5-times increased likelihood of readmission. It also prolongs hospital stay between 5 and 20 days, with a substantial increase in hospital costs. The high prevalence and the financial burden associated with SSI have led investigators in many countries to develop infection control systems. The application of protocols for the prevention of SSI has proved to be effective in reducing postoperative infections.

Objectives: To assess the prevalence of surgical site infection and to determine antimicrobial susceptibility pattern of bacterial isolates from SSI's.

Materials and Methods: A total of 1282 frank pus and pus swabs were processed on MacConkey agar and sheep blood agar as per standard microbiological methods. Identification of the organisms, ESBL production and carbapenem resistance was done as per CLSI guidelines.

Results: E.coli (15.8%) was the commonest isolate followed by S.aureus(13.6%), Pseudomonas aeruginosa(7.78%) and Klebsiella pneumoniae(7.72%). ESBL positivity was seen more in E.coli than Klebsiella pneumoniae . Carbapenem resistance was commonest in Acinetobacter baumanii(68.2%) followed by Klebsiella pneumoniae(31.3%) and E.coli(15.3%).

Conclusions: The most common isolate in wound infection was E. coli followed by S. aureus, Pseudomonas aeruginosa and K. pneumoniae. These isolates showed high frequency of resistance to most of the antibiotics including carbapenems.

Antimicrobial susceptibility testing for *Staphylococcus aureus, Staphylococcus intermedius* and *Staphylococcus hyicus* isolated from bovine milk in small dairy farms in Brazil

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Chronic mastitis cases irresponsible to treatment are increasingly in dairy farms. The treatment of bovine mastitis is complicated by the overuse of antibiotics, and the generation of drug-resistent-bacteria. Many antibiotic treatments for mastitis are often not justified with a cost/benefit analysis [1]. This study aimed to isolate and identify coagulase positive staphylococci (CPS) species from seven Brazilian small dairy herds and establish the antimicrobial profile. A single aseptic milk sample (20 mL) was collected from all California Mastitis Test (CMT) positive quarters. Identification of *Staphylococcus* spp. was performed using conventional microbiology. Of the 102 CMT positive milk samples, 38 (37.2%) was CPS. Thirty-three (33) samples was identified as *Staphylococcus aureus (S. aureus)* (86.8%), four (4) samples as *Staphylococcus intermedius (S. intermedius)* (10.5%) and one single sample as *Staphylococcus hyicus (S. hyicus)* (2.6%). The distribution of CPS was different among herds: *S. aureus* was found in four herds, *S. intermedius* in two herds and *S. hyicus* was found in just one herd. Antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines [2]. Commercially available antibiotic disks were used for antimicrobial susceptibility testing: vancomycin (Oxoid®), ampicillin (Cecon®), oxacillin (Sensifar®), enrofloxacin (Cecon®), cephalexin (Cecon®), gentamicin (Cecon®), cotrimoxazole (Cecon®), penicillin (Cecon®), neomycin (Cecon®), and tetracycline (Cecon®). The percentage susceptibility of *S. aureus* towards vancomycin was 100% while that for cephalexin was 97%; cotrimoxazole was 93.9%; oxacillin was 90.9%; enrofloxacin and gentamicin were 87.8%; neomycin was 81.8% and tetracycline was 69.7%. The percentage resistance of *S. aureus* was 93.9% for penicillin and ampicillin. For *S. intermedius*, the percentage susceptibility was 100% for vancomycin, enrofloxacyn, cephalexin, cotrimoxazole and tetracycline; 75% for oxacillin and 50% for gentamicin and neomycin; high rate of resistance to penicillin (100%) and ampicillin (75%) were demonstrated. The antimicrobial susceptibility profile for the single sample of *S. hyicus* isolated was 100% for vancomycin, oxacillin, enrofloxacin, cephalexin, gentamicin, cotrimoxazole, neomycin and tetracycline, while 100% was resistant to ampicillin and penicillin. The increasing resistance to antibiotics is a major concern to the treatment of mastitis; therefore, a detailed bacteriological diagnosis and susceptibility testing is required to overcome global problem of antibiotic resistance.

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Antimicrobial susceptibility, virulence factors and enterotoxigenic genes of food isolates of coagulase-positive *Staphylococcus*

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This work aims to evaluate a set of coagulase-positive *Staphylococcus* (CPS) isolates for (1) the ability to produce virulence factors (coagulase, hemolysin and deoxyribonuclease), (2) the presence of enterotoxigenic genes, (3) and the antimicrobial profile.

Staphylococcus aureus is the specie of the *Staphylococcus* genus more frequently related with cases and outbreaks of food poisoning, caused by staphylococcal enterotoxins [1]. About 95% of staphylococcal food poisoning are caused by enterotoxins SEA, SEB, SEC, SED and SEE [2]. Thus, a multiplex PCR assay was used in this work to verify the presence of gene fragments of SE (*sea*, *seb*, *sec*, *sed* and *see*) and the *femA* gene fragment, specific for *Staphylococcus aureus*, using CPS isolates obtained from raw milk cheeses (n=95), milk (from cow, sheep and goat; n=16) and other foods (n=10) mainly of animal origin. Five coagulase-negative *Staphylococcus* (CNS) was also studied.

From a total of 121 CPS isolates, 31.4% amplified *femA* gene fragment but not any of the five staphylococcal enterotoxins (SE) gene fragments evaluated in this study, specially in the milk group isolates. The *sec* gene fragment was the only amplified between cheese isolates and was also amplified between milk isolates and other food isolates. The *sea* and *see* genes were also amplified between milk isolates and other food isolates. Gene fragments of SEB and SED were not amplified. Isolates without amplification of *femA* were submitted to biochemical tests for identification to the specie level.

In addition to the evaluation of the presence of enterotoxigenic genes and the *femA* gene, another objective of this study was to evaluate the susceptibility profile of the 76 CPS and 4 CNS isolates to the nine antibiotics (trimethoprim-sulfamethoxazole 25 µg, ampicillin 10 µg, amoxicillin 10 µg, penicillin G 10 IU, enrofloxacin 5 µg, neomycin 30 µg, erythromycin 15 µg, streptomycin 10 µg and cefazolin 30 µg), through the application of the Kirby-Baeur technique. Although 60 of the CPS isolates show no resistance to all antibiotics studied, was observed multiresistance to two or three antibiotics in 13 of the isolates, with a higher percentage of multidrug resistance to Penicillin G and Ampicillin (7 isolates) and Penicillin G, Ampicillin and Amoxicillin (4 isolates). Concerning the origin of the isolates, the highest percentage of resistance was detected in isolates from cow's milk, where 100% of the isolates were resistant to Penicillin G and Ampicillin and 25% to Amoxicillin. These data lead us to question the inappropriate use of antibiotics in animal milk production.

Keywords: Coagulase-positive *Staphylococcus*; Enterotoxigenic genes; Multiplex PCR; Antibiotics resistance.

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Antimicrobial treatment of nonspecific men's urethritis as a promising method for the treatment of infertility

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Increase of unit weight of conditionally pathogenic microflora in etiological structure of infectious inflammatory diseases of urogenital tract at infertile men noted nowadays [1, 2]. Aim of this research is to examine clinical and microbiological features of antimicrobial treatment of infertile men with nonspecific urethritis. 45 infertile men with inflammatory urogenital diseases were inspected. Clinical and laboratory inspection included anamnesis collection and bacteriological study of separated urethra's part. All patients got a antimicrobial treatment with antibiotics and antimycotics. The effectiveness of treatment was evaluated clinically and with bacteriological method. Statistical processing of the data was performed using Student *t*-test.

Clinically examined men had the following symptoms: frequent and painful urination, nycturia, perineal pain, skin rash, hyperaemia and edema of urethra's mucous membrane. Prostatitis and vesiculitis were revealed in 82% of cases as a result of ultrasonic diagnostics. During microbiological study of urethra's separated part 77 microorganisms strains were figured out. 75% of them were gram-positive bacteria strains, 22% of them were gram-negative bacteria strains, and 24% were *Candida*. Gram-positive microorganisms included genus of *Staphylococcus* sp. (76%), *Streptococcus* sp. (19%) and *Corynebacterium* sp. (5%). In 57% of cases staphylococci were coagulase-positive (76% - *St. aureus*, 24% - *St. intermedius*). Coagulase-negative staphylococci were presented with *St. haemolytics* in 47% of cases, *St. saprophyticus* – in 26% of cases and *St. epidermidis* – in 16% of cases. Among streptococci appeared *Str. Viridans* in 45% of cases, *Str. mitis* – in 9% of cases, *Str. sanginis* – in 9% of cases. All figured out corynebacteria were presented with nonpathogenic species of natural normal flora. Gram-negative microorganisms were represented with *Enterobateriaceae* in 76,5% of cases, and *Neisseria* in 23,5% of cases. *Escherichia coli* were singled out in 77% of cases, *Citrobacter diversus* – in 8% of cases. *Neisseria* was represented with *N. lactamia*, *N. sicca et al*. All figured out yeast-like fungi were represented with *Candida albicans*.

After the antimicrobial therapy 77% of men had no opportunistic microorganisms, others had decreased number of staphylococci ($p<0.05$ – to comparison before treatment), *St. aureus* were not found. After 7 days of therapy 89% of patients had no pain symptoms *etc*. Signs of inflammation decreased in 94% of patients. All patients after antimicrobial therapy has become fertile.

There fore ous research shows that urethra of infertile men with nonspecific urethritis more frequently has staphylococci. *Enterobacteriaceae* and yeast-like fungi appear less frequently in the case of nonspecific urethritis. However their presence influences greatly on the flow of inflammatory process. Thus, the research found high efficiency of the proposed antimicrobial therapy without specific drugs which increase fertility in infertile men.

Keywords: antimicrobial therapy; fertility; inflammatory

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Application of targeted delivery methods for optimization of distribution of concentration of rifampicin

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Introduction. The maintaining of high concentrations of rifampicin in blood plasma and certain tissues of the body for a long period of time is crucial point for effective treatment of patients with multidrug-resistant tuberculosis. It might be achieved by using targeted delivery method based on an incorporation of the drug into autologous erythrocyte ghosts. In this study we analyzed pharmacokinetic profiles of rifampicin encapsulated into erythrocyte ghosts ("pharmacocytes") in comparison to administration of a free form of rifampicin.

Material and methods. The experiments were conducted on albino rats with mass of 150.0-180.0 g (n = 148). Animals were randomly divided into two groups. Each group was receiving a different type of intravenous injection via the tail vein. Group A (control) received intravenously 40 mg of free rifampicin, while group B received an injection of 1 ml of pharmacocytes loaded with 40 mg of rifampicin. At fixed time points after injection (30, 60, 180, 360, 720, 1440 and 2160 min) serum samples were collected. Homogenates of liver, spleen, lung, heart, kidney and skeletal muscle were obtained in control group 12 hours after injections, and in study group 36 hours after injections respectively. Concentration of the tested substance in the collected organs and blood plasma were measured by ELISA test. Modeling was performed using Borgia 1.03 software.

Results. We observed increased half-life period ($T_{1/2}$) for rifampicin encapsulated into erythrocyte ghosts comparing to the control group. $T_{1/2}$ for free rifampicin was 3.08 ± 0.5 hours, while administration of pharmacocytes with loaded rifampicin led to increase of the half-life period up to 5 fold (15.86 ± 1.6min). The increased time of presence of rifampicin in the body (administered in the form of pharmacocytes) could be explained by the reduction of clearance (CL_{el}) by 5 fold, and augmentation of the mean residence time (MRT) by more than 9 fold.

It has been revealed, that the pharmacocytes help to maintain the concentration of rifampicin in the serum during long period (from 3 to 18 hours after intravenous administration). The highest level of concentration of rifampicin was observed, when it was administered in the form of a free drug (16 μg/ml), while for pharmacocytes loaded with rifampicin the maximal level was 12.8 μg/ml. The concentration of rifampicin administered in the form of pharmacocytes in the first 6 hours was below the level that was detected after the injection of free rifampicin, and subsequently exceeded that.

Based on calculation of the level and therapeutic activity of the drug (>4 μg/ml), the therapeutic range of rifampicin administered in the free form is maintained up to 8 hours. At the same time the administration of rifampicin in the form of pharmacocytes can prolong its therapeutic range in the blood up to 30 hours.

In addition, we have observed an increase of the concentration of rifampicin in lung tissue when it was administered in the form of pharmacocytes.

Conclusions. Pharmacocytes have shown the potential to maintain a high concentration of rifampicin in the serum by increasing the half-life period, reducing its clearance (CL_{el}) and increasing the mean residence time (MRT). Apart from that, the pharmacocytes are capable to improve the deposition of the drug in lung tissue.

These data suggest that pharmacocytes may promote a high concentration of rifampicin in the blood and peripheral tissues through targeted delivery to the sites of inflammation. The application of pharmacocytes can lead to significant increase of antibacterial activity of the rifampicin, which might have a direct effect on the treatment of patients with multidrug-resistant tuberculosis.

Keywords: multidrug-resistant tuberculosis; rifampicin; targeted delivery; erythrocyte ghosts

Beyond infectious diseases: impact of antibiotic use on the changing trend of esophageal adenocarcinoma

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For unclear reasons, the incidence of esophageal adenocarcinoma (EA) arising out of Barrett's esophagus and reflux esophagitis has risen more than 600% in the United States since the 1970s[1]. Although specific host factors might predispose one to disease risk, such a rapid increase in incidence must be predominantly environmental. The widespread use of antibiotics since 1950s could have contributed to this drastic change. Exposure to antibiotics may occur during treatment for infectious diseases or through consumption of food or use of hygiene products containing antibiotics. American livestocks consume 15-17 millions of pounds of antibiotics per year for growth promotion and improvement of feed effeciency. One hypothesis to explain the increase in EA is disappearing *Helicobacter pylori*[2]. *H. pylori*, discovered in early 1980s as the cause of gastritis and gastric cancer, plays a protective role for gastroesopahgeal reflux disorders. Although not directed at *H. pylori*, antibiotic exposure prior to 1980s could unintentionally eradicate *H. pylori* leading to the observed decrease in the incidence of gastric cancer and increase in EA. Another factor accounts for the rising incidence of esophageal adenocarcinoma is obesity. Obesity has had a drastic increase in prevalence since since 1970s, similar to EA in trend. In mice, sub-therapeutic antibiotics consistently increase the relative abundance of Firmicutes at the cost of decreasing the abundance of Bacteroidetes[3]. In humans, Firmicutes and Bacteroidetes are the two main phyla in the colonic microbiome. Overweight people have more Firmicutes but fewer Bacteroidetes than lean controls[4]. Weight loss on diet therapy enrichs Bacteroidetes and depletes Firmicutes. These findings suggest that exposure to antibiotics changes the colonic microbiome in favor of gaining body weight. Antibiotic use could also have altered the microbiome in the distal esophagus. The human microbiome had not been the main focus of disease studies until the laugh of the NIH human microbiome project in 2008. Case control studies showed microbiome is altered in the distal esophagus in patients with reflux disorders including EA. The microbiome in esophageal diseases is more diversified than in controls[5]. This effect is not only seen in the esophagus but also oberved in the mouth and stomach. Overall, esophageal diseases tend to be associated with depletion of Gram-positive bacteria and enrichment of Gram-negative bacteria. *Streptococcus* is the most abundant Gram-positive bacteria in the foregut, comprising nearly 80% of the bacterial population. The relative abundance of *Streptococcus* tends to decrease along the disease progression from normal to reflux, Barrett's esophagus, and adenocarcinoma, in both the mouth and esophagus. Changes in the major non-streptococcal taxa are also observed. Microbiome alteration often extended to the mouth and sometimes to the stomach and rectum. The altered microbiome could play a more direct role in the initiation and progression of reflux disorders than *H. pylori* or obesity by induction of chronic inflammation and/or activation of carcinogens[6]. Microbiome studies open a new avenue to the understanding of the etiology and pathogenesis of reflux disorders and could potential lead to finding new measure for cancer prevention via normalization of the microbiome.

Keywords: Esophagus; adenocarcinoma; microbiome; antibiotics; *Helicobacter*; obesity

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Blueprint of the serotype distribution and antimicrobial resistance in human salmonellosis in Belgium (2009-2013)

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Although there has been a steady decrease in cases of human salmonellosis in the European Union, it remains the second most frequently reported zoonosis (20.4 confirmed cases per population of 100,000) and the most frequent cause of foodborne outbreaks [1]. Non-typhoid salmonellosis is usually self-limiting and normally resolves without the need of antimicrobials, but life-threatening invasive infections may occur in vulnerable patients. The causative agent, *Salmonella enterica* subsp. Enterica, is subdivided into 1,531 serotypes, which differ greatly in their natural reservoirs, their ability to provoke infections and their resistance to antimicrobials [2]. The severity of inflicted disease also differs substantially among serotypes, with case fatality rates ranging over 100-fold and proportions of hospitalizations varying between 14 and 67% for non-typhoidal strains [3].

Invasive Salmonella infections mandate the need for chemotherapy. The increasing rates of resistance against traditional agents caused a shift to fluoroquinolones (FQ) and third-generation cephalosporines (CSP) in empiric treatment [3]. However, a plethora of studies report high-level FQ and CSP resistance emerging in different parts of the world [e.g., 4], and global travel and food trade increase the likelihood of acquiring infection from nondomestic sources. As such, it is crucial to closely monitor trends in resistance, and prevalence of mobile and non-mobile genetic determinants underlying resistance to these first-line drugs.

In the five-year period spanning this study, the Belgian Reference Centre for *Salmonella* received 16,544 human *S. enterica* strains, mainly isolated from faeces (94.4%), blood (2.4%) and urine (1.5%) samples. In this vast collection, we identified 377 different serotypes. Despite subtle (non-significant) annual fluctuations, the landscape clearly dominated by serotypes Typhimurium and Enteritidis which were retrieved in on average 54.2% and 19.2% of all samples, respectively.

Upon antibiotic susceptibility analysis of a subset of strains (N=4,561), distinct trends appear for different serotypes. Quinolone resistance, assessed using nalidixic acid (NalR) and ciprofloxacin (CipR) and observed in 16.4% and 4.4% of the strains, respectively, is mainly mediated by serotype-dependent mutations in GyrA residues Ser83 and Asp87 (92.2% non-wild type). An additional ParC_Ser80Ile mutation lead to CipR in 95.5% of clonal Kentucky isolates. Plasmid-mediated quinolone resistance alleles QnrA1 (N=1), QnrS (N=9), QnrD1 (N=4) or QnrB (N=4) were only found in 3.0% of 533 NalR isolates.

Although no resistance to carbapenemases was noted, 1.6% of the S. enterica strains are resistant to cefotaxime (CTXR), a third-generation cephalosporin. In these isolates we identified a broad range of Ambler class A and C β-lactamases (e.g., SHV-12, TEM-52, CTX-M-14, CTX-M-15) commonly associated with Enterobacteriaceae. Our results show that mobile resistance determinants remain rare in *S. enterica*, but clonal resistant serotypes arise and continued (inter)national surveillance is mandatory to understand the origin and routes of dissemination thereof.

Keywords: Salmonella; ESBL; Fluoroquinolones; qnr

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Characterization of clinical methicillin sensitive *Staphylococcus aureus* isolates with reduced susceptibility to chlorhexidine

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In clinical practice decontamination and disinfection are the most important intervention measures to prevent bacterial infection from spreading. *Staphylococcus aureus* (*S. aureus*) is commensal to humans but have the ability to become pathogenic due to developing resistance to antimicrobial agents and harbouring virulence factors on mobile genetic elements. It is important to analyse the clinical *S. aureus* isolates for the presence of genes that confer resistance to disinfectants. More often surveillance programs are limited to methicillin resistant *S. aureus* (MRSA). However evidence suggests that infection caused by methicillin-susceptible *S. aureus* strains (MSSA) is on the rise since 2003 (http://www.smrsarl.scot.nhs.uk/reports.asp). Therefore more attention should be paid to MSSA isolates that cause infections in hospitals and community. In this study our focus is on the characterization of methicillin sensitive *S. aureus* (MSSA) in Kuwait.

In total 58 MSSA were collected from clinical specimens in 2013. *qacA/B*, *qacG*, *qacH*, *qacH2, norA*, *smr*, *blaZ, PVL* and *agr* genes were amplified by PCR. Minimum inhibitory concentrations (MICs) were determined for 10 antibiotics and chlorhexidine. *spa* typing and pulsed-field gel electrophoresis (PFGE) were used to identify the genetic variations between the isolates.

The prevalence of the genes tested were as follow; *qacA/B* 12%, *norA* 86%, *smr* 21% and *blaZ* 91%. *qacG*, *qacH*, *qacH2* were not detected. The bacteriophage-encoded Panton–Valentine leukocidin (PVL) toxin was present in 96.6%. *agr*-1Sa was detected in 46.5% while *agr*-2Sa and *agr*-3Sa were present in 21% and 24% respectively. The minimum inhibitory concentration (MIC) for chlorhexidine for *qacA/B* positive isolates ranged from 4-15mg/l.

We did not find any correlation between antibiotic resistance and the presence of biocide resistance genes however the MIC of chlorhexidine was higher for *qacA/B* positive isolates. Genotypic analysis revealed that the *qacA/B* are prevalent in isolates that are clonal. These isolates were collected from different speicimens (blood, skin and groin). Moreover; the presence of PVL toxin and *agr*-dependent colony spreading may also play a role during infections caused by these strains. Disinfectants usage in community and hospitals may be a contributing factor in clonal expansion. These bacterial sub-populations are likely to persist and survive in environmental niches where biocide delivery is compromised. Biocide resistance genes may be among the biological attributes that give these strains a competitive edge to spread in an environment that is actively selecting.

Keywords: Methicillin sensitive *S. aureus* (MSSA); *qacA/B*

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Cloning and Expression of Synthetic Genes Encoding the Broad Antimicrobial Spectrum Bacteriocins SRCAM 602, OR-7, E-760 and L-1077, by Recombinant *Pichia pastoris*

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In this study we have evaluated the cloning and functional expression of previously described broad antimicrobial spectrum bacteriocins SRCAM 602 [1, 2], OR-7 [3], E-760 [4] and L-1077 [5], by recombinant *Pichia pastoris*. Synthetic genes, matching the codon usage of *P. pastoris,* were designed from the known mature amino acid sequence of these bacteriocins and cloned into the protein expression vector pPICZαA. The recombinant derived plasmids were linearized and transformed into competent *P. pastoris* X-33, and the presence of integrated plasmids into the transformed cells was confirmed by PCR and sequencing of the inserts. The antimicrobial activity, expected in supernatants of the recombinant *P. pastoris* producers, was purified using a multi-step chromatographic procedure including ammonium sulfate precipitation, desalting by gel filtration, cation exhange-, hydrophobic interaction-, and reverse phase-chromatography (RP-FPLC), but a measurable antimicrobial activity was only detected after the hydrophobic interaction and RP-FPLC steps of the purified supernatants. MALDI-TOF MS analysis of the antimicrobial fractions eluted from RP-FPLC revealed the existence of peptide fragments of lower and higher molecular mass than expected. MALDI-TOF/TOF MS analysis of selected peptides from eluted RP-FPLC samples with antimicrobial activity indicated the presence of peptide fragments not related to the amino acid sequence of the cloned bacteriocins.

Keywords: bacteriocins; heterologous production; recombinant *Pichia pastoris*

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Determining presence of *Listeria monocytogenes* and serological typing of the isolates in kashar cheese samples sold in Istanbul

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Listeria monocytogenes is a human food-borne pathogen responsible for gastroenteritis, and more severe manifestations including septicemia, central nervous system infections, and materno-fetal infections leading to abortions. Agent is identified as one of the main food-borne pathogens and responsible for sporadic and endemic listeriosis outbreaks by the medical literatures. Although rare, listeriosis is of public health concern because of its high case-fatality (20–30%) and the potential of *L. monocytogenes* to cause large outbreaks targeting predominantly pregnant women neonatals and immunodeficient individuals. *Listeria monocytogenes* is a gram positive and facultative anaerobic microorganism. Because the agent can live in the soil, it can be isolated from vegetables, dairy and dairy products, drinkable and / or waste water and can easily contaminate from animal to animal and / or from animal to human by feco-oral way. The main food sources of human listeriosis are unpasteurized / weakly pasteurized milk and dairy products (especially cheese) and meat originated foods. Especially foods directly derivated from milk like unaged or aged produced under insufficient hygienic conditions / short aged cheese products, cream, unpasteurized butter and / or the foods that are produced in anti – hygienic conditions are the primary risky food groups for the existance of *Listeria monocytogenes.*Serotyping the species of *Listeria monocytogenes* with conventional methods are important, too. For isolation and identification of *Listeria monocytogenes*, conventional microbiological methods are still accepted as referance methods. In addition to this, serological typing procedures are being used for determining existance of the agent and identification of the isolates in an increasing rate and accepted and adviced by the Europian Community. In this study, 100 kashar cheese samples collected from open bazaars were analyzed for *Listeria monocytogenes* and the results showed that 7 samples were positive. According to the serological tests, 3 samples were identified as seotype 1/2b (human originated), 2 samples were identified as serotype 1/2a (cattle orginated), 1 sample was identified as 4a (food originated) and 1 sample was identified as 4c (non – spesific). As conclusion it was decided that different originated *Listeria monocytogenes* serotypes may bring about serious threats for public / consumers' health.

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Drug-resistant tuberculosis in Poland in 2012

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Tuberculosis still remains a major problem in the world, with approximately 8 million new infections and 2,5 million to 3 million deaths per year. Drug resistant tuberculosis, and particularly multidrug-resistant tuberculosis is an increasing health problem and a serious challenge to TB control programmes. Information about susceptibility patterns of *Mycobacterium tuberculosis* isolates against antituberculosis drugs is an important aspect of tuberculosis control, and surveillance and analysis of local rates of TB drug resistance is helpful in the detection and monitoring of the extent of MDR strains. In 1994, the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) launched a global project on anti-tuberculosis drug resistance surveillance. To date, WHO published five reports presenting global incidence of tuberculosis with primary and acquired drug resistance. In Poland, in the period 1997 to 2012 was performed 5 retrospective studies covering the whole country.

The aim: To determine the prevalence and patterns of primary and acquired drug resistance among Mycobacterium tuberculosis isolates recovered from tuberculosis patients in Poland in 2012

Materials and methods. Prospective survey based on the questionnaires and strains of *M.tuberculosis*. Cultures was obtained from all regional centers participating in this co-operative study. 4190 questionnaires and cultures were obtained from patients who excreted TB bacilli during the 12-month period from 1 January 2012 to 31 December 2012. Drug resistance tests were performed on the L-J medium and MGIT system.

Results. Of the 4136 patients included, 2951 (71,39%) were males and 1185 (28,7%) were females, giving a sex ratio of 2.5:1. Of the 4136 patients, 3596 (86,9%) had received no previous treatment (new tuberculosis cases), while 540 (13,1%) had received treatment (previously treated tuberculosis cases). The prevalence of MDR-TB in the sample as a whole was 1,1%, whereas it was 0,6% among the new cases of tuberculosis and 4,4% among the patients with a history of tuberculosis treatment.

Keywords: drug resistance, *Mycobacterium tuberculosis*

Efficient national surveillance for healthcare-associated infections

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Detecting novel healthcare-associated infections (HCAI) as early as possible is an important public health priority. However, there is currently no evidence base to guide the design of efficient and reliable surveillance systems. Here we address this issue in the context of a novel pathogen spreading primarily between hospitals through the movement of patients.

Using a recently developped mathematical modelling approach [1] we compare the current surveillance system for a HCAI that spreads between hospitals due to patient movements as it is implemented in Scotland with a gold standard to determine if the current system is maximally efficient or if it would be beneficial to alter the number and choice of hospitals in which to concentrate surveillance effort.

We validate our model by demonstrating that it accurately predicts the risk of methicillin-resistant Staphylococcus aureus bacteraemia cases in hospitals in Scotland. Furthermore, the model predicts that relying solely on the 29 (out of 182) 'sentinel' hospitals that currently contribute most of the national surveillance effort results in an average detection time of 117 days. This can be reduced to 87 days by optimal selection of the same number of hospitals. Alternatively, the same detection time (117 days) can be achieved using just 22 optimally selected hospitals. Increasing the number of sentinel hospitals to 38 (all teaching and general hospitals) reduces detection time by just 13 days; however decreasing the number to 7 sentinel hospitals (all teaching hospitals) increases detection time substantially to 268 days (Figure 1).

Our results show that the current surveillance system as it is used in Scotland is near optimal for detecting novel pathogens when compared to a gold standard. However, efficiency gains are possible by better choice of sentinel hospitals, although there would be little advantage in increasing the number of hospitals involved in surveillance. Similar studies could be used elsewhere to inform the design and implementation of efficient national, hospital-based surveillance systems that achieve rapid detection of novel HCAIs for minimal effort.

Keywords: surveillance; healthcare-associated infections; MRSA; hospital-network; patients-referral; stochastic simulation model

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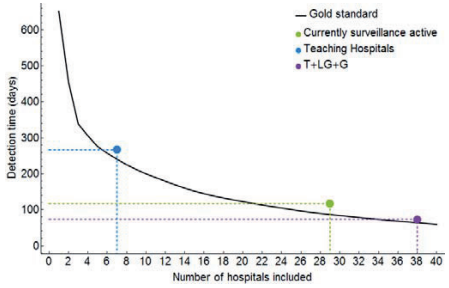

Figure 1. Mean detection time of a novel nosocomial pathogen, following emergence in a single randomly selected hospital versus number of hospitals participating in a sentinel surveillance programme. T+LG+G = teaching + large general hospitals + general hospitals.

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Examination of thioridazines potentiating effect on chlorhexidine against Methicillin-resistant *Staphylococcus aureus*

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Introduction

Resistant bacteria and in particular Methicillin resistant *Staphylococcus aureus* (MRSA) are on the rise throughout Europe. In Denmark, which is a former low incidence country, we have seen a dramatic rise in cases since 2003[1], which has led to more public and political attention to the dangers of resistant bacteria. MRSA is a well-known pathogen in both local and airway infections as well as sepsis. Also MRSA has shown a significant ability for biofilm formation in both urinary catheters as well as in direct venous access catheters [2].
The aim of this study was to investigate whether the phenothiazine thioridazine has the same potentiating effect on chlorhexidine, that we have earlier shown on dicloxacillin [3,4], if so we believe this effect can be used in the treatment of MRSA colonization and infection in the outer nasal cavity and in catheters.
In the present study we have explored the synergistic effect of thioridazine further in combination with common chlorhexidine against five clinical isolates of MRSA.

Methods

A sub inhibitory concentration of both chlorhexidine and thioridazine was chosen using macro tube dilution. A bacterial overnight (ON) culture was diluted to 0.02 optical density at 600 nm (OD600) compared to sterile medium and grown to early exponential phase at 37 degrees, as determined by OD600 of 0.2. The bacterial culture was then divided and different concentrations of both chlorhexidine and thioridazine were added. The highest concentration which saw no inhibitory effect on bacterial growth was then used in a viability assay. Five strains (one methicillin sensitive strain: 25923 and four MRSA strains: USA300, 52944 (CC398), 51726 (CC398), and the mupirocin resistant 4828) were grown as described, in the presence of the chosen sub inhibitory concentrations of both drugs independently and in combination. The bacterial growth was recorded at every hour by OD600 measurement, and the number of colony-forming-units per milliliter was counted five times in the 24 hours. Effective synergy was defined as a growth inhibition of $2*\log^{10}$ CFU/ml in the combination treatment compared to either drug alone. All results were repeated for improved reliability.

Results

All five strains were tested with fixed sub inhibitory concentrations of thioridazine, chlorhexidine and in combination in a 24 hour viability assay. We saw a significantly increased killing effect of the combination treatment compared to either drug alone with a growth inhibition of $6*\log^{10}$ to $8*\log^{10}$ as measured by CFU count after 24 hours.

Conclusion

With the remarkable growth inhibition seen in all five strains tested, we conclude that thioridazine effectively potentiates chlorhexidine in the killing of differing strains of both methicillin sensitive and resistant *Staphylococcus aureus* as well as the mupirocin resistant strain 4828. With respect to the different strains tested, we expect to see the same drug combination synergy against most *Staphylococcus aureus* strains. How this effect can be harnessed in vivo, is a matter for future studies.

Areas of further study

Ongoing studies continue examining the effect of the drug synergy on MRSA in vitro biofilm formation and degradation.

Keywords: MRSA; Thioridazine; Chlorhexidine; Combination therapy

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Experimental evaluation of the action of antihistaminic drug methdiazine singly and in combination against *Mycobacterium tuberculosis*

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Tuberculosis (TB) continues to be one of the most fatal infections of the present time despite a well planned therapeutic regimen recommended by the World Health Organisation (WHO). In 2011, there were an estimated 8.7 million new cases of TB (13% co-infected with HIV), of whom 1.4 million people died [1]. The occurrence of multidrug resistant strains of *Mycobacterium tuberculosis* in particular and other mycobacteria in general requires surveillance and control at a global level. Failure to cure multidrug resistant tuberculosis (MDR-TB) with the currently available antitubercular drugs led to a search for newer and potent drugs to treat such cases, and thereby prevent this emerging multidimensional problem. Different studies aimed at discovering new antimycobacterial agents have revealed moderate to powerful action to several compounds belonging to various pharmacological groups. These include chlorpromazine, promazine, thioridazine, trifluprom azine and other phenothiazines [2]. Many such agents also possess strong antimicrobial action against Gram positive and Gram negative bacterial pathogens.

The antihistaminic drug methdilazine (Md) possessing remarkable antimicrobial action against many crucial pathogens was looked for its activity against *M. tuberculosis* and several other mycobacteria. The minimum inhibitory concentration (MIC) of Md and common antimycobacterial drugs were determined with the help of tube dilution test in Kirchner's Liquid Medium (KLM) in which most of the test organisms were inhibited at 10-25 µg of Md.

In vivo test was carried out by producing systemic infection in two groups of inbred Swiss Albino mice. Both the groups were challenged with $9x10^9$ cells of *M. tuberculosis* $H_{37}Ra102$ suspended in KLM. One of the groups was administered Md (dose 10 mg/kg body weight/day) for six weeks. Thereafter the viscera of all the animals were autopsied, examined for macroscopic lesions and portions of each organ were processed for histological studies. Statistically significant protection was observed in the batch of mice that received Md. According to chi-square test, *in vivo* data were highly significant ($p<0.01$). *In vitro* studies for determination of combined action of Md with known antitubercular antibiotics revealed that it was distinctly synergistic with streptomycin and antagonistic with rifampicin.

Keywords: Methdilazine, Antihistamine, *Mycobacterium tuberculosis*, Antimycobacterial, Synergism

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Haemobiogram after intramuscular administration of amoxicillin to sheep

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There are many bacterial infections affecting sheep that necessitates antibiotic intervension. Amoxicillin is among commonly used antibiotics in such case for its broad spectrum of activity. However, the side alterations in blood and organ function that may be associated during or after treatment are questionable. Therefore, the aim of the present study was to assess the possible alterations in blood parameters and organ function biomarkers of sheep that may occur following intramuscular injection of amoxicillin. Amoxicillin has been administered intramuscularly to 10 sheep at a dosage regimen of 7 mg/kg of body weight for 5 successive days. Two types of blood samples (with and without anticoagulant) were collected from the jugular vein pre- and post-administration of the drug. Amoxicillin significantly ($P < 0.001$) increased total leukocyte count and ($P < 0.05$) absolute eosinophilic count when compared with those of the control samples. Aspartate aminotransferase, alkaline phosphatase and cholesterol were significantly ($P < 0.05$) higher than the corresponding control values. In addition, amoxicillin significantly ($P < 0.05$) increased blood urea nitrogen and creatinine but decreased phosphorus level when compared with those of prior-administration samples.

These data may indicate that although the side changes caused by amoxicillin are minor in sheep, yet the liver and kidney functions should be monitored during its usage in therapy and it should be used with care for treatment of sheep with renal and/or hepatic impairments.

Keywords: Amoxicillin, biogram, haemogram, sheep.

Helicobacter pylori-targeted biomaterials to prevent gastric cancer

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Introduction: *Helicobacter pylori* (*H. pylori*) is a Gram negative bacterium that colonizes the stomach of about 50% of human population.[1] Although infection may be asymptomatic in some individuals, if persistent can cause several gastric disorders including gastric carcinoma.[2] Inefficiency of antibiotic treatment in 20% of the cases[3] led to the urgent need of alternative therapies. *H. pylori* adhesion to the gastric mucosa is mediated by bacterial adhesins and glycan structures expressed by gastric epithelial cells: the blood group antigen binding adhesin (BabA) recognizes Lewis b (Leb) and H-type 1 and sialic acid binding adhesin (SabA) binds sialyl-Lewis a (sLea) and sialyl-Lewis x (sLex).[4,5] This work aims to develop a biomaterial which can be orally administrated and bind *H. pylori* in the stomach, removing them through the gastrointestinal tract. It has been previously shown that 170 µm chitosan microspheres partially crosslinked with genipin are stable in acidic pH, have mucoadhesive properties, are not cytotoxic, are retained in the stomach of C56BL/6 mice for at least 2h and bind unspecifically different strains of *H. pylori*.[6,7] We herein evaluate the capacity of glycan-decorated chitosan microspheres (GlyR-Mic) to bind *H. pylori* through specific glycan-adhesin interactions and compete for bacterial adhesion with mice and human gastric mucosa.

Experimental Methods: Chitosan microspheres (Ch-Mic; d=170µm) were produced and GlyR (Leb or sLex) immobilized by "click chemistry". Immobilization of Leb and sLex was confirmed by transmission electron microscopy (TEM) after immunogold labeling with 1ary monoclonal Ab (BG6 or KM93) and 2ary immunogold conjugate Ab. Adhesion of FITC-*H. pylori* (with different BabA and SabA expression) to microspheres (2h; 37ºC; OD600i=0.04) was visualized by confocal microscopy. Competition assays for *H. pylori* adhesion between Mic and gastric mucosa were performed using two in vitro models and one ex vivo model, all expressing Leb. Regarding in vitro studies, Mic were added before (to test their prevention capacity) or after (to evaluate their removal capacity) the FITC-*H. pylori* incubation with sections of paraffin embedded gastric mucosa from mice and humans. Bacterial adhesion was detected by fluoeresecence microscopy and quantified using ImageJ software. An ex vivo model was designed to evaluate the ability of Mic to remove/prevent ^{35}S-radiolabeled *H. pylori* adhesion to fresh mice gastric mucosa, quantified using a luminescence counter. Statistical analysis was performed using Welch – ANOVA and T-test ($p<0.01$) for results from in-vitro and ex-vivo studies, respectively.

Results and Discussion: Glycan-adhesin specific *H. pylori* adhesion was confirmed since BabA+/SabA- *H. pylori* strain adhered to Leb-Mic but not to sLex-Mic. Unlike sLex-Mic, Leb-Mic were able to compete with Leb carbohydrates expressed in all the gastric mucosa models tested using a BabA+ *H. pylori* strain. Moreover, Leb-Mic presented higher bacterial removal and prevention rates than Ch-Mic. Regarding the in vitro model of mice and human gastric mucosa, statistically significant differences were observed for bacteria removal rates of ~60% and ~43%, and prevention rates of ~32% and ~35% using Leb-Mic, respectively. The ex-vivo model using fresh mice gastric stomach also suggested Leb-Mic ability to both remove (~65%) and prevent (~78%) *H. pylori* adhesion from/to these gastric sections.

Conclusion: *H. pylori* adhesion to Leb- and sLex-Mic is ligand specific, and efficiency studies revealed Leb-Mic increased capacity to compete with mice and human gastric mucosa for *H. pylori* adhesion comparing to chitosan microspheres without glycans. These results highlight the potential of glycan nanoengineered chitosan microspheres as alternative or complementary treatment to *H. pylori* gastric infection.

Keywords: gastric infection; chitosan; microspheres; bacterial adhesion; glycans; adhesins.

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Identification of bats that act as reservoirs or hosts for viral diseases by the sequencing of mitochondrial DNA b gene

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The identification of species that act as reservoirs or hosts of zoonotic agents is essential for control and epidemiological surveillance of the important illness in public health. According to World Health Organization (WHO) viruses are the main etiological agents of emergent and reemergent zoonoses around the world, as Rabies virus. Identification of the reservoirs for zoonoses can help to clarify how the pathogens are maintained in nature, leading to more effective disease control and avoiding indiscriminate extermination of wild animals. Bats are the main reservoir of Rabies virus and other infectious agents as other viruses, fungi, parasites, bacteria or even unconventional agents. The aim of the present study was to perform genetic sequencing of the mitochondrial DNA b gene (mtDNA cyt-b) of bats from different species. The mtDNA cyt-b gene contains species-specific information and has been used routinely because there are thousands of sequences of this gene of vertebrates available in public database, as GenBank. The diversity of bats and the important role they play in the spread of diseases make the need for correct identification of these animals. This study was conducted using 56 tissue samples sent to rabies diagnosis from bats. The PCR and the DNA sequencing was carried out according Carnieli et al. [1] using the set of primers 05A (sense: 5'-CGACTAATGACATGAAAAATCACCGTTG-3') and 14A (antisense: 5'-TATTCCCTTTGCCGGTTTACAAGACC-3') primers [2]. By sequencing the mtDNA cyt-b gene and posterior phylogenetic analysis four families (Molossidae, Vespertilionidae, Noctilionidae and Phyllostomidae), twelve genera and nineteen different species of bats were identified. The Basic Local Alignment Search Tool (BLAST) was used to confirm species identity. Our results were concordant with those obtained by other authors, showing effectiveness of the method described to genetic identification of bats. The phylogenetic tree generated with the genetic sequences of the mtDNA cyt-b gene has confirmed the quality of sequences and phylogenetic relationships evidenced by the genetic-tree topology are consistent with the results of different authors. In addition the same protocol used to identify bats was tested with tissue samples of other species of mammal from three superorders of the infraclass Placentalia (Xenarthra, Euarchontoglires and Laurasiatheria), except Afrotheria superorder. The results were similar to those obtained with tissue samples of bats. Thus, one can say that the same method can be used to identify other species of mammal. The very wide variety of bats and their great ability to adapt to different environments are reflected in the many different species already identified on all continents, except Polar Regions. The colonization of different ecosystems and different eating habits of the bats may be the cause of the large number of viruses from different family's identified recently in the order Chiroptera, such as several viruses of the genus Lyssavirus (Rabies virus, European bat lyssavirus, Aravan virus and Khujand virus) and others of the families Adenoviridae, Bunyaviridae, Arenaviridae, Paramyxoviridae, Herpesviridae, Flaviviridae, Rhabdoviridae, Coronaviridae and so on. In conclusion the sequencing of the mtDNA cyt-b gene can be used as an important tool for the genetic identification of different species of mammal.

Keywords bats; viral diseases; mitochondrial DNA cytochrome b gene

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Identification of Candida species using CHROMagar and their evaluation of susceptibility testing with Sensititre Yeast One colorimetric antifungal microdilution panel

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The aim of this study was to investigate the identification of *Candida* species using CHROMagar and conventional methods and the in vitro susceptibility to nine antifungal agents against yeasts isolated from various clinical specimens of hospitalized patients in the coronary and surgical ICUs of the Istanbul University Cardiology Institute during a one year period.

A total of 44 isolates were obtained from 16 patients. Yeasts were isolated from blood, urine, endotracheal aspiration fluid, sputum and wound. All yeasts were identified by conventional methods and using CHROMagar Candida (CA) (Becton Dickenson). When suspected from identification of *Candida* species by API ID 32C (Bio Merieux, France) was confirmed. Susceptibility to anidulafungin, micafungin, caspofungin, 5-flucytosine, posaconazole, voriconazole, itraconazole, fluconazole and amphotericin B was evaluated using colorimetric microdilution panel (SENSITITRE YeastONE Trek Diagnostic Systems, Cleveland, OH, USA).

The most common isolated yeast was *Candida albicans* (54%), followed by *Candida tropicalis* (27%), *Candida glabrata* (11%), *Candida parapsilosis* (2%), *Candida lusitaniae* (2%), *Candida sake* (2%) and *Geotrichum capitatum* (2%). Resistance varies depending on the species and the respective antifungal agents. All of *C. albicans* isolates was found to be susceptible to anidulafungin, micafungin, caspofungin, 5-flucytosine and amphotericin B. But, 11 of *C. albicans* isolates were resistant to voriconazole (MIC≥8 µg/ml), itraconazole (MIC≥16µg/ml), fluconazole (MIC≥256 µg/ml) and posaconazole (MIC≥8 µg/ml). Also, three of *C. albicans* isolates was resistant to itraconazole (MIC≥2 µg/ml) and susceptible-dose depend (SDD) to fluconazole (MIC=16-32 µg/ml). All of *C. tropicalis* was found to be susceptible anidulafungin, micafungin, caspofungin, 5-flucytosine, posaconazole, voriconazole, fluconazole and amphotericin B. But, three of *C. tropicalis* were resistant to itraconazole (MIC≥1 µg/ml) and also, two C. tropicalis were susceptible-dose dependent to itraconazole (MIC≥0.25-0.5 µg/ml). Two of *C. glabrata* isolates were resistant to itraconazole (MIC≥2 µg/ml) and also, S-DD to fluconazole (MIC=16-32 µg/ml). Three of *C. glabrata* were resistant to itraconazole (MIC≥16 µg/ml) and fluconazole (MIC≥128 µg/ml) and these *C. glabrata* isolates were also susceptible to the other antifungals.*C. parapsilosis* was found to be susceptible to studied all antifungals. *C. lusitaniae* was S-DD to itraconazole (MIC=0.25 µg/ml) and susceptible to other studied antifungals. *C. sake* was S-DD to fluconazole MIC=16 µg/ml and susceptible to other antifungal. *Geotrichum capitatum* was non susceptible (NS) to anidulafungin (MIC≥8 µg/ml), micagungin (MIC≥8 µg/ml) and caspofungin (MIC≥8 µg/ml). Also, this isolate was S-DD to voriconazole (MIC≥2 µg/ml), itraconazole (MIC≥0.5 µg/ml) and fluconazole (MIC=16 µg/ml) and susceptible to the other antifungals.

Resistance to antifungal agents is an alarming sign for the emerging nosocomial fungal infections in our unit.

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Improvement of modified karmali agar by addition of tazobactam for detecting *Campylobacter* spp. in chicken carcass rinse

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Tazobactam, ESBL inhibitor, was added to karmali agar and investigated for improving the selectivity and specificity of commercial karmali agar. The modified agar showed optimum activity at 4 mg of tazobactam for 1 L of karmali agar concentration. Normal karmali agar and modified karmali agar (T-karmali agar) were evaluated with 120 whole chicken samples. All samples were rinsed with 400 ml buffered peptone water by gentle shaking for 1 min and then this rinse fluid samples were enriched with 2x Bolton enrichment broth at 42°C for 48 h under microaerobic condition. After incubation, a loopful of enrichment broth was streaked onto unmodified and modified karmali agar, followed by incubation at same condition as described above and then presumptive colonies was subcultured on 5% Columbia blood agar and final identification was performed by colony PCR. The isolation ability of T-karmali was higher than unmodified normal karmali agar (T-karmali, 16 out of 120, karmali, 10 out of 120). Furthermore, the selectivity of the T-karmali agar was significantly higher than that of karmali agar (18 out of 120, 99 out of 120) and growth index of background flora (T-karmali, 0.18 ; Karmali, 2.43) was also better than normal karmali agar. We conclude that karmali agar supplemented with tazobactam can effectively inhibit competing flora, allowing to isolate *Campylobacter* spp. successfully from chicken carcass.

Keywords: Campylobacter;Tazobactam; ESBL; Karmali; Chicken carcass

In vitro adherence of *Staphylococci* to polymeric and biologic hernia mesh implants

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Background: Prosthetic mesh infection is one of the most serious complications that can occur following hernia surgery, being the first 24 h of exposition a highly risk period for the bacterial adhesion and mesh surface colonization. The aim of the present work was to compare *in vitro* the behaviour of several meshes for hernia repair when the materials were exposed to bacterial contamination with two *Staphylococci* strains.

Methods: Nine commercially available hernia meshes were used and classified according to the nature of the material (see table). Ten sterile samples of the each material (1x1cm) were immersed in 3 mL of Lysogeny broth and inoculated with 1 mL of a 10^6 CFU suspension of *Staphylococcus aureus* ATCC25923 and *Staphylococcus epidermidis* ATCC12228 (n=5 each). After 24 h of incubation at 37 ºC, the mesh fragments were collected. For each material and contaminating strain, 3 samples were carefully washed and sonicated at 40 KHz for 10 minutes. The product of the sonication was serially diluted and plated to quantify the bacterial adhesion to the mesh surface. The remaining fragments were cut in half, fixed and analyzed under scanning electron microscopy (SEM). Biomeshes were also processed for light microscopy, to evaluate the bacterial colonization through the mesh wall, using gram staining and immunostaining (anti- *S. aureus*, *S. epidermidis*) protocols.

Results: There were no relevant differences in terms of bacterial adhesion to the mesh surface between *S. aureus* and *S. epidermidis*, with the only exception of *Perm*, which showed a higher adhesion when inoculated with *S. epidermidis* ($p<0.05$). For the two strains, the meshes with the lowest adhesion were *Op*, *Surg* and *Precl*.
Regarding the polymeric meshes contaminated with *S. aureus*, *BioA* exhibited the highest adhesion, specially relevant when compared to *Op*, *Surg* and *Precl* ($p<0.001$). From the biomeshes, *Perm* and *Tuto* behaved similarly and showed a lower adhesion than *St*. *Sis* was the one with the lowest bacterial recovery.
With *S. epidermidis*, *BioA* and *TIGR* were the polymeric meshes which the highest adhesion. From the biomeshes, *Perm* and *St* exhibited big amounts of bacteria attached to the surface. As observed previously, *Sis* showed the lowest adhesion.
S. epidermidis were more likely to form biofilms on the different surfaces than *S. aureus*, as revealed with the SEM analyses. *S. aureus* attached to the polymeric materials exhibited planktonic growth while the bioprostheses showed niches of bacteria growing in the pores of the collagen matrices. Both gram staining and immunostaining revealed a similar and discreet colonizarion of *S. aureus* and *S. epidermidis* into the wall of the biomeshes, with the exception of *Sis*, which evidenced a higher penetration due to its multilaminar structure.

Conclusion: Polymeric meshes such as *Op*, *Surg* and *Precl* showed very low levels of bacterial adhesion. By contrast, both *TIGR* and *BioA* behaved similarly to those biologic meshes, allowing the bacteria to settle in niches and colonize the whole surface of the graft. *Op*, *Surg* and *Precl* probably constitute better candidates than biomeshes to be used in contaminated fields.

Mesh	Material (composition and origin)	Structure	Manufacturer
Optilene® (*Op*)	Polypropylene (Polymeric)	Reticular, monofilament	B Braun
Surgipro® (*Surg*)	Polypropylene (Polymeric)	Reticular, monofilament	Covidien
TIGR® (*TIGR*)	Resorbable glycolide, lactide, trimethylcarbonate (Polymeric)	Reticular, multifilament	Novus Scientific
Preclude® (*Precl*)	Non-porous polytetrafluoroethylene (Polymeric)	Laminar	Gore & Assoc.
Bio-A® (*BIOA*)	Resorbable polyglicolide, TMC (Polymeric)	Laminar	Gore & Assoc.
Permacol™ (*Perm*)	Porcine dermal collagen matrix (Biologic)	Laminar, crosslink	Covidien
Surgisis® (*Sis*)	Porcine small intestinal submucosa matrix (Biologic)	Multilaminar, noncrosslink	Cook Ireland
Strattice™ (*St*)	Porcine dermal collagen matrix (Biologic)	Laminar, non-crosslink	LifeCell EMEA
Tutomesh® (*Tuto*)	Bovine pericardium matrix (Biologic)	Laminar, non-crosslink	Tutogen Medical

Table: Prosthetic materials utilized in the study

Keywords: Mesh infection; Bacterial adhesion; Biofilms; *Staphylococcus aureus*; *Staphylococcus epidermidis*.

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In vitro and *in vivo* analyses of the antipsychotic phenothiazine compound triflupromazine as an antimicrobial agent

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Extensive use of antibiotics had led to the emergence of multidrug resistance among pathogenic bacteria. This ever growing problem had been discussed and studied in many investigations in recent years. Although many new anti-infective compounds are being reported, acquisition of mutations and drug resistant plasmids has restricted their therapeutic usage. However, several studies have revealed significant antimicrobial property of drugs belonging to different pharmacological classes. These newer antimicrobials from non-conventional sources have been designated as "Non-antibiotics"[1]. Again, some of these antimictrobial agents have been revealed to show significant synergism with known antibiotics. From all these studies done extensively by Kristiansen, Molnar, Amaral and Dastidar and their colleagues [2], it has been observed that antipsychotics, containing three benzene rings, are endowed with the most potent antimicrobial action. The present study describes the antimicrobial potentiality of such a non-antibiotic – the antipsychotic drug triflupromazine (Tp). This drug possesses a methyl-thio substituent at position 10 and a fluorine moiety at position 2. Significant antimicrobial activity was shown against 279 strains of Gram-positive and Gram-negative bacteria by this non-antibiotic compound.. The Minimum Inhibitory Concentration (MIC) of the drug ranged between 2-50 µg/ml for *Staphylococcus aureus* and 5-100 µg/ml for shigellae and vibrios. Disc diffusion assays were performed between this phenothiazine and antibiotics, which showed a highly effective synergism when Tp was combined with streptomycin. Fractional Inhibitory Concentration (FIC) index of the duo was found to be 0.375 which confirmed the significant synergism. Furthermore, triflupromazine was also subjected to *in vivo* experiments in Swiss Albino mice, challenged with 50 median lethal dose of *Salmonella typhimurium* NCTC 74. Both alone or in combination, Tp manifested a significant protection to the mice (*P<0.001*) and also reduced the infection in internal organs. Thus, the present study suggests that Tp has the potential for being developed into a powerful antibacterial agent, the efficacy of which may be enhanced further with a suitable synergistic combination.

Keywords : triflupromazine, phenothiazine, antimicrobial, microbicide, non-antibiotic, synergism

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In vitro effect of silver nitrate and hypertonic sodium chloride against protoscoleces of hydatid cyst in a short period, up to five minutes

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Hydatidosis is one of the most important parasitic zoonoses. Its selective treatment is surgery. Since there is a risk of cyst rupture during surgery and the formation of secondary cysts, surgeons aspirate some of the cyst's fluid before opening it and inject a protoscolicidal agent in order to prevent secondary cyst formation in the case of cyst rupture. However, the application of these substances seem doubtful because the protoscolicidal effect of the substances and the necessary time for their effect are questionable and also most of these substances could create some complications and have some side effects on the patient. Hence, we aimed to compare the protoscolicidal effect of silver nitrate 0.5% and hypertonic sodium chloride 20% with cetrimide 0.5% as positive control which showed a high protoscolicidal effect and normal saline 0.9% as a negative control in short periods of up to 5 minutes.

Sheep cysts of the liver and lung were gathered from Shiraz slaughter house and in the parasitology laboratory of the Shiraz Faculty of Medicine, 3000-4000 protoscoleces with a viability of over 90% were separately exposed to 1 milliliter cetrimide 0.5 %, hypertonic sodium chloride 20 % , silver nitrate 0.5 % and normal saline at three different time periods of 1, 2 and 5 minutes. Afterwards, their viability was evaluated with eosin 0.1 % and the obtained results were analyzed using ANOVA and LSD methods.

In vitro observations showed that the protoscolicidal effect of cetrimide 0.5 % was 100 % from the very first minute and showed a significant difference ($P<0.05$), compared to other tested substances. At 2 minutes of exposure, the protoscolicidal effect of cetrimide 0.5 % was also significant ($P= 0.003$). At 5 minutes, there was no significant difference between cetrimide 0.5% and hypertonic sodium chloride 20% regarding their protoscolicidal effect ($P=1$), while at this exposure time the difference between cetrimide 0.5 % and silver nitrate 0.5 % was significant regarding their protoscolicidal effect ($P=0$).

These findings showed that in *in vitro*, hypertonic sodium chloride 20 % at 5 minutes, has a high protoscolicidal effect while silver nitrate 0.5 % does not have a complete protoscolicidal effect up to 5 minutes. *In vivo* studies are necessary in order to investigate the side effects of these substances in the body.

Key words: protoscoleces, silver nitrate, hypertonic sodium chloride, protoscolicidal agents

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In vivo Effect of Combination of Ceftazidime and Ciprofloxacin on Antibiotic Resistant *E coli* isolate from Urine Specimen of Seropositive HIV patients in North Central, Nigeria

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BACKGROUND: Antibiotics combination therapy provides justification of delaying the emergence of antibiotic resistance, minimizing the risk of drug toxicity and broadens the spectrum of antibiotic activities. Studies on the effect of combination of ceftazidime and ciprofloxacin on antibiotic resistant *E. coli* isolated from urine of sero-positive HIV patients attending South Atlantic Medical Centre, Nasarawa State University, Keffi was carried out. **METHOD:** *Escherichia coli* was isolated from urine using Standard Protocol and antibiotic susceptibility was carried out using standard procedure. The Minimum Inhibitory Concentration of ceftazodime alone, ciprofloxacin alone and combination of ceftazidime and ciprofloxacin using standard protocol. The time-kill assay was carried out using standard procedure. The synergistic effect combination of ciprofloxacin and ceftazidime was observed in all the isolates demonstrated by checkerboard titration method and synergism and indifference demonstrated in combination of ciprofloxacin and ceftazidime was observed in all the phenotypes. **RESULTS:** Irrespective of all the resistant phenotype, the effect of combination of ceftazidime and ciprofloxacin was significant at 0hr($P=0.0012$), 2hr(0.0161), 4hr($P=0.0000004$) 6hr($P=0.000003$), 8hr(0.0003), 10hr($P=0.00007$) respectively and insignificant at 12hr($P=0.3742$) **CONCLUSION:** in view of synergism demonstrated in combination of ceftazidime and ciprofloxacin, implies that the combination of the drug may be useful for therapy of *E.coli* infection and further work in the mechanism of synergism demonstrated in the combination of the two drug should be carried out.

Keyword: Synergism, ceftrazidime

Investigation of the synergistic antifungal activities of the novel cationic steroid antibiotics CSA-8, CSA-13, CSA-44, CSA-131 and CSA-138 against *Candida* species isolated from various cultures in a Turkish Hospital

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Opportunistic yeast infections, particularly those caused by Candida species, are the most prevalent fungal infections of humans and are a serious concern for patients with compromised immune systems, such as transplant recipients, human immunodeficiency virus-infected patients, and cancer patients especially with hematologic malignancies and febrile neutropenia attacks. As chronic infections are difficult to treat, attempts have been made to discover new antimicrobial agents targeting novel sites that may circumvent resistance. The ceragenins, designed to mimic the activities of antimicrobial peptides, are a new class of antimicrobial agent. Ceragenins are not peptide based, are not salt sensitive, and are relatively simple to prepare and purify on a large scale. Among them, CSA-13, which stands for cationic steroidal antimicrobial is a lead ceragenin and is highly active against Gram-positive and Gram-negative bacteria.

In this study, in vitro antifungal combination activities of the various novel cationic steroid molecules such as CSA-8, CSA-13, CSA-44, CSA-131 and CSA-138, were assessed against *Candida* strains from various specimens submitted to the Clinical Microbiology Laboratories of Group Florence Nightingale Hospitals in Turkey. MICs and MFCs were determined by microdilution technique according to CLSI guidelines. Antibiotic combinations were assessed by using the checkerboard technique.

Susceptibility testing demonstrated that the MIC_{50} (µg/ml) values of CSA-8, CSA-13, CSA-44, CSA-131, CSA-138 and amphotericin B were 16, 1, 2, 1, 1 and 1, respectively. The MFCs were equal to or two-fold greater than those of the MICs. With a FIC index of ≤ 0.5 as borderline, synergistic interactions were mostly seen with CSA-13- amphotericin B combination (35 %). No antagonism was observed.

According to our results, especially CSA-13, CSA-131 and CSA-138 appear to be good candidates in the treatment of *Candida* infections. The CSAs exhibited pronounced synergistic activity in combination with the key antifungal drug, amphotericin B. Therefore, CSAs are promising candidates for further research as antifungal drugs and as agents for combination treatment. Future studies should be performed to correlate its safety, efficacy and pharmacokinetic parameters of these molecules.

Keywords: Candida; cationic steroid antibiotics; combination

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Invitro antimicrobial susceptibility of *Propionibacterium acnes* isolated from acne patients in India

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Background: *Propionibacterium acnes* is an anaerobic bacterium mainly implicated in the development of acne vulgaris. Rampant use of topical and systemic antibiotics for acne vulgaris has resulted in reports of resistance worldwide due to selective pressure. The alarming rise in antimicrobial resistance in *P.acnes* poses a serious threat to management of patients. There are no previous reports of antimicrobial susceptibility data of *P.acnes* available from India.

Objectives: This study aims to determine the susceptibilities of *P. acnes* to commonly used antibiotics in the treatment of acne and provide resistance rates in India

Methods: Samples were collected from acne lesions of 100 patients. All samples were cultured into sheep blood agar, MacConkey agar and Brain Heart Infusion agar with 5g/ l glucose (BHIg) and 2mg/l Furazolidone under aerobic and anaerobic conditions. Species identification of all isolates was done by conventional methods and final identification was performed using VITEK^R 2 Compact (C) (Biomeriux, North Carolina / USA) system. Antibiotic susceptibility of aerobes was determined by Kirby- Bauer disc diffusion method. For anaerobes, Minimum inhibitory concentration (MIC) for penicillin, erythromycin, clindamycin, ciprofloxacin, nadifloxacin, and tetracycline was determined by E- test (ABO Sweden) and MIC of minocycline was determined by agar dilution on BHIg using spot inoculation. MIC results were interpreted as per EUCAST and CLSI guidelines (M27-A7).

Results: *P.* acnes was the most common anaerobe (66%) isolated. Among aerobes, *Staphylococcus aureus* (65%) was predominant, followed by *S. epidermidis* (5%), and *Klebsiella sp* (4%). Resistance to penicillin was observed among 6.7% isolates (EUCAST breakpoints) and to ciprofloxacin among 3% isolates (CLSI breakpoints). Resistance (%) rates using EUCAST and CLSI breakpoints were 10.6 and 6.1, 7.6 and 0, 7.8 and 0 for erythromycin, clindamycin and minocycline respectively. Tetracycline resistance was observed in 9.2% isolates irrespective of the interpretative criteria used. MIC values showed bimodal distribution for all antibiotics and were lowest for penicillin (0.03-32 µg/ml). MIC_{50} and MIC_{90} values for nadifloxacin (0.25 and 1 µg/ml) were found to be two fold lower than ciprofloxacin (0.5, 1 µg/ml). Similarly MIC_{50} and MIC_{90} values for minocycline (0.125 and 0.5 µg/ml) were also two to three fold lower than those of tetracycline (0.38 and 1 µg/ml).

Conclusion: Topical and systemic anti-acne antibiotics (penicillin, macrolides, lincosamides, fluoroquinolones and tetracyclines) continue to maintain activity against *P.acnes* isolated from acne lesions in India. Resistance to individual agents is now present within 10% of isolates and therefore the situation should continue to be monitored.

Isolation of a Multi-drug resistant (Manual ESBL and Modified Hodge Test Negative) and KpC positive Salmonella Group E from a 5-year old male with Severe Combined Immunodeficiency (SCID) in a Private Tertiary Hospital in Davao City, Philippines.

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History: A 5-year old male was admitted last February 2013 for multiple infections secondary to Severe Combined Immunodeficiency. He was treated with different antibiotics for Mycobacterial, Fungal and Multiple opportunistic Bacterial Infections. Patient complained of gastrointestinal pain and mild diarrhea. The first request for stool culture was made on May 6, 2013 and the Microbiology Laboratory identified the organism by VITEK 2 compact (Biomeriux) as *Salmonella* Group. Polyvalent slide latex agglutination (Sifin Germany) was used and typed it as Group E *Salmonella*. Sensitivity results were resistant to all Cephalosporins, Fluoroquinolones, and Aminopenicillins and Penicillins with beta lactamase inhibitor. Carbapenems were all sensitive and attending physician started Imipenem. After 15 days, the second culture was repeated. *Salmonella* group E was isolated with the same sensitivity pattern but this time gave an intermediate result on Ertapenem. The third stool culture was done after five days. *Salmonella* group E was isolated with same sensitivity pattern but carbapenems were all reported resistant. Manual ESBL and Modified Hodge tests were performed according to the CLSI and ESCMID guidelines. Both yielded negative. Sample was sent to Milan, Italy for Molecular Typing. Results showed that the organism was positive for the Carbapenemase class KpC. Patient was treated successfully with Colistin.

Conclusion: The investigators were able to isolate the first *Salmonella* non-typhi (Group E) that is highly resistant to Carbepenems, Cephalosporins & other anti-Salmonella drugs. *Salmonella spp*. is generally sensitive to a wide variety of antibiotics. ESCMID and CLSI recommend only a few antibiotics to be tested against *Salmonella typhi* and *Salmonella non-typhi*. Ampicillin, Co-trimoxazole, a flouroquinolone and a 3rd generation Cephalosporin antibiotics in cases of extra intestinal Salmonellosis must be reported in the sensitivity panel. This case reports the 1st strain of *Salmonella* non-typhi that is highly resistant to these anti-microbials and responded only to Colistin.

Keywords: Carbapenemase, ESBL and Modified Hodge Test

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Isolation of clinical strains of *Staphylococcus epidermidis* from a Portuguese hospital and assessment of their relationship between biofilm formation capacity and antimicrobial resistance

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Staphylococcus epidermidis has been documented as an emergent pathogen responsible for many healthcare-associated infections (HAIs). These infections are an increasing cause of major concern not only due to the high distribution of methicillin resistance, but also due to their ability to form biofilm, which increases their persistence, impairs patient's quality of life and leads to failed treatment and extra costs. Portugal has one of highest incidence rates of HAIs in Europe. However, bacteriological information that may shed light on the clinical significance of *S. epidermidis* Portuguese isolates and provide data for control as well as epidemiological measures is missing. In order to fill this gap, the aim of this study was to isolate and determinate the antibiotic resistance profile of clinical strains of *S. epidermidis* and ensure its association with phenotypic and genotypic biofilm-associated determinants.

Of the 89 studied patients, 52 (58.4%) were men and the mean age was 45 years old. Bloodstream infections (69.7%) were the most frequently reported infections during the study period and almost a third of all infections were catheter-related. The majority (85.4%) of the clinical isolates were *mecA*-positive and among those, 92.1% were also resistant to 3 or more of the antimicrobial agent groups tested and hence considered multidrug-resistant (MDR). Resistance also reaches higher levels among β-lactam antibiotics (96.4%) and erythromycin (79.8%). Notwithstanding, positive associations were found between MDR and MRSE strains, between MDR strains and prescription of at least one antimicrobial agent and between patients under antibiotic therapeutic and MRSE strains. Regarding the phenotypic and molecular features, the majority (64%) of the clinical isolates were considered biofilm producers and all strong producers were carriers of the *icaA* gene, although equal distributed among MRSE and MSSE strains. The genetic combination most frequently observed was $icaA^{+}aap^{+}$ (41.6%) followed by $icaA^{+}aap^{+}bhp^{+}$ (21.3%). Additionally, strains with the genetic combination $icaA^{+}aap^{+}bhp^{+}$ were positively associated with both MRSE and MDR phenotype.

Our results confirmed the impact of *S. epidermidis* on hospital-acquired infections and highlight the burden of antimicrobial resistance, mainly multidrug resistance that reached alarming levels in this tertiary-care hospital. Moreover, this data raised concerns regarding antimicrobial strategies previously adopted. In addition, an association between the carriage of some virulent-associated genes and biofilm phenotype was clear, mainly regarding the carriage of *icaA* gene that demostrated to be essential in the biofilm process of *S. epidermidis* clinical strains.

Keywords: *Staphylococcus epidermidis*; antibiotic resistance; phenotypic and clinical features.

Isolation of *Listeria monocytogenes* of Karun River (Environmental Sources wild and urban) by Culture and PCR Assay

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Background
Listeria spp. are Gram-positive, different anaerobic, non-spore-forming, rod-shaped bacteria. This bacterium infects a wide variety of animal hosts in addition to humans, including other mammals (sheep, cattle, goats, pigs, rabbits, mice), birds, fish and freshwater. Only *L. monocytogenes*is is the primary human pathogenesis. Listeriolysin O (LLO) is a major pathogenesis factor in this bacterium, which is encoded by *hlyA* gene.

Objectives
The purpose of this research was to isolation of *Listeria monocytogenes* from different parts of the Karoun River in Iran by culture and PCR assay.

Materials and Methods
A total of 150 water samples Karun river (environment wild and urban) collected over of 3-month sampling period (from March to May 2014). These isolates were identified by conventional (Typical colonies were identified by the morphological characteristics and biochemical tests) and PCR techniques as belonging to *L. monocytogenes***.** Finally, *hlyA* and *inlA* gene was determined by PCR method.

Results
A total of 150 water samples Karun river (wild and urban environment), 4 samples (wild environment) and 16 (urban environment) were culture positive and all these samples by PCR (*hlyA* and *inlA* genes) were confirmed.

Conclusion
Consumption of water contaminated with *Listeria monocytogenes,* can cause human disease.

Key words: *Listeriamonocytogenes*, Cultural method , PCR assay, *hlyA* and *inlA* genes, Karun river

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Laboratory diagnosis of purulent otitis by analysing enzyme activity of blood serum for the ability to destroy peptidoglycan

Viktoryia Ziamko; Vitaly Okulich

Background. Endogenous antimicrobial peptides are small molecules, built from amino acids. They are effective against a broad range of pathogens including bacteria, fungi and viruses. Most of them are represented by cationic granule-associated polypeptides with an affinity for components of microbial cell wall, for example an affinity for peptidoglican (PG).

Methods. Isolation of PG from cell wall of gram-negative bacteria was carried out by method proposed by V. Lvov, B. Pinegina and R. Khaitov in our modification. *E. coli* ATCC 25922 was used as a culture. The received PG, labeled with 2% solution of Congo red was used as a substrate for determining activity of enzymes, that destroy PG. Blood was taken from patients with purulent otitis and from healthy donors. Enzymes in blood serum destroyed PG, Congo red became soluble, changed its color from colorless to red with a maximum spectrum of absorption at a wavelength of 495 nm. Absorbance of the solution was measured in a multichannel spectrophotometer F300 in the wavelength of 492 nm.

The result was expressed in picokatals (pkat) and was calculated by using the following formula, obtained after construction of the calibration graph for the breeding of Congo red:

$$Y = [-0{,}001 + 0{,}026 \times Eop] \times 9{,}921$$

Y - the desired result
Eop - optical density of the sample minus optical density of control

Since the analysis of the date distribution showed their nonparametric distribution, statistic processing was performed by using the Kolmogorov-Smirnov test, the differences were considered significant at $p < 0.05$.

Results. In patients with purulent otitis activity of enzymes destroying PG was significantly higher than in donors. After complement inactivation, enzyme ability significantly reduced.

Conclusions. The technique allows to determine antimicrobial enzyme activity of blood serum for ability to degrade peptidoglycan.

It was found that enzyme ability to destroy peptidoglycan was significantly higher in patients with purulent otitis, than in donors.

The ability of serum to destroy peptidoglycan after inactivation of complement decreases.

Keywords: Endogenous antimicrobial peptides; peptidoglycan; enzyme activity.

Reference:

Yanagi S., Ashitani J., Imai K. Significance of human b-defensins in the epithelial lining fluid of patients with chronic lower respiratory tract infections // ClinMicrobiol Infect. - 2007. -Vol.13. - P. 63-69.

Lantibiotic Nai-107 rescues Drosophila melanogaster from fatal injection with *Staphylococcus aureus*

Thomas Thyge Thomsen

Since the golden era of antibiotic drug development during the 1950`s and 60`s, drug resistance development has been of growing concern globally. Today multidrug resistance is a huge burden to societies around the globe, as many lives are lost to infections previously treatable. Furthermore, multidrug resistance has financial costs in the billions of dollars on public budgets around the globe. New antibiotics are lacking and few are under development, as antibiotic drug development is costly and with limited developmental success. Therefore, it is of interest to develop alternative methods for screening of lead compounds before taking them into expensive clinical trials, as this might help bring down costs of development. Previously, Drosophila has been suggested as a model for screening of antibacterials, and has been applied limited (Chamilos et al., 2011; Ben-Ami et al., 2013; Tzelepis et al., 2013). Here we examine the potential of Drosophila as a model for testing antimicrobial efficacy and toxicity of antimicrobial peptides in vivo. A panel of peptides with known antibacterial activity was tested to assay the usefulness as Drosophila as an initial in vivo platform. Of the compounds tested Lantibiotic Nai-107 currently undergoing preclinical trial, rescues adult flies from fatal injections with *Staphylococcus aureus* 8325-4 ($p < 0{,}0001$).

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Management of Chronic Periodontitis using Metronidazole local drug delivery device as an adjunct to subgingival debridement: A clinical, microbiological & molecular study

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Background: Modern concepts of treating inflammatory periodontal disease aims at changes in the sub gingival ecosystems within the periodontal pockets in order to alter the complex microbial community into a micro biota compatible with periodontal health. Systemic antimicrobial therapy though effective, involves a relatively high dose with repeated intakes over a prolonged period of time to achieve the required inhibitory concentrations in the sulcular fluid. The adjunctive use of local drug delivery may provide a beneficial response especially in specific areas where conventional forms of therapy might fail. The aim of this study was to evaluate the efficacy of a Local drug delivery system containing Metronidazole as adjunct to mechanotherapy in the treatment of Chronic Periodontitis.

Methods: There were two groups divided as group A (Scaling + Metronidazole) and group B (Scaling alone). A microbiological analysis was carried out to determine the efficacy of these systems in changing the pathogenic flora in deep pockets. Further a Multiplex Polymerase chain reaction (PCR) was carried out to confirm the presence of Actinobacillus actinomycetemcomitans (Aa) Porphyromonas gingivalis (Pg) and Tannerella forsythensis (Tf) in the flora associated with Chronic Periodontitis.

Results: In group A, there was a clinical improvement which correlated with an improvement in microbiological parameters and these results were maintained 90 days following therapy. In Group B, the flora showed a shift towards baseline at the end of 90 days.

Conclusions: According to this study the local drug delivery system when used as adjunct to mechanotherapy resulted in greater improvement in microbiological parameters when compared to mechanotherapy alone.

Key Words: Mechanotherapy; local drug delivery

Microbiological analysis of bacterial and fungal Late onset sepsis in Neonatal Intensive Care Unit Cairo University- Egypt

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Sepsis remains among the leading causes of death in both developed and underdeveloped countries. Late onset sepsis (LOS manifests after the first week of life and is generally acquired from postnatal environment. The diagnosis of LOS is problematic because of the overlap between clinical signs associated with physiological disturbances and those of bacteraemia or fungemia. The aim of the study is to assess the patterns of microorganisms causing Late Onset sepsis in NICU Cairo University and to detect the prevalence of fungal infections in both blood and bronchalveolar lavage (BAL) of septic neonates. A prospective study including 100 septic neonates receiving intravenous antibiotics for a week or more with no signs of clinical improvement. All included neonates were subjected to full history taking and laboratory investigations including: CBC where I: T ratio was calculated, C-Reactive protein. Blood culture and bronchoalveolar lavage (BAL) samples were collected simultaneously. Culture and sensitivity for both bacterial and fungal infections. Blood cultures detected: CONS in 24%. Candidemia was proved in 32% of cultures using Sabaraud Dextrose Agar (SDA) Media. By PCR 56% (18/32) of all isolated candida species were identified as C.albicans while only 50 % (16/32) were correctly identified with conventional laboratory methods. BAL Cultures detected: Candida in 10%, Klebsiella and Pseudomonas in 8% of samples. Antibiotic sensititivity patterns of Gram negative organisms in blood cultures and BAL showed that most of them were sensitive to imipenem; and to a lesser extent to amikacin. Antibiotic sensitivity of Gram positive organisms; including CONS showed 100% sensitivity to vancomycin (VA). Forty eight percent of patients died (30% having positive candida culture) and 52% discharged (2% having positive candida culture) CONS were the most prevalent isolated bacterial pathogen in septic neonates. C. albicans was the most common isolated candida species. In accordance effective infection control strategy is strongly recommended, and implementation of a proper antibiotic policy, in order to reduce the colonization of candida spp. and the emergence of resistant bacterial strains. Identification of candida infection by PCR using ITS sequencing, is a reliable method that can be used as an accurate and sensitive alternative to conventional identification methods.

Key words; neonatal sepsis, fungemia, bactremia, candidemia, NICU

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Microbiology and clinical outcomes of intra-abdominal infections in a tertiary hospital ICU, one year period

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Introduction and Objectives: Intraabdominal infection present high rates of intra-hospital mortality. Types of pathogens and their drug resistance patterns may determine the outcome of peritonitis. The present study determines the microbiology of peritonitis, antibiotic resistance in commonly isolated bacterial pathogens. The objective was to describe the most common pathogens and their resistance to empirically antibiotics most commonly used; and clinical outcomes.

Methods: This is a retrospective study conducted in the ICU of CHU Badajoz in patients admitted to our Unit with the diagnosis of primary, secondary and tertiary peritonitis. We describe the results including microbiological spectrum antibiotic resistance profile and sensitivity. Microbiological samples were obtained mostly from direct aspiration during surgical procedure and /or paracentesis.

Results: 31 patients, 5 admitted with a diagnosis of primary peritonitis (16%), secondary peritonitis 21 (68%) and tertiary peritonitis 5 (16%). The mean age was 56 years (SD ± 12), 24 males (77.4%). The mean SOFA was 5.5 (SD ± 2.5) and APACHE II of 13.5 (± 5.2). Bacteria were isolated in 25 samples, from which 17 (68%) were gram-negative bacilli (GNB) and 9 (36%) of them were non-fermenting (NF-GNB) and 8 (32%) were fermenters (F-GNB). Gram-positive cocci (GPC) were isolated in 8 (32%) of which 6 (24%) were enterococci and 2 (8%) coagulase-negative Staphylococcus. Most of NF-GNB presented resistance to carbapenems, aminoglycosides and cephalosporins 3rd and 4th, and sensitive to colistin in the case of A. Baumanii, the remaining to fosfomycin and piperacillin / tazobactam. F-GNBs were resistant to cephalosporins and aminoglycosides, and sensitive to quinolones, carbapenems and fosfomycin. Regarding GPC more than 50% were resistant to oxacillin and aminoglycosides, and 50% showed vancomycin MIC ≤ 1. There were 16 deaths, and in 7 (44%) of them GNB were isolated.

Conclusions: In our group of peritonitis, the vast majority of infections were due to GNB bugs, with similar percentage of NF-GNB and F-GNB. We found high rates of resistance to 3rd and 4th cephalosporins and aminoglycosides, being the NF-GNB carbapenemase resistant, so its use should not be empirically recommended. Bacterial cultures are fundamental for the knowledge of local flora and their resistance profile, in order to improve antibiotic therapy protocols and a more suitable treatment.

Keywords: Peritonitis1, Microbiology2; Drug Resistance3

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Molecular analysis of class I integron genes in clinical Staphylococcus isolates

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Introduction and Objectives: Staphylococcus is an important Nosocomial infectious agent which is notorious for rapidly gaining antimicrobial resistance genes. Integrons are a series of mobile genetic elements that are able to express gene cassettes encoding various antibiotic resistances. This study aimed to identify integron class I gene cassettes in clinical Staphylococcus isolates recovered from patients in Sanandaj, Iran hospitals.

Materials and Methods: A total of 200 Staphylococci spp. was recovered from nose and throat swabs of patients (ICU and infection wards) in Toohid and Beasat hospitals in Sanandaj, Iran. Following bacterial DNA extraction, Class I Integron gene was detected by PCR.

Results: Out of the 200 Staphylococci spp. , 81 (40.5%) isolates were carriers of class I integron . The integron expressing isolates included 35 cases (23.5%) of *Staphylococcus epidermidis*, 37 cases (40.1%) of *Staphylococcus aureus*, and 9 cases (36%) of Staphylococcus *saprophyticus.*

Conclusion: Results indicated that frequency of class I integron gene is quite high among clinical Staphylococcus isolates in Sanandaj area. For control of antibiotic resistance spread, screening of clinical samples for these genes and elucidation of their genetic diversity is crucial.

Key words: *Staphylococcus aureus*, Coagulase negative Staphylococcus, integrons class I

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Molecular sub-typing and genetic characteristics of *Campylobacter* isolated in China

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In order to obtain the genetic mechanism and molecular sub-typing characteristics of the erythromycin resistant *Campylobacter* isolates in China, a total of 222 *Campylobacter* isolates from diarrheal patients and food producing animals were selected in this study. The agar dilution method, according to CLSI (2006) was used to investigate the Minimum Inhibitory Concentrations（MIC） of erythromycin for each isolate. PCR and DNA sequencing were used to identify the mutations in the gene of 23s rDNA and the present of *ermB* gene was detected by PCR for the entire erythromycin resistant isolates with MIC equal or over 32 ug/ml. Multi-locus sequence typing (MLST) was performed for the entire resistant isolates. Overall, 113 isolates (51%) were resistant to erythromycin, of which 109 were *C. coli* and 4 were *C. jejuni*. Eighty two isolates (72.6%) of the resistant isolates show A2075G mutation in the 23S rDNA gene, including 79 *C. coli* and 3 *C. jejuni*. None A2074C were found in this study. The *ermB* gene was identified in 27 isolates (23.9%), 26 were *C. coli* and 1 *C. jejuni*. One isolate was found having both *ermB* gene and 23S rDNA A2075G mutation. There are four isolates which have neither *ermB* gene and nor 23S rDNA mutation. Forty ST types were identified among the 113 isolates and ST872（21 *C. coli*）, ST860（11 *C. coli*）, ST6322（9 *C. coli*）and ST1145（8，7 *C. coli* and 1 *C. jejuni*）were predominant among the resistant isolates. All of the ST6322 (9 isolates) and ST1145 (8 isolates) isolates identified in this study have the *ermB* gene.

Keywords: *Campylobacter*; erythromycin resistance; genetic mechanisms; MLST

Molecular tests for the detection of drug-resistant tuberculosis

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Drug-resistant tuberculosis, and particularly multidrug-resistant tuberculosis (MDR-TB) and extensive drug resistant TB (XDR-TB) as an increasing health problem and a serious challenge to TB control programmes. To control MDR-TB, drug resistance patterns should be available to guide the therapy of the patient. However, phenotypic drug susceptibility testing (DST) is a time-consuming process because it requires culturing, which may take up to two months or longer. To address such delay in TB diagnosis, molecular diagnosis aspects need to be considered for the early detection of *M. tuberculosis* which involves the detection of the mutation in specific genes imparting resistance against rifampicin (RIF) and/or isoniazid (INH), mostly used as the first line anti-tubercular drugs. Rapid diagnosis of MDR-TB will permit an earlier start with second-line drug treatment for patients with MDR-TB and may thus decrease the risk of treatment failure, relapse and continuing transmission of MDR-TB.

In this study, we compared the performance of the GenoType® MTBDRplus to a broth-based DST assay for detecting INH resistance, RIF resistance.

Materials and Methods: We analyzed 60 strains of *Mycobacterium tuberculosis*. For all the strains made conventional drug resistance detection and molecular analysis of mutations in the genes determining the occurrence of drug resistance.

Results Among the 60 strains, 48 (80%) showed an identical pattern of resistance obtained by both methods. 56% of the strains showed resistance MDR, one strain was resistant to rifampicin, 9 were identified as INH-resistant and four strains were sensitive to all the antimycobacterial drugs. Forty six of 51 (90,2%) INH-resistant strains and 39 of 42 (92,9%) RMP-resistant strains were found to have a mutation in the analyzed katG gene fragment or inhA locus and rpoB gene fragment.

Keywords: MDR-TB, molecular diagnosis

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Monovalent D-Mannosides as FimH Antagonists – A Novel Therapy for Urinary Tract Infections

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Urinary tract infections (UTIs), primarily caused by uropathogenic *Escherichia coli* (UPEC), affect millions of people each year and account for significant morbidity and high medical costs. These infections are initiated by the adhesion of the lectin FimH, located at the tips of bacterial type 1 pili, to oligomannosides of the glycoprotein uroplakin on the uroepithelium. Since bacterial resistance to antibiotics is a major problem of recurrent infections, blocking of bacterial adhesion with soluble carbohydrates or analogs thereof is a promising therapeutic approach to UTI.

We have synthesized various families of monovalent FimH antagonists, explored their activity in a panel of *in vitro* assays and determined their pharmacokinetic properties.[1,2] In addition, the interaction of the antagonists with the FimH CRD could be refined by X-ray crystallography. The most promising candidates were validated in an *in vivo* PK/PD study. Efficacy studies in the UTI mouse model resulted in a significant reduction of bacterial infection in bladder and kidneys, demonstrating the high therapeutic potential of FimH antagonists.

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Phenotypic and genotypic detection of Extended Spectrum Beta-lactamases into *Escherichia coli, Klebsiella pneumoniae* and *Enterobacter* spp. inpatients at a university hospital in southern Brazil

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Enterobacteriaceae are opportunistic pathogens responsible for approximately 70% of urinary infections and 50% of septicemia. Furthermore, it has become a challenge in the treatment of infectious diseases, especially in hospitals, due to the easiness with which acquire resistance genes. The production of Extended Spectrum β-lactamases (ESBL) is the most important mechanism of resistance in Enterobacteriaceae. Among the several ESBLs described in the literature, ESBL like TEM, SHV and CTX-M are the most widespread worldwide. The aim of this study was to evaluate by phenotypic and genotypic methods, the resistance profile of strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter* spp. ESBL-producing isolates from inpatients at the university hospital located in Florianópolis/SC, Brazil. 145 strains isolated from blood, urine, tracheal aspirates and wound inpatients were identified. Antimicrobial susceptibility profile were performed on the Vitek 2 (bioMérieux) according to the manufacturer's instructions and phenotypic ESBL detection by disk approximation test were performed by Kirby-Bauer's method. ESBLs genotypic survey was taken for bla_{TEM}, bla_{SHV} and $bla_{\text{CTX-M}}$ genes by two multiplex PCR reactions. The identity of the amplified product of each gene was confirmed by Sanger sequencing. The genetic similarity of isolates was performed by RAPD-PCR. Posteriorly, whole-genome sequencing of six *K. pneumoniae* strains were performed on the MiSeq Sequencing System (Illumina, Inc) with Nextera™ DNA Sample Prep Kit and MiSeq Reagent Kit v3 (paired-ends reads 2 x 75pb) to assess the genetic profile of these isolates. The reads from sequencing were mapped using bowtie 2.2.3 against an antimicrobial resistance genes collection and to all bacteria complete genome and plasmids database, available on NCBI ftp server. The total isolates, 66.9% were *E. coli*, 25.5% were *K. pneumoniae*, 4.1% were *Enterobacter cloacae* and 3.4% were *Enterobacter aerogenes*. The ESBL prevalence in isolates was 27.6% and 32.5% of these were *E. coli*, 60% were *K. pneumoniae* and 7.5% were *E. cloacae*. The bla_{TEM} gene was found in 82.5% (33) samples, bla_{SHV} was detected in 60% (24) isolates and $bla_{\text{CTX-M}}$ was detected in 42.5% (17) 17.5% (7) and 12.5% (5) for CTX-M group 1, group 2 and group 9, respectively. Additionally, 77% of ESBL-positive isolates expressing more than one enzyme type. The sequencing analysis confirmed the species *Klebsiella pneumonia*. Eleven plasmids were identified by assembly mapping, namely pKpn114, pEA1509.B, pIGMS32, pNE1280, pGNB2, pBRST7.6, pB1023, pSL476.3, pEA1509.B, pCKO3, pCSA2, all of them described in different species of *Enterobacteriaceae*, which are related to the dissemination of different resistance genes. This finding underscores the promiscuous plasmid transfer occurring between different bacterial species and the diversity of plasmids carrying the most varied resistance genes. RAPD genotyping, *K. pneumoniae* samples were more similar to each other than *E. coli* isolates, and two of the three *E. clocae* stems had the same electrophoretic profile. However, despite some slight differences, most isolates were classified at least as "possibly related" according to the criteria of Tenover. Therefore, the results obtained in this study suggest the existence of a strain of *K. pneumoniae* endemic in this hospital, spread across all sectors. This scenario is quite alarming, considering that different plasmids were found in genotypically similar strains by RAPD, confirming the unrestrained transfer of plasmids in strains of *Enterobacteriaceae* .

Keywords: ESBL, Enterobacteriaceae, disk approximation, multiplex PCR, whole-genome sequencing, RAPD .

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Phenotypic and molecular characterization to determine the antimicrobial profile in *Acinetobacter baumannii*: support for clinical practice management

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Acinetobacter baumannii is an emerging bacterial pathogen worldwide, which causes a variety of healthcare-associated infections (HAI), such as bacteremia, urinary tract infection, surgical site infection, skin and soft tissue infection, ventilator-associated pneumonia and bloodstream infections, some of them associated with high morbidity and mortality. *A. baumannii* has a unique ability to survive in hospital environments and to develop antibiotics resistance, causing major hospital outbreaks and creating great therapeutic challenges for the current antibiotic era. In general, the success of *A. baumannii* can be attributed to several factors, like its ability to form biofilms, desiccation tolerance on abiotic surfaces and besides that, horizontal transfer has been shown to be an important process for exogenous DNA acquisition in *A. baumannii*, specially for antibiotic resistance genes, maintained at a high frequency in the population through the intense selection imposed by antimicrobial therapy processes. In Brazil the major gene clusters encoding OXA-type beta-lactamases were identified in *A. baumannii* strains, giving them a multiresistant profile. This study aimed to establish the antimicrobial resistance pattern of *A. baumannii* isolated in a university hospital located in Florianópolis/SC, Brazil, by using phenotypic and molecular characterization. *A. baumannii* isolates were obtained from routine clinical samples of 31 inpatients. Bacterial identification, the minimal inhibitory concentration (MIC) and antimicrobial susceptibility profile were performed on the Vitek 2 (bioMérieux) according to the manufacturer's instructions. The presence of the genes *bla*OXA-23-like, *bla*OXA-51-like, *bla*OXA-58-like, *bla*OXA-72-like and *bla*OXA-143 was verified by Polymerase Chain Reaction (PCR), with specific primers (Neoprospecta Microbiome Technologies S/A). Subsequently, whole-genome sequencing of eight *A. baumannii* strains were performed on the MiSeq Sequencing System (Illumina, Inc) with Nextera™ DNA Sample Prep Kit and MiSeq Reagent Kit v3 (paired-ends reads 2 x 75pb) to assess the genetic profile of these isolates. The reads from sequencing were mapped using bowtie 2.2.3 against an antimicrobial resistance genes collection and to all bacteria complete genome and plasmids database, available on NCBI ftp server. The multidrug resistant (MDR) profile was found in 97% (30) of isolates (resistance to 10 out of the 14 antimicrobials tested), whereas 10% of these (3) presented an extensive drug resistant (XDR) profile (complete or intermediate resistance to 12 out of the 14 antimicrobials tested). Only 3% (1) of the isolates did not show a MDR profile. The *bla*OXA-23-like and *bla*OXA-51-like genes were identified in all isolates by PCR, while the remaining genes were not identified. The sequencing analysis confirmed the species *Acinetobacter baumannii*, as well as the presence of the *bla*OXA-23-like and *bla*OXA-51-like genes in all sequenced isolates. The search for resistance genes allowed the identification of *bla*TEM-1 gene in all isolates, which confers resistance to penicilin and cephalosporins antibiotics. The IS*Aba* insertion sequence was also identified, which encode transposases and have been found to affect the expression of neighboring genes, like *bla*OXA-23. Furthermore, four plasmids were identified by assembly mapping, namely p1BJAB0868, p1ABTCDC0715, pAB0057 and p1ABIBUN, all of them described in *A. baumannii* strains. These results corroborate the findings of other Brazilian studies that demonstrate the MDR/XDR profiles and the presence of OXA-like genes in *A. baumannii* strains that colonize healthcare settings in Brazil. The complete genome sequencing confirmed that the molecular detection of resistance genes is effective and it provided important information, such as the presence of plasmids that can promote horizontal gene exchange. Although one of the isolates (community origin) showed an antibiogram profile of sensitivity to most classes of the investigated antibiotics, the genotypic data demonstrated the opposite. In this case, the MDR profile could emerge after antibiotic selection pressure, or MDR profile showed by other isolates could be explained by other factors like Over-Expressed Efflux Pumps. Considering that the patterns of expression of resistance genes are highly variable and subject to micro-environment conditions of the host, the exclusive use of phenotypic tests in the clinical management could lead to type II errors (false negatives) that contribute to the increase and spread of

antimicrobial resistance. Thus, it seems to be crucial to implement techniques, such as based on molecular biology, to assist the clinical and therapeutic management of patient.

Keywords: *A. baumannii*, healthcare-associated infections, OXA-type beta-lactamases, whole-genome sequencing.

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Prevalence and characterization of extended-spectrum-β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in ready-to-eat vegetables

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Objectives: To determine the prevalence and characteristics of extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) in ready-to-eat (RTE) vegetables.

Methods: A total of 189 RTE vegetables samples (91 sprouts and 98 mixed salads) were collected in a retail market in South Korea from October 2011 to February 2012. The samples were screened for ESBL-producing *E. coli* and *K. pneumoniae*, and were confirmed using Brilliance ESBL agar and Vitek2, respectively. ESBL genes and CTX-M genetic platforms were characterized by PCR and sequencing.

Results: The prevalence of ESBL-producing *E. coli* and *K. pneumoniae* was 10.1%. Of these, 94.7% were from the sprout samples. All isolates were resistant to cefotaxime, and most ESBL producers were resistant to non-β-lactam antibiotics, including gentamicin, trimethoprim/sulfamethoxazole, and ciprofloxacin (73.7%, 63.2%, and 26.3% respectively). TEM-1, SHV-1, -2, -11, -12, -27, -28, -61 and -120, and CTX-M-14, -15 and -55 β-lactamases were detected alone or in combination. The genetic platforms of all CTX-M producing isolates were ISEcp1-blaCTX-M-orf477 and ISEcp1-blaCTX-M-IS903 in CTX-M groups 1 and 9, respectively.

Conclusions: To our knowledge, this is the first report of the prevalence and characterization of ESBL-producing *E. coli* and *K. pneumoniae* isolated from RTE vegetables. The results of this study indicate that RTE vegetables may play a role in spread of ESBL genes.

Keywords: ESBL; RTE-Vegetables; Prevalence; *E. coli*; *K. pneumoniae*

Prevalence and genetic relatedness of ESBL-producing *E. coli* from pig holdings and humans in the Dutch-German border region

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Objectives: The spread of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* is a global threat. Besides its increase in the clinical setting, livestock is considered to be a potential zoonotic reservoir of multidrug-resistant bacteria. The aim of this study was to compare the prevalence and types of ESBL-*Escherichia coli* in pig holdings and hospitalized patients from the border region of Germany and the Netherlands.

Methods: A total of 305 ESBL-*E. coli* detected by selective medium for ESBL-producing bacteria were included in the study: 199 clinical *E. coli* isolates from patients of four hospitals in the Dutch-German border region (Groningen, Enschede, Oldenburg, and Münster) collected from January to June 2011; and 106 *E. coli* collected from 47 different pig farms (dust and manure samples) on the German side of the border region between February and September 2013. Susceptibility testing was studied by VITEK®2 automated systems according to EUCAST breakpoints. Microarray assays were performed to determine the ESBL-type (Check-MDR CT103 array®, Clondiag® Chip). The molecular epidemiology was determined by the Diversilab® (DL) bacterial typing system. Analysis was done using the Pearson correlation, isolates with a similarity <90% were considered different and isolates with a similarity >95% indistinguishable. Those human and animal isolates which cluster together by DL will be analyzed by whole genome sequencing and the information will be added (not included).

Results: A total of 67.7% of human clinical ESBL-*E. coli* isolates were resistant to ceftazidime, 72.4% to amoxicillin/clavulanic acid and 66.7% to piperacillin/sulbactam. Animal-associated ESBL-*E. coli* isolates were 23% resistant and 12.4% intermediate to ceftazidime. Besides, they were resistant to ampicillin/sulbactam (84.4%) and piperacillin/sulbactam (4.7%). The most prevalent ESBL gene among human *E. coli* was CTX-M-group-1/group-15 (57.8%) alone (14.6%) or combined with TEM non-ESBL (19.6%) and/or other resistant genes (CMY and OXA-1-like, 23.6%). Second most prevalent (23.1%) was the CTX-M-9-like gene alone (4%) or together with TEM non-ESBL (9%) and/or other resistant genes (CMY and OXA-1-like, 10.1%). *E. coli* isolates from animal origin presented the following ESBL genes: CTX-M-1-like (n=46, 43.4%), CTX-M-15-like (n=7, 6.6%), CTX-M-9-like (n=6, 5.7%), these genes were also found combined with TEM non-ESBL in 25.5% (n=27), 3.8% (n=4) and 6.6% (n=7), respectively. Additionally, five human isolates (4.7%) had OXA-1-like genes (three together with CTX-M-15-like and two with CTX-M-9-like). Two *E. coli* isolates had a plasmid-AmpC CMY-II-like gene together with a TEM non-ESBL gene. Molecular typing of 199 human *E. coli* isolates revealed 63 singleton clusters and 33 clusters, with one large cluster formed by 42 isolates from different hospitals. Animal *E. coli* isolates showed 22 clusters formed by more than one isolate: eight clusters with isolates harbouring the same ESBL gene and isolated from the same farm and ten clusters combined isolates from geographically unrelated farms having the same ESBL gene. The analysis of clinical and animal-associated *E. coli* isolates together showed a total of eight clusters combining isolates from both origins, two clusters with clinical *E. coli* isolates from Dutch hospitals and six clusters with clinical isolates from the German side.

Conclusions: The most prevalent ESBL gene found among *E. coli* isolates from pig holdings was CTX-M-1 like, unaccompanied or together with TEM non-ESBL. In samples from hospital origin the most frequent ESBL genes in *E. coli* isolates were CTX-M-group-1 and 15, accompanied with TEM non-ESBL, CMY and OXA-1-like genes. Some *E. coli* isolates from human and animal origin were genetically related, which could indicate possible transmission of resistant bacteria between animals and humans.

Keywords: ESBL; livestock; *E. coli*

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Pseudomonas aeruginosa diversification at early infection stages in cystic fibrosis lungs

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Cystic fibrosis (CF) is an autosomal recessive lung disease caused by a defect in the cystic fibrosis conductance regulator (CFTR) gene. The CFTR protein lack causes a defective chloride secretion creating an osmotic gradient that, consequently, provokes water hyper-reabsorption and abnormal thick and sticky sputum. The accumulated sputum is rich in nutrients being, thus, a good environment for microbial colonization. CF lungs are infected with a complex microbial flora, provoking acute and chronic infections that result in decline of the lung function and premature death of patients. The lungs colonization by *P. aeruginosa* in younger patients is less frequent however is directly associated with rapid lack of lung function and reduced chances of survival. While *P. aeruginosa* diversity and mechanisms of adaptation and evolution have been intensively studied at chronic stages, it is less clear the mechanisms used by *P. aeruginosa* to establish an infection in CF lungs and unclear the existence of bacterial diversification and its impact in infection establishment and progress.

This study aimed to investigate whether phenotypic diversity is present at early stages of CF infection and how it impacts in microevolution to chronic stages. Moreover, it was aimed to determine the role of early antibiotic treatments as a driven and selective force in *P. aeruginosa* populations towards diversification.

Three strains of P. *aeruginosa* were cultured in artificial sputum medium with and without sub-inhibitory concentrations of ciprofloxacin (CIP) for ten days. Afterwards, the diversity of the bacterial populations was assessed along time in terms of colony morphology. Each morphotype detected was further characterised regarding 6 virulence-associated traits and sensitivity to 10 clinical relevant antibiotics.

Results demonstrated the existence of population diversity at early stages of infection with and without antibiotic exposure. According the level of diversity, *P. aeruginosa* populations reacted differently to CIP concentrations. More diverse populations were able to resist to increased CIP concentrations in contrast with less diverse populations. Moreover, CIP treatments changed the population diversity and dynamics. CIP exposure favoured the emergence of mucoidy morphotypes (moist and mucoid variants) and small colony variants. The phenotypic diversity presents within *P. aeruginosa* populations was analyzed more in depth by isolating each colony morphology variant and measuring its antibiotic sensitivity, pyocyanin and hemolysin production, motility, auxotrophy and biofilm formation ability. The bacterial characteristics among CF early isolates significantly vary, however there is a trend towards high virulence potential. The examination of the virulence traits exhibited by colony morphotypes demonstrated that: bacteria had still limited ability to form biofilms, typical of early infection stages; hemolysin and pyocyanin production was variable during early infection; and bacteria still exhibited their ability to swim, swarm and twitch. Concerning antibiotic sensitivity, the majority of the colony variants that composed the bacterial populations exhibited sensitivity to antibiotics. Furthermore, it was observed that morphotypes have a degree of individuality, i.e., each morphotype gather particular combinations of characteristics no repeated in the population by other morphotype. According to the ecological model “insurance hypothesis”, this diversity ensures population to maintain or enhance their functioning against environmental fluctuations, typically antibiotic exposure, host immune defences, oxygen depletion and pH alterations.

In conclusion, according to these data *P. aeruginosa* diversification may exist in early in vivo CF infections. The functional importance and role within population of bacteria-associated colony morphology remains quite unexplored, however the findings of this study suggest that the level of bacterial diversification of populations at early stages can be an important indicator of the infection course and severity in CF airways.

Keywords: *Pseudomonas aeruginosa*; cystic fibrosis; clonal diversification, phenotypic variation

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Rapid Changes in Serotype and Antimicrobial Resistant Profile of Penicillin-nonsusceptible Pneumococci by Introduction of PCV7

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In Japan, 7-valent pneumococcal conjugative vaccine (PCV7) has been available commercially from February 2010 and incorporated into the routine vaccination schedule since April 2013. To investigate the impact of the introduction of PCV7, we have continuously collected penicillin-nonsusceptible *Streptococcus pneumoniae* (PNSSP) clinical isolates from a university hospital. In this study, the distribution of serotype and antimicrobial susceptibility were analysed among PNSSP isolated from pediatric nasal cavities before and after the introduction of PCV7.

A total of 301 PNSSP isolates from pediatric nasal cavities were collected from a university hospital between 2007 and 2012. These isolates were characterized by capsular typing based on the sequence of *cps* regions and antimicrobial susceptibility test determined by a broth-dilution method according to Clinical and Laboratory Standard Institute (CLSI). For antimicrobial resistant and nonsusceptible isolates, their mechanisms were determined genetically.

Two periods, before introduction of PCV7 (2007-2009) and after introduction of PCV7 (2010-2012), were included in this study. The proportion of vaccine serotypes decreased from 72.2% in 2007-2009 to 66.4% in 2010-2012, and significantly decreased to 52.1% in 2012 as compared to before introduction ($P<0.01$). Meanwhile, non-vaccine serotypes 23A and 15A increased in 2010-2012. Since these serotypes were not included in the 13-valent pneumococcal conjugate vaccine (PCV13), which was incorporated into the routine vaccination schedule in November 2013 instead of PCV7, the coverage rate of PCV13 decreased from 86.1% in 2007-2009 to 76.9% in 2010-2012. In this study, fluoroquinolone-resistant strains were not detected but isolates with high MIC for norfloxacine were detected and these isolates had an amino acid substitution I460V in ParE. The proportion of macrolide-resistant strains which harbour *mefA* and/or *ermB* were very high throughout the study periods (95.8% in 2007-2009, 97.8% in 2010-2012), in particular all isolates showed macrolide resistance in 2012.

It has been estimated that the PCV7 vaccination rate was <10% in 2010, 50-60% in 2011, and 80-90% in 2012 since an official program encouraged parents to get children vaccinated for PCV7. Our study indicated that a vaccination rate of approximately 50% could affect the serotype distribution of PNSSP in pediatric nasal cavties, suggesting that serotype distribution may change drastically after incorporation into the routine vaccination. Moreover, PNSSP tended to possess multidrug resistance.

Keywords: 7-valent pneumococcal conjugate vaccine (PCV7); PCV13; macrolide resistance

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Relationship between ciprofloxacin resistance and extended-spectrum beta-lactamase (ESBL) production in Escherichia coli Isolates from female patients with urinary tract infections in Turkey

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E. coli is the most common cause of urinary tract infections (UTIs) in women. The resistance of *E. coli* to commonly prescribed antibiotics is increasing worldwide. The present study was conducted to investigate the antimicrobial resistance patterns, relationship between ciprofloxacin resistance and ESBL production in *E. coli* isolates from female patients with UTIs

Between March and June 2014, a total of 131 *E. coli* isolates were isolated from urine samples of female patients with UTIs in Istanbul University, Cerrahpasa Medical Faculty hospital, Istanbul. ESBL production was detected in 23 (17,5%) isolates. Thirty-three isolates (25%) were resistant to ciprofloxacin (MICs ≥32 µg/ml). Fifteen (65,2%) of ESBL-producing isolates were resistant to ciprofloxacin, and ESBL was positive 8% of ciprofloxacin-susceptible isolates. ESBL-producing isolates were significantly more frequent among ciprofloxacin-resistant *E. coli* isolates than among ciprofloxacin-susceptible isolates ($p < 0.05$). Twenty-two (66,6%) of ciprofloxacin-resistant isolates belonged to women over the age of forty.

Ciprofloxacin-resistant and ESBL-producing isolates were highly resistant to ampicillin, cefuroxime, cefotaxime, ceftazidime (100%), amoxicillin/clavulanic acid, cefepime and trimethoprim-sulfamethoxazole (73,3%). The resistance is lower to amikacin (20%) and nitrofurantoin (6,6%). There was no resistance of carbapenems and fosfomycin.

These results suggest that ciprofloxacin should be used more cautiously in female patients with suspicion of an ESBL-producing *E. coli* UTI.

Keywords: Escherichia coli; Urinary tract infections; Ciprofloxacin resistance; ESBL

Remarkable antibiotic resistance of *Pantoea agglomerans,* opportunistic bacteria in patients with immunodeficiency

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Pantoea agglomerans has been commonly reported as epiphyte, endophyte or as plant pathogen, although it has also been involved in animal and human pathogenic processes associated to skin injuries caused by thorns, wood slivers, or wooden splinters. *P. agglomerans* can also occur as an opportunistic human pathogen, and has been recovered from joint fluids of patients with arthritis, synovitis, or osteomielitis [1].

The aim of this research was to isolate, identify and determine antibiotic sensitivity in opportunistic bacteria isolated from 25 HIV^+ patients from different Mexican hospitals.

Patients belonged to two groups; 21 HIV^+ patients with acute respiratory syndrome; 4 HIV^+ patients with tuberculosis symptoms. Serial (three days) sputum samples were obtained from all patients and processed in accordance with the guideline for tuberculosis bacteriological diagnoses. Obtained isolates were characterized and grouped by API 20E and API 50CH test strips followed by comparison to BioMerieux profile index. Antibiotic sensitivity was determined by disk diffusion method according to NCCLS Standards. Tested antibiotics included β-Lactams, aminoglycosides, cephalosporins, macrolides, nitrofurans, quinolones and sulfonamide. Identification was achieved by sequence analysis of 16S rRNA gene.

Pantoea agglomerans was the most common isolate, obtained in 36% of patients (9 out of 25). Strains showing 100% sequence similarity were regarded as same, selected strains of each group were used for antibiograms. From the 60 antibiotic resistance determinations (10 antibiotics x 6 strains), the overall resistance was 77%, while susceptibility scored for only 8%.

Antibiotic resistance of 6 selected *Pantoea agglomerans* strains

Antibiotic	NR5	NR9	NR17	NR24	NR43	NR31
Amikacin	R	I	R	S	S	R
Ampicillin	S	R	R	R	R	R
Carbenicillin	R	R	R	R	R	R
Cefotaxime	S	R	R	I	R	R
Ceftriaxone	R	R	R	I	R	R
Chloramphenicol	R	I	I	R	R	I
Gentamycin	R	I	I	R	S	R
Netilmicin	R	R	R	R	R	R
Nitrofurantoin	I	R	R	R	R	R
Pefloxacin	R	R	R	R	R	R

R: Resistant; I: Intermediate; S: Susceptible

Keywords: *Pantoea agglomerans* ; antibiotic resistance

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Retreatment of relapsing small intestinal bacterial overgrowth with Rifaximin polymorph α is effective and safe

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Background: The incidence of small intestinal bacterial overgrowth (SIBO) is increasing, mainly because of the increase of pharmacological risk factors (1, 2). Although SIBO can be successfully eradicated by Rifaximin, its recurrence is easily predictable, as long as the risk factors persist as long-term therapy with proton pump inhibitors (IPP), chronic atrophic gastritis, lactose intolerance (3) etc. Re-treatment therefore can be a clinical challenge.

Material & Method: One hundred and forty four patients treated on long-term treatment with PPI for gastro-esophageal reflux disease (GERD), successfully eradicated from SIBO with high dose Rifaximin were followed-up for 1 year for relapse investigation. At the end of follow-up, or before if symptoms suggested it, glucose hydrogen breath test (GHBT), (Quintron, Milwaukee, WI, USA) was performed to each patient and symptoms were recorded. All relapsed patients were retreated with Rifaximin 1200 mg/die for 2 weeks, as in the first course. The outcome of therapy was assessed both clinically and by means of GHBT, 2 months after the completion of Rifaximin course.

Results: Out of a cohort of 144 patients (M 84; mean age 46±14) successfully eradicated from SIBO with Rifaximin, 52 patients (M 32; mean age 45±13) relapsed (36%), mainly because of continuation of treatment with PPI for GERD (52/59, i.e. 88%). Forty-seven out of 52 patients retreated with Rifaximin showed negative GHBT along-side symptoms remission (90%), indicating a successful eradication from SIBO. No relevant side effect was registered.

Conclusions: 1) Relapse rate of SIBO within 1 year is high if treatment with PPI is not discontinued (88%). 2) Retreatment with Rifaximin 1200 mg/die for 2 weeks results to be effective and safe.

Key words: SIBO; Relapse; Retreatment; Rifaximin.

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Risk factors for fecal carriage of carbapenemase producing Enterobacteriaceae (CPE) among intensive care unit patients from a tertiary care center in India

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Introduction: - Carbapenem resistant Enterobacteriaceae (CRE) have emerged globally and have become a major threat to public health. The carbapenem resistance in enterobacteriaceae is mediated by a variety of mechanisms, the predominant being production of diverse carbapenemases and in particular NDM-1 enzyme is a matter of concern. Gastrointestinal tract may serve as a reservoir for CRE which may spread between patients and commensal flora. Hence active surveillance among high risk patients is important for prevention in acute care facilities.

Aims and objectives: - the present study was initiated to study fecal colonization with carbapenemase producing Enterobacteriaceae (CPE) amongst ICU patients and non hospitalized patients attending out patients department (OPD) using culture based methods.

Material and methods: - Case control study was undertaken, cases were enrolled from ICU and rectal swab were collected on Day-1 and again from same patient on Day-4. Exclusion criteria included prior admission to ward or and other hospital. To study risk factors for colonization demographic data, clinical history, current antibiotic therapy, including carbapenems, invasive procedures and co-morbid conditions were recorded through chart review in a predesigned proforma. Stool samples collected from non hospitalized patients visiting OPD with no prior history of hospitalization in last 6 months served as control .

Screening for CRE was done by in house prepared selective media using: - MacConkey agar with imipenem 1µg/ml (MacI), MacConkey agar with cefotaxime 1µg/ml (**MacC ESBL**), MacConkey agar plates with standard Imipenem, Meropenem and Ertapenem Disk (10µg Disk), applied at the 4-, 8-, and 12-o'clock positions (**MacD**) and Two step broth enrichment method using Ertapenem disc as per CDC protocol (CDC TSB) and incubated at 37°c overnight.

Isolates growing on any one of the selective media were identified by conventional tests and subjected to antibiotic susceptibility. All Enterobacteriaceae with ertapenem MIC>0.5mg/L were subjected to MIC for meropenem, imipenem, colistin, tigecycline, phenotypic test and PCR for ESBL, AmpC and carbapenemases. All CRE with ertapenem MIC>0.5mg/L and positive PCR for carbapenemase gene (NDM, KPC, VIM, IMP, OXA 48 and OXA 181) were considered carbapenemase producing Enterobacteriaceae (CPE). Risk factors for colonization with CPE in ICU patients were compared using $\chi 2$ or Fisher exact test, as appropriate. All p values were two tailed. p value < 0.05 was considered statistically significant. Adjusted odds ratios and 95% confidence intervals (CIs) were computed for the significant factors. Variables that were present in more than 10 % of ICU patients with CRE colonization with p value < 0.05 were entered into backward stepwise logistic regression models in multivariate analysis using SPSS version 20.0 (SPSS, Chicago, IL)

Results: - Colonization with CPE was observed to be 8/122 (6.6%) among controls and 11/100(11%) among ICU patients on day 1. There was a significant increase in CPE on day 4 (22/100) (p value 0.002). A total of 50 CPE were isolated from 34 patients. MIC range of CPE isolates were 0.38 mg/L -64 mg/L for both meropenem and imipenem. 8/50 CPE isolates were susceptible to both meropenem and imipenem (MIC ≤ 1mg/L). CPE isolates were susceptible to colistin (MIC range 0.125mg/L to 1mg/L) and tigecycline (MIC range 0.064mg/L to 1.5mg/L). Carbapenemase gene was isolated predominantly from *Escherichia coli* (78%) followed by *Klebsiella sp.(16%)*. Amongst the controls NDM-1 was the only carbapenemase gene detected. Amongst ICU patient's carbapenemase gene were diverse with predominance of NDM-1 followed by OXA-48, OXA-181 and KPC.

In Univariate logistic regression model, use of carbapenems (p value 0.02), aminoglycosides (p value 0.02), ventilator (p value 0.01) and surgical procedures (p value 0.048) were associated with a risk of carrying carbapenemase genotype in CRE. A multivariate logistic regression model was constructed to adjust for confounding variables. Use of aminoglycosides and ventilator were the only variables independently associated with carriage.

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Discussion: - This study shows high prevalence of gut colonization with CRE and predominant carbapenemase gene was NDM-1. Fecal colonization with CRE increased with ICU stay suggesting horizontal transfer. Colonization among 6.6% non-hospitalized patients suggests that these genes are disseminated in community. This may be a source of endogenous community and hospital acquired infection infuture. Therapeutic option for CRE include colistin and tigecycline.

Strong synergism of peptides derived from fish (*Pleuronectes americanus*) show activity *in vitro* and *in vivo* against *Klebsiella pneumoniae*

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Antimicrobial peptides (AMPs) are innate immune system components, which are present in several living organisms [1]. This molecules have been focused as an alternative for traditional antibiotics due to their potent antimicrobial activities against a broad range of microorganisms including those resistant to classical antibiotics. In spite of pharmaceutical companies dynamism for new drugs discovery, have been virtually impossible to pharmaceutical market follow the phenomen of multidrug resistance shown by many microorganisms [2]. By this way, antimicrobial peptides stand out as promising pharmacological alternatives, since they can be used alone or in combination to classic antibiotic. In this view, the present study showes the activity of two synthetic analogues, named Pa-MAP 1.5 and Pa-MAP 1.9 from *Pleuronectes americanus* fish Both analogs had chemically synthesized, being purity degree checked by MALDI-ToF mass spectrometry. Moreover, peptides were assayed alone and in combination according their antibacterial activities, against Gram-negative *Klebsiella pneumonia* as well as their hemolytic activity. Data *in vitro* assay demonstrate a strong synergistic activity between peptides, at a concentration of 4/4μg.ml^{-1} for each peptide. Nevertheless at this concentration they did not caused any cytolytic effects. The *in vivo* results showed that combined peptides were capable to pronounced decrease *K. pneumoniae's* UFCs in blood count, as well in black C57/bl6 mouse's lung. The animals were treated with the combined peptides via intravenously using concentrations of 36 mg.Kg^{-1}, 18 mg.Kg^{-1} and 9 mg.Kg^{-1} for Pa-MAP 1.5; and 4 mg.Kg^{-1}, 2 mg.Kg^{-1} e 1 mg.Kg^{-1} for Pa-MAP 1.9. In order to understand these peptides interaction *in silico* three-dimensional models were constructed by molecular modeling. Results of *in silico* modeling suggest amphipathic α-helical structures for both peptides. When molecular peptide docking was performed with anionic membranes, conjugated peptides showed high affinity rates, thus corroborating with in vitro and *in vivo* data presented.

Keywords: Antimicrobial peptides; *Klebsiella pneumoniae.*

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Structure –Function Analysis of Synthesized Antimicrobial Peptide

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Antibiotics are used to treat various infectious and contagious diseases in both human and veterinary medicines. Moreover, many of these antibiotics used routinely in healthy livestock and poultry to promote growth. It is reported that out of the total antibiotics used in worldwide, 50% of the antibiotics used as growth enhancer. Indiscriminate use of antibiotics contributes significantly to emerge antibiotics resistance microorganisms and subsequently spread of all those. Antibiotic resistant microorganisms have been posing increasingly serious concern and new approaches to development of novel classes of antimicrobial agent are need of the hour. Considering all these facts present study was designed to characterized antimicrobial peptide gene from tongue epithelial cells of buffalo for prediction of amino acids to use as template for synthesis of antimicrobial peptide and its evaluation by structurally and functionally.

Three overlapping peptides were synthesized using Solid Phase method using the deduced amino acid sequence from the cDNA (Accession No. DQ458768) of tongue epithelial cells of buffalo. Antimicrobial sensitivity test and cyto-toxicity was studied using disc diffusion test and Fluroscent Activated Cell Sorter (FACS) respectively. Circular Dichroism spectroscopy of all these synthesized peptides were carried out on JASCO J-810 spectropolarimeter to analyze the structure of the synthesized peptide. The study revealed broad spectrum antimicrobial activity, dose dependent cyto-toxicity and dominant β-structure for all these three peptides in different solutions. The study reveals the similar structure and functional property of LAP with already characterized synthetic Antimicrobial Peptide from mammary gland cDNA (Accession No. DQ 886701). Thus, it can be concluded that tongue epithelial cell expressed a potent antimicrobial peptide which can be exploit for synthesis of novel antimicrobial agent.

Study of antibiotic sensitivity of microorganisms isolated from the fungal-bacterial associations of respiratory tract

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Currently, data about the participation of *Candida* in inflammatory respiratory diseases is sometimes varied, and information on the microbial associations is insufficient [1, 2]. Practical interest is taken to studying the sensitivity of *Candida*, isolated in inflammatory diseases of the respiratory tract, to antimycotic agents. In this regard, the aim of our study was to evaluate the effect of *Candida* in inflammatory diseases of respiratory tract and sensitivity to antimycotic agents. We studied 277 samples of sputum of patients with community-acquired pneumonia, as well as 389 samples of discharge of patients with inflammatory diseases of the upper respiratory tract. Isolation of *Candida* from clinical specimens was performed with bacteriological method using Sabouraud's medium. Identification was performed by culture and biochemical characteristics. Blood and yolk-salt agars, Endo and thioglycolic mediums were used to isolate the accompanying bacterial flora. Determination of sensitivity of the isolated microorganisms was performed with disk diffusion method.

During the studies, it was found that *Candida* was found in 35% of sputum samples, and in 15% of samples of discharge of the upper respiratory tract. With more frequent isolation of *Candida* from the sputum, in monoculture they were more frequent in the discharge of the upper respiratory tract (27%) than in the sputum (9%). As during the study of sputum samples the most stable and most sensitive strains of *Candida* were isolated from associations with the streptococci, it was interesting to study the sensitivity of this streptococci to antibiotics. Streptococci isolated from sputum in association with *Candida*, sensitive to 3 antimycotics, detected at 10^5 colony-forming units per 1 ml (CFU/ml) and more, and wherein all showed resistance to 2 agents. Streptococci isolated in association with *Candida*, sensitive to 2 agents, only in 50% of the samples were detected in a diagnostically significant amount of 10^5 CFU/ml and more. In these associations, streptococci were resistant to 3 agents in 33% of samples, to 5 drugs in 33% of samples and to 7 agents in 33% of samples. In association with *Candida*, sensitive to one agent, streptococcus were found at 10^5 CFU/ml and more, and a half of these strains was resistant to 3 agents and another one was resistant to 6 agents. In associations with *Candida*, resistant to 3 agents, streptococci were found in amount of 10^5 CFU/ml and more, half of them was resistant to 2 agents, and another half to 6 agents. When studying the properties of microorganisms isolated from the discharge of the upper respiratory tract, it was found that all of streptococci from association with *Candida*, sensitive to 3 drugs were detected at 10^5 CFU/ml or more. Half of streptococci strains showed resistance to 4 agents, and the other half was resistant to 2 agents. In 50% association with *Candida*, sensitive to 2 drugs, streptococcus were found at 10^5 CFU/ml or more. Those strains of streptococci which quantity was 10^5 CFU/ml or more, in all cases, were resistant to 3 agents. In associations represented by *Candida*, resistant to 3 agents, streptococci were found in amount of 10^6 CFU/ml or more, and they all showed the resistance to 2 agents.

Thus, these data suggest the participation of fungal-bacterial associations in inflammatory diseases of the respiratory tract. In the discharge of the upper respiratory tract and sputum it was revealed not a pure culture of the pathogen, but the combination of different microbes, most of *Candida* with representatives of *Streptococus* and *Staphylococcus*. Participants of fungal-streptococcal associations exhibited high antimicrobial resistance. Revealed facts may indicate the possibility of mutual influence of microbes-associates and exchange of genetic information between them. It's necessary to take into account the possible role of each of associates in the pathological process to ensure the effective treatment of inflammatory diseases of the respiratory tract.

Keywords: fungal-bacterial associations; antimicrobial; mutual influence; microbes-associates

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Study of Antimicrobial resistance pattern among pediatric patients in emergency department (PED) in an Egyptian hospital- A step forward to start antimicrobial stewardship program

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Background: Bacterial resistance to commonly used antimicrobial agents is growing up day by day in both community and hospital settings. This is problematic especially in PED and it is mainly correlated with an antibiotic abuse. Therefore a rational choice of an empiric therapy requires properly collected information of both global and local resistance patterns. **Objective:** This study was initiated to benchmark prevailing resistance rates for the most common bacterial pathogens in PED of Cairo University Specialized Pediatric Hospital (CUSPH) aiming to be able to design local data- based antimicrobial stewardship in PED. **Methods:** A cross sectional study performed on 970 patients who were selected from 1085 cases admitted to PED in the period from 1st of august 2011 – 1st of august 2012. Clinical samples were collected from included patients according to the type of infection. All specimens were processed, cultured and the isolates were identified according to the standard microbiological techniques. Antimicrobial susceptibility (AST) of all isolates was determined following the performance standards set by clinical laboratory standards institute (CLSI). **Results:** Patients with pneumonia were the commonest among our cases representing 79%, followed by sepsis and UTI showing 18% and 2.8% respectively. The predominant organism isolated from patients with pneumonia was *pseudomonas* (27.7%), while *CONS* (42.9%) and *E.coli* (63.6%) were the most prevalent among patients with sepsis and UTI respectively. Antibiotic sensitivity pattern in pneumonia cases was; ciprofloxacin (CIP) 43.4%, imipenem (IPM) 42.3% and meropenem (MEM) 37% for Gram negative organisms, while Gram positive organisms showed 100% sensitivity to vancomycin (VA), teicoplanin (TEC), clindamycin (DA) and erythromycin (E) and 66% to doxycycline (DO). In cases of sepsis; Gram negative organisms were highly sensitive to: ciprofloxacin (CIP) 67%, aztreonam (ATM) 53.6% and gentamycin (GN) 41.3%, Imepenem(IPM) 40.6%, and Amikacin (AK) 36% and Gram positive organisms were 100% sensitive to vancomycin (VA), 86.5% to teicoplanin (TEC) and 42.5% to clindamycin (DA) .Gram negative organisms isolated from patients with UTI showed highest sensitivity to: ciprofloxacin (CIP) 83.3%, meropenem(MEM) 44.3% and amikacin (AK) 25%, while Gram positive organisms were 100% sensitive to vancomycin (VA) and gentamycin (GN). Methicillin resistance was encountered in 75% of Staphylococci aureus and 99% of CONS isolates. All E-coli (100%) and 73% of Klebsiella spp. strains exhibited phenotypic extended-spectrum ß-lactamase resistance (ESBL) patterns. Regarding community acquired infections (CAI), the recommended antibiotic therapy included, ciprofloxacin (CIP), imipenem (IPM), meropenem (MEM) and amikacin for Gram negative organism, while in Gram positive organisms vancomycin (VA), teicoplanin (TEC) and clindamycin (DA) were the most advised. **Conclusion:** There are an alarming increase in MDR organisms among paediatric patient population, enhancing demands to review strictly the infection control measures aiming to limit further development and spread of bacterial resistance. Establishment of antibiotic stewardship guided by the collected data in our study, which should be updated periodically according to microbiology laboratory reports to monitor variations in pathogen occurrences and emerging antimicrobial resistance, especially because a new agents such as fluoroquinolones are recently used to a greater extent in this age group.

Key words: Antimicrobial sensitivity pattern; MDR; PED; Infection control; HAI; nosocomial infections; antimicrobial stewardship

Study on the production of HA antigen reagent for quality control of pademic influenza vaccine

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The vaccination is the best way to prevent pademic influenza. For pandemic influenza vaccine production, WHO recommends new vaccine strain(s) annually because influenza viruses undergo frequent antigenic drift in their surface antigen proteins.[1]

The hemagglutinin(HA) of the influenza virus is the major surface antigen inducing protective immune responses, and HA content determination is required for production and quality control of the influenza vaccine.

The single radial immunodiffusion(SRID) assay is the standard test method for HA content determination and reference reagent is essential for this assay.[2] Reference reagents are developed and supplied by essential regulatory laboratories(ERLs).[3] But this is very time consuming step for vaccine production and quality control, therfore it is difficult to manage the pandemic outbreak promptly.

WHO recommends that national regulatory authorities(NRAs) do some researches to minimize pandemic impacts.[4] In order to shorten HA reference reagent production period, we prepared various antigen reagent from different HA subtypes by using recombinant technology and generated 5 HA vectors(H1N1, H5N1, H7N3, H7N9, H9N2) for HA protein production. The produced HA proteins will be evaluated for SRID assay for vaccine qulity control test.

Keywords: influenza vaccine; reference reagent

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Synergetic effect of lactobacillus extract and disinfectant against biofilm *Staphylococcus aureus* cells isolated from oral cavity of Tunisian children

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In this study antimicrobial activities of probiotic *Lactobacillus plantarum* extracts (LPEs) was investigated against a panel of oral *S. aureus* strains (n= 9). *S. aureus* ATCC 25923 was used as a positive control. Their ability to modify bacterial resistance to tetracycline, benzalchonium chloride and clohrhexidine in vitro were performed. Minimal inhibitory and bactericidal concentrations (MICs and MBCs respectively) of LPEs, antibiotic and disinfectant against *Staphylococcus* strains was determined using successful dilutions. Synergetic activities of *L. plantarum* extract with disinfectant and antibiotic against *S. aureus* strains were evaluated and its capacity to reduce oral pathogenic biofilm formation was assessed. MICs and MBCs of, tetracycline, benzalchonium chloride and clohrhexidine were determined in presence of a sub-MIC of the LPEs (1/2 MIC). Results demonstrated that lactic acid bacteria extract exhibited a selective antimicrobial activity against oral bacteria. Its synergetic effect resulted in at least a 4-fold potentiation of the tested antibiotics and antiseptic. It is therefore suggested that LPE could be used as a source of natural products with resistance-modifying activity. Further investigation is needed to assess their clinical relevance.

Keywords: Antibacterials, *Staphylococcus aureus*, Biofilm, Antibiotics, Synergetic, Lactobacillus

The evaluation of pathogen bacteria profile of "çiğ köfte" (raw meatball) and its lettuce marketed in populous cities of Turkey

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Çiğ köfte (raw meatball) is a traditional food of Turkey and it is very widely consumed all over our country. Because çiğ köfte is a traditional food, it is produced generally in houses and small local sales points with conventional methods and unfortunately there is not a production standart for çiğ köfte. Based on its ingridents (minced raw meat, onion, garlic, boiled wheat, parsley, mint, black pepper, red pepper, salt, tomato paste / sauce) çiğ köfte is produced and consumed daily and hygiene conditions of both raw materials and production is very important for the health of consumers. In this study, it is aimed to expose the microbiological profile of "çiğ köfte" (raw meatball). For this purpose, total of 3.000 çiğ köfte and the lettuce (that are served with çiğ köfte) samples were collected from 10 cities that are located in 5 different geographical regions of Turkey (150 samples of çiğ köfte and 150 samples of lettuce from every city) and the samples were analyzed for 4 different parameters (*Listeria monocytogenes, Eschersichia coli, Bacillus cereus,* and *Staphylococcus aureus,*). According to the results, it was exposed that the samples were contaminated with different microbiological parameters at different levels and also it was determined that some samples were very risky for consumers' health. Besides, the correlations among çiğ köfte and lettuce (which is always seviced with çiğ köfte) samples for the analyzed parameters were investigated and positive correlations were determined among çiğ köfte and lettuce samples. The results showed that the presence of a microbiological parameters in çiğ köfte or lettuce induced to contamination of the same parameter to the other product primarly via staff who works in production processes. Seasonal factors were also explored and it was found out that the different climate conditions were affected some of the analyzed parameters. As a result it was conluded that to generate a production standard for çiğ köfte and to involve the aforementioned standard with effective contamination varibales as lettuce that are served with çiğ köfte and staff are necessary for both protecting consumers' health and introducing çiğ köfte to international markets.

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The occurrence and effect of some antibiotics on *Streptococcus mutans* in dental caries in Jos

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Antibiotic sensitivity test was carried out on *Streptococcus mutans* isolated from patients with tooth decay at the Dental Clinic of plateau State Hospital Jos, to establish their antimicrobial susceptibility. Samples were collected from 100 patients with tooth decay by Scraping the Sulcus part of the decayed tooth, out of which 74% were identified to have *Streptococcus mutans*. The Molar teeth were more affected with 56% compared to the Premolars and the roots with 35% and 9% respectively, and the number of cases with dental caries was found to be highest between ages of 21 – 40 with more females 54% than male 46%. Obtained results shows *Streptococcus mutans* to be susceptible to *Ampicilin* 84%, *Amoxyl* 90%, *Ciprofloxacin* 85%, *penicillin* 78%, *Ampiclox* 55% and *Streptomycin* 30%, but resistant to *Erythromycin, Gentamycin* and *Cefuroxine*. The level of dental health in a community depends on the extend to which people seek dental care and apply preventive measures

The profile of consumers' habits and hygiene analysis of the animal based foods form purchasing to consumption period in the cities of Aegean Region, Turkey

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An adequate and correct alimentation is a basic right for human race in order to achieve mental, physical and physiological development both for individuals and societies. Unfortunately, although the resources of the world have enough potential to nourish all the population, hundreds of millions of human beings are facing with famine, starvation and lethal diseases. Animal based foods are very important for people to live a healthy life in many ways. Variables as rapid urbanization, development of tourism sector, growing of the food and animal trade all over the world, changing food consuming habits, and developed production technics and socio-economic conditions cause to increase the incidence of food infections and intoxications and important losses of food, animals and human beings. Correct and adequate procedures, such as a food security system must be applied especially to animal based food in order to minimise the risks for consumers' health. In spite of application of the correct procedures during the production period of the animal based food, a lot of different variables of the consumers may cause the safe animal based food to important risk factors.

The purpose of this study is to determine consumption patterns of the public in all the cities of Aegean region (İzmir, Aydın, Muğla, Uşak, Denizli, Manisa, Kütahya, Afyonkarahisar) with regard to food of animal origin (milk and dairy products, meat and meat products, water products, honey, poultry and poultry products) and to exhibit whether consumption habits (brand choice, if the product is prepacked or not, how and under which conditions the products are preserved until consumption, why the household members prefer certain products etc.) are effective over the microbiological quality of the food of animal origin and to put forward these relationships in statistical modelling in order to show the correlation among consumption habits, product hygiene and public health. A total of 1600 households have been visited and to each household, 73 questions in 6 different categories have been asked. From each household, one relevant food product has been taken and the collected samples have been analysed with regard to 10 different microbiological parameters. According to the results, it has been determined that in Aegean region, the individual demographic variables and consumption habits of food of animal origin of the consumers are closely related to the hygiene of the products they consume.

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Therapeutic Enhancement of Newly Derived Bacteriocins Against *Giardia Lamblia*

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Trials for identifying efficient anti-giardial agents are still ongoing. Nowadays, bacteriocins have attracted the attention as potential antimicrobial compounds. For the first time, the current study evaluated the therapeutic efficacy of bacteriocins newly derived from newly isolated Egyptian strains of probiotics *Lactobacilli; L acidophilus* (P106) and *L. plantarum* (P164) against *Giardia lamblia*. Bacteriocins' efficacy was evaluated both *in vitro*; by growth inhibition and adherence assays, and *in vivo*; through estimation of parasite density, intestinal histopathological examination and ultrastructural analysis of *Giardia* trophozoites. *In vivo* bacteriocins' clinical safety was assessed. In vitro results proved that 50 µg of *L acidophilus* bacteriocin induced reduction of the mean *Giardia lamblia* trophozoites by 58.3±4.04%, while at lower concentrations of 10 µg and 20 µg of both *L. acidophilus* and *L. plantarum*, non significant reduction of the mean parasite density was achieved. In vitro trophozoites adherence was susceptible to the tested bacteriocins at all concentrations studied with variable degrees, while the highest adherence reduction was demonstrated by 50 µg of *L acidophilus* bacteriocin. In vivo, oral inoculation of 50 µg/mouse *L. acidophilus* bacteriocin for five successive days resulted in a noteworthy decline of the intestinal parasite density, along with amelioration of intestinal pathology of infected mice. Ultrastructural examination proved that five doses of *L. acidophilus* bacteriocin showed marked changes in cellular architecture of the trophozoites with evident disorganization of the cell membrane, adhesive disc and cytoplasmic components. This is the first reported study of the safe anti-giardial efficacy of *L. acidophilus* P106 derived bacteriocin, hence highlighting its great promise as a potential therapeutic safe alternative to existing commercial drugs.

Toxin gene profile, phenotype and antimicrobial resistance of *Bacillus cereus* in Korean fermented soybean products

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Korean fermented soybean products, such as *doenjang, kochujang, ssamjang,* and *cho-kochujang*, can harbor foodborne pathogens such as *Bacillus cereus* (*B. cereus*).The aim of this study was to investigate the toxin gene profiles, biochemical characteristics, and antibiotic resistance pattern of *B. cereus* strains isolated from Korean fermented soybean products. Eighty-eight Korean fermented soybean products were purchased from retail markets in Seoul, South korea, betwwen May and November 2013. Twenty-five grams of each sample were suspended in 225 ml of Butterfield's phosphate-buffered water followed by homogenization for 2 min. 0.1 ml of homogenate was serially diluted (10-fold) in 0.85% saline, and then 0.1 ml of each dilution was inoculated onto Bacara® agar. Plates were incubated at 30°C for 24 h. Suspected colonies were biochemically confirmed by using Vitek 2 BCL kit. Toxin gene profile was evaluated by PCR targeting enterotoxin genes (*nheABC, hblCDA, cytK, entFM*) and emetic toxin gene (*EM1*). Isolates were conducted biochemical test (starch hydrolysis, salicin fermentation, hemolysis, lecithinase production and motility test). Antimicrobials were ampicillin, gentamicin, cefepime, ciprofloxacin, imipenem, chloramphenicol, tetracycline, oxacillin, penicillin, erythromycin, clindamycin, vancomycin, cefotetan, trimethoprim-sulfamethoxazole and rifampicin. Eighty-seven *B. cereus* strains were isolated from 47 positive samples, and all isolates carried at least one enterotoxin gene. The detection rates of *hblCDA, nheABC, cytK*, and *entFM* enterotoxin genes among all isolates were 34.5%, 98.9%, 57.5%, and 100%, respectively. Fifteen strains (17.2%) harbored the emetic toxin gene all of which were positive for at least one of the enterotoxin genes. Most strains tested positive for salicin fermentation (62.1%), starch hydrolysis (66.7%), hemolysis (98.9%), motility test (100%), and lecithinase production (96.6%). The *B. cereus* strains were highly resistant to β-lactam antibiotics such as ampicillin, penicillin, cefepime, imipenem, and oxacillin. These results indicate that *B. cereus* in Korean fermented soybean products have the potential to cause diarrheal or emetic gastrointestinal diseases

Keywords: *Bacillus cereus*; Enterotoxin; Emetic toxin; Fermented soybean product; Food poisoning

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Trend of bacteria isolated from patients with acne vulgaris in a Japanese university hospital

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Propionibacterium acnes and *Staphylococcus epidermidis* are isolated from acne lesions. *P. acnes* is an important factor causing inflammation and antimicrobial treatment against *P. acnes* is a standard treatment for acne vulgaris. In recent years, an increase in the number of antimicrobial-resistant *P. acnes* has become a major problem. However, few articles have reported on the antimicrobial susceptibility in *P. acnes*. Therefore, in this study, the antimicrobial susceptibility and resistant factor of *P. acnes* and *S. epidermidis* were investigated.

A total of 69 *P. acnes* and 58 *S. epidermidis* strains were collected from 91 patients with acne vulgaris from a university hospital in Japan from 2009 to 2010. The antimicrobial susceptibilities were determined by an agar dilution procedure for CLSI. Resistant factors in *P. acnes* were analyzed by reserved *erm*(X) and the 23S rRNA mutation causing macrolide resistance, the 16S rRNA mutation causing tetracycline resistance and DNA gyrase substitution causing fluoroquinolone resistance.

In *P. acnes*, 13 strains (18.8%) were clindamycin resistant, including one strain carrying *erm*(X), which showed high-level resistance. Three isolates which showed low susceptibility to doxycycline had the 16S rRNA mutation. Additionally, another three isolates, which showed low susceptibility to nadifloxacin, had DNA gyrase GyrA novel substitution. Therefore, the ratio of antimicrobial-resistant *P. acnes* in Japan was lower than in foreign countries, but resistant strains are clearly increasing in Japan. Additionally, it is suggested that the acquirement of skin bacterial resistance was affected by antimicrobial use because macrolide-resistant *S. epidermidis* were frequently isolated (81.8%) from 11 patients from whom macrolide-resistant *P. acnes* was isolated. It is necessary to pay attention to the spread of antimicrobial-resistant bacteria in the future.

Keywords: *Propionibacterium acnes*, antimicrobial resistance, acne vulgaris

Reference: Nakase K., Nakaminami H., Takenaka Y. et al. *J. Med. Microbiol.*, 63:721-8 (2014)

Use of computer tool in antibiotic prescription

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The reporting of susceptibility testing to antibiotics, whenever a pathogen is identified, it is usually accompanied by the values of MIC (Minimum Inhibitory Concentration) collected for each combination of species/antibiotic, in combination with the interpretation of the test (S, I or R).

With the increasingly common use of automated analyzers and the simultaneous increase of antibiotic molecules used, it has become very common that the requesting physician may use the lowest value between the MIC indicated as the only discriminating factor to decide the antibiotic therapy.

So, in order to eliminate the potential misleading of only the absolute value of MIC, from which can also result a high possibility that the molecule prescribed may be ineffective, it is now recommended to use for this purpose the ratio Breakpoint/MIC. Today, this ratio is not routinely available on microbiological reporting.

Therefore, in this paper we propose a computer-readable form, on-line connected with the EUCAST database, able to provide immediately medical practitioner with the ratio BP/MIC more favorable, selecting this between the molecules on which the sensitivity is detected.

In order to evaluate whether the use of this computer tool could improve the effectiveness of antibiotic prescription, we took into account the established antibiotic therapy at a hospital ward with a high incidence of infectious diseases, re-evaluating the same therapy after applying the computer tool algorithm on all the molecules sensitive for each pathogen microorganism detected.

Number of examined antibiotic reports was 263, excluding pathogens presenting complete multiresistence to all the antibiotic molecules or sensitivity to colistin only.

In 62% of cases, following the delivery of antibiotic sensitivity test, no therapy was enforced or modified.

The correlation between the antibiotic prescribed or maintained and that indicated by the computer tool was correct in 22% of cases only.

These preliminary results seem to strongly encourage the introduction and routine use by the physician prescriber of this specific computer tool, so as to limit and circumscribe the known effects related to prolonged and inappropriate antibiotic therapy.

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Virulence genes distribution and antibiotic resistance patterns of *Escherichia coli* strains isolated from patients with community acquired urinary tract infections in central Mexico.

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A urinary tract infection (UTI) is defined as microbial infiltration of the otherwise sterile urinary tract and is one of the most common bacterial infections worldwide. Only in the United States it has been estimated an annual occurrence of more than 8 million UTI cases, many of which result in a visit to a physician, and require a regimen of antibiotics. Furthermore, it has been estimated that 1% of UTI patients require hospitalization and these infections have an annual cost of approximately $2.14 billion. In Mexico, UTIs are the most frequent bacterial infection seen among the adult population with an estimated rate of 3,500 cases per 100,000 inhabitants for 2010, but the number of UTI patients that requiring antibiotic treatment and hospitalization is unknown. UTIs encompass infections of the urethra (urethritis), bladder (cystitis), ureters (ureteritis), and kidney (pyelonephritis). Uropathogenic *Escherichia coli* (UPEC) is the main infectious agent causing UTIs worldwide. Studies conducted in industrialized countries have shown that UPECs strains have increasingly become resistant to first line antibiotic therapy. Also there have been described several molecules implicated in UPEC virulence such as fimbria and adhesin for adherence to the uroepithelium, toxins that cause tissue damage, iron acquisition systems and molecules that participate in the bacterial intracellular communities formation. Unfortunately, in Mexico UTIs continue to be treated empirically, urinary bacterial cultures are not requested, thus studies of UPECs prevalence, antibiotic susceptibility and distribution of virulence genes are scarce. Therefore, the aims of this study were 1) to conduct a pilot study of UTI infections in two regions of central Mexico, Tulancingo, Hidalgo and Mexico City, 2) to isolate UPEC strains and further characterize them by antibiotic susceptibility tests to nine antibiotics (including those for UTI treatment in Mexico) and by two PCRs that together identify 10 previously described UPEC virulence genes, developed in our laboratory. From 61 urine samples, from 51 women UTI patients (84%) and 10 (16%) men, *E. coli* strains were recovered. One *E. coli* strain was collected from 58 urine samples and two *E. coli* strains were collected from 3 patients. Of the 58 single *E. coli* isolates 79% were resistance to Ampicillin, 71% to Amoxicillin/Clavulanic Acid, 52% to Ciprofloxacin, 50% to Trimethoprim/Sulfamethoxazole, 40% Cefepim, 36% Ceftriaxon, 28% Amikacin, 24% Gentamicin and 5% Nitrofurantoin. Of the 58 single *E. coli* strains 57 harboured one or more genes encoding for different virulence factors, of these: 95% for the adherence fimbria type 1 (*fim*A), 81% for antigen 43 (*agn*43), 74% for yersiniabactin (*fyu*A), 62% for kapsule II (*kps*M II), 57% for salmoquelin (*iro*N), 41% for adhesin P (*pap*C), 28% for hemolysin toxin (*hly*A), 24% for cytotoxic necrotizing factor toxin (*cnf*1), 21% for vacuolating toxin (*vat*) and 17% for aerobactin (*iut*A). The most frequent gene profile identified (13%) among these strains was: *fim*A, *fyu*A, *pap*C, *agn*43, *kps*M II and *iro*N. Furthermore, 61% of the strains isolated from severe clinical cases carried *pap*C. Regarding the three urine samples from which two UPEC strains were isolated, it was observed that the antibiotic resistance patterns were similar or identical between the two *E. coli* strains isolated from the same patient, in spite of this, their gene profile was very different. Overall our results showed that the majority UPEC strains isolated from UTI patients harboured several virulence genes, that most strains were resistant to first line antibiotics, also that more than one *E. coli* strain should be collected from each urine samples. We also revealed the importance of undertaking these studies in order to provide sound treatment to patients and to diminish therapeutic failure.

Keywords: UPEC; antibiotic resistance, virulence genes

Why are we unable to change antibiotic prescribing over time? A sociological analysis of the factors underpinning antibiotic use hospitals

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Introduction:
In Australian hospitals, up to 48% of antibiotics prescribed are inappropriate (1, 2). The challenges of managing antibiotic use and increased resistance are not restricted to Australia, posing problems globally. The European Union's (EU) European Surveillance of Antimicrobial Consumption Network records data from the EU, illustrating that while 29% of hospital in-patients receive antibiotics, only 50% are concordant with clinical guidelines (3). The Centers for Disease Control and Prevention has invested significantly in monitoring in-patient antimicrobial use in US hospitals, including recent initiatives seeking to promote systematic reporting of antimicrobial use and roll-out national point prevalence surveys of hospitalised patients (4). Such initiatives emerge from concerning US data illustrating high levels of inter and intra-institutional variability in antibiotic use within US hospitals (5). The inability to change prescribing behavior in a sustainable manner, substantially limits the success of antimicrobial stewardship (AMS) interventions. Why do short term improvements in prescribing decline over time? Emerging research internationally suggests that prescribing may be significantly mediated by social and behavioural dynamics (6, 7).

Methods:
Semi-structured interviews with 30 doctors were performed. The interviews were focused on sensitivity towards resistance and the self-reported individual and interpersonal factors influencing antibiotic decision-making. NVivo10 qualitative data analysis software was used in conjunction with the framework approach.

Results:
'Sub-optimal' antibiotic prescribing can be a logical choice within the social context of the hospital. The 'rules of the game' are heavily weighted in favour of the management of immediate clinical risks, time pressures, reputation and concordance with peer practice vis-à-vis longer-term population consequences. The interviews illustrate that antimicrobial resistance is a principal of limited significance in the hospital, and identify the key interpersonal and inter-professional reasons for this.

Conclusion:
This study illustrates how antibiotic prescribing practices are shaped by social and behavioural factors such as individual confidence, time pressures, and hierarchical relationships between doctors. If these issues are not addressed in the development of AMS programs, there is likely to be limited efficacy in modifying prescribing behaviour in the long term. Attempts to impose guideline concordance without acknowledging the driving forces behind the cultures of prescribing may be expensive and ineffective.

Disclosure of Interest Statement:
No disclosures**.**

Keywords: antibiotic prescribing; antimicrobial stewardship; qualitative research

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Strengthening of innate immune system as antimicrobial strategy

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Antibacterial mechanisms and immunomodulatory activities of chicken cathelicidin-2

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The increased prevalence of antibiotic resistance is a serious threat to both public and animal health. Host defense peptides may be an alternative to conventional antibiotics. Here we report mechanisms of antimicrobial killing of chicken cathelicidin-2 (CATH-2) against Gram-positive and Gram-negative bacteria. In addition, we investigated immunomodulatory properties of CATH-2.

CATH-2 induced killing of *E. coli* and MRSA at MIC values of 2.5 to 10 μM. However, perturbation of the membrane potential, measured with $DiSC_3(5)$, already occurred at concentrations as low as 0.19 μM. Live-imaging with confocal fluorescence microscopy demonstrated that CATH-2 was localized mainly on the membrane of *E. coli* showing a preference for the bacterial septum of dividing cells. In addition, propidium iodide influx into the cell showed permeabilization of the bacterial membrane upon CATH-2 binding. Further investigating into the bactericidal action of CATH-2 was performed using transmission electron microscopy. Dose-dependent morphological changes were observed for CATH-2-treated *E. coli* and MRSA in comparison with untreated bacteria. Intracellular granulation and cytoplasmic retractions were detected at sub-MIC concentrations of CATH-2, whereas at MIC values and higher, membrane rupture and cell lysis were observed.

In a chicken macrophage cell line (HD11), CATH-2 induced the transcription of the chemokines CXCLi2/IL-8, MCP-3, and CCLi4/RANTES in a dose-dependent fashion. However, no effect of CATH-2 on the transcription of the pro-inflammatory cytokine IL-1β was observed. In addition, LPS-induced IL-1β transcription and nitric oxide production was inhibited in the presence of CATH-2.

In conclusion, CATH-2 exerts its antimicrobial killing effect not only on the bacterial membrane, but may also have an intracellular mode of action. In addition, CATH-2 has immunomodulatory properties and CATH-2-derived peptides may be used as paradigms to develop alternatives to antibiotics.

Keywords: chicken cathelicidin; antimicrobial; immunemodulation; bacteria; membrane potential; chemokines; LPS

Antimicrobial activity of trout hepcidin during the innate immune response in fish

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Hepcidin is an antimicrobial peptide (AMP) produced by the liver and head kidney of salmonids. This AMP is a cysteine-rich peptide with a highly conserved β-sheet structure, consisting in a prepropeptide with a conserved signal peptide, an acidic propiece and a mature peptide. Hepcidin expression and secretion is regulated by iron and pathogen associated molecular patterns (PAMPs), however the functions of the mature peptide are poorly characterized. This work contributes to elucidate the potential role of hepcidin during the innate immune response in trout by the characterization of its expression and antimicrobial activity against a typical intracellular pathogen of salmon. For this, we produced the synthetic peptide of the mature region by solid-phase peptide synthesis using the Fmoc/t-butyl strategy. The correctly-folded hepcidin was obtained by DMSO oxidation under acidic conditions and the beta-sheet structure was confirmed by circular dichroism. We showed that the synthetic oxidized hepcidin display bactericidal effect *in vitro* against the Gram-negative bacteria *Piscirickettsia salmonis*. Moreover, antimicrobial activity assays suggest that hepcidin acts intracellularly through independent mechanisms of membrane damage, evidenced by Sytox permeation assay. Confocal microscopy analysis of *P. salmonis* cultures exposed to trout hepcidin coupled with rhodamine revealed the intracellular location of the peptide. This result supports the hypothesis of a non-membranolytic mechanism of action of hepcidin but rather directed to intracellular targets. The ability of hepcidin to degrade DNA through hydrolysis was further demonstrated using a plasmid degradation model. In order to get new insights of the role of hepcidin in the innate immune response, we assessed its expression and secretion both *in vivo* and *in vitro*. We were able to detect the production of hepcidin in trout liver in response to intraperitoneally injected LPS. *In vitro*, the monocyte/macrophage-like trout cell line RTS11 increase the expression of hepcidin when challenged with *P. salmonis*. Overall, results obtained in this study provide new evidence that trout hepcidin might has a major role in fish innate immunity. Further understanding of its antimicrobial function could lead the development of new strategies in the prevention of bacterial infections.

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Asoxime (HI-6) is able to modulate immunization efficacy by keyhole limpet hemocyanin in mouse model

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Compound HI-6 (or named asoxime in some sources) is used as an antidote to nerve agents because it causes return of acetylcholinesterase activity. In the present work, link between HI-6 and cholinergic anti-inflammatory pathway via alpha 7 acetylcholine receptors is hypothesized. Laboratory BALB/c mice received HI-6 and/or keyhole limpet hemocyanin (KLH) as an antigen. Controls received saline or a combination of Freund´s complete adjuvant and KLH. Antibody production was investigated after either 21 or 65 days when either single or repeated dose of antigen was applied. When considered antibodies production, HI-6 significantly improved vaccination efficacy when KLH was given in a dose of 1 mg/kg. The effect was dose dependent: repeated HI-6 produced no on further improvement of the vaccination. A combination of HI-6 and KLH produced a vaccination of almost the same efficacy as that for Freund´s complete adjuvant. The findings point at the suitability of HI-6 for improving vaccination efficacy at the level of immunity regulation by the nervous system.

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Characterisation of anti-bacterial factors in marine fish blood by cell-based assay and by proteomic approach

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In recent decades, *Edwardsiella tarda* (*E.tarda*), a causative agent of many serious diseases in both freshwater and marine fish, has aroused increasing concerns around the world. Understanding the molecular mechanism of natural defense against these pathogenic bacteria is critical for the prevention and control of the diseases. Due to the primitiveness of immune system, fish rely mainly on innate immunity to defense against the invasion of pathogens, and blood proteins act as indispensible factors in early innate immune response. The objective of this study is to characterize the bactericidal factors in fish blood.

Sera extracted from marine medaka (*Oryzias melastigm*a) or turbot (*Scophthalmus maximus*) were mixed with *E.tarda*, and bacterial cell numbers after treatment were counted to assess the anti-microbial activities of these sera. Mock treatments by using culture medium or heat-inactivated sera were used as controls. The number of *E. tarda* bacteria significantly decreased after a four-hour exposure to fish sera (60.57% and 33.67% for medaka serum and turbot serum, respectively), but no bactericidal effect was observed for heat-inactivated sera. This suggests that the anti-bacterial activity in fish blood is mediated by heat labile molecules. We conjugated fish serum proteins with fluorescent dyes before exposing to bacteria, and showed that *E.tarda* were coated with fluorescent label before lysis, implying the binding of serum proteins onto bacterial cells. While some of these anti-bacterial molecules, such as complement proteins, are well established, a systematic identification of anti-bacterial factors in fish blood has not been achieved. Towards this goal, we have developed a proteomic approach for the isolation and characterization of the *E.tarda*-binding proteins in fish serum. Bacteria were incubated with Turbot serum and, after the removal of unbound proteins, subjected to 2D gel analysis. When compared to a reference 2D gel containing *E.Tarda* proteins, we identified more than 80 spots that were present only in serum treated bacteria. These spots likely represented fish serum proteins pulled down by their interactions with the bacteria. Mass spectrometry of these spots indicated that these proteins include immunoglobulins, complement component C3 and Wap65-2, all of which are immune related proteins. This suggests the use of live bacteria to affinity-purify serum components, combined with proteomics, is an effective approach in discovering potentially novel factors in fish innate immunity.

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Deregulation of iron metabolism during *Listeria monocytogenes* infection in mice is not dependent on hepcidin expression

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Background: As iron is vital for all cells, host sequestration of iron (nutritional immunity) provides a significant barrier to bacterial infection. Consequently, anaemia may be a complication of chronic infectious diseases. Despite the extensive research over the last decade in iron metabolism and its deregulation during infection, the mechanisms by which anaemia occurs remain to be known.

Hepcidin, which was firstly described as an antimicrobial peptide, is an iron-regulatory hormone, predominantly produced in hepatocytes, that has been considered a fundamental element orchestrating host response upon bacterial infection and thus in the development of the anaemia of inflammation and chronic infection. Our group showed that host iron overload, leads to increased susceptibility to *Mycobacterium avium*. On the other hand, *M. avium* infection leads to moderate anaemia, without any alteration on hepcidin levels. *Listeria monocytogenes* is a Gram-positive intracellular pathogen that has been used as a model to study innate and adaptive immunity. Its ability to acquire iron is essential for infection. **Aim:** In this work, we aimed to test the alterations on iron metabolism in the host and its possible association with hepcidin expression in liver during intravenous infection with *L. monocytogenes*. **Methods:** C57bl6 male mice were infected with $1x10^4$ CFU *L. monocytogenes* or vehicle and sacrificed 24h, 48h, 72h and 96h later. Bacterial load was quantified, blood was collected to evaluate the erythron and serum iron parameters. Liver was harvested for the determination of gene expression by RT-PCR. **Results:** From the analysed haematological parameters, we were able to observe a decrease on red blood cells (RBC) number and on mean corpuscular volume over the experiment. In addition, *L. monocytogenes* infection induced the reduction of serum-iron over the first 72h of infection, accompanied by the decrease of transferrin saturation (sTRF). In the last time point analysed (96h), serum-iron and sTRF values recovered to nearly basal levels. In terms of gene expression analysis, *L. monocytogenes* infection was shown to induce a transcriptional up-regulation of transferrin (48h and 72h post-infection) and interleukin-6 (IL6) 48h after infection, however with no significant impact on hepcidin levels. **Conclusions:** From the obtained data, although *L. monocytogenes* infection induces a deregulation on iron metabolism and an up-regulation of IL6 transcription no impact on hepcidin expression was observed. Thus, suggesting that a hepcidin-independent mechanism might be involved in the iron metabolism deregulation. This point will be investigated in future work.

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Keywords: hepcidin; bacterial infection; iron metabolism, *Listeria monocytogenes*

Direct and indirect roles of RIG-I for antiviral defense against hepatitis B virus in human hepatocytes

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Hepatitis B virus (HBV) is a hepatotropic DNA virus belonging to the *Hepadnaviridae* family, which can cause both acute and chronic disease. It is a major global health problem: Approximately 2-300 million individuals in the world are chronically infected with HBV. HBV can not only cause hepatic inflammation but also potentially develop into cirrhosis or cancer in the liver. Host immune responses during HBV infection have been studied, however, the innate sensing mechanism to detect HBV infection in host still remained to be fully clarified. In this study, we report that RIG-I (retinoic acid-inducible gene-I), which is known to be a cytoplasmic RNA sensor for many RNA viruses, functions as an innate sensor to induce antiviral responses against HBV infection in human hepatocytes. Interestingly, we observed that type III but not type I interferons (IFNs) were predominantly induced in vitro and in vivo during infection with HBV genotypes A, B and C, which is dependent on the RIG-I pathway. We also characterized the mechanism how RIG-I recognized HBV infection, and determined the unique region of HBV pregenomic RNA, which is targeted for the RIG-I-mediated sensing. In addition, further investigation revealed that RIG-I has a hitherto unknown role as an antiviral factor that directly inhibits viral replication. In this presentation, we would also like to report some result regarding possible therapeutic application of a virus-derived RNA, which is based upon an HBV infection model with human hepatocyte-chimeric mice.

Keywords: Hepatitis B virus; RIG-I; innate immunity

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Effect of inactivated influenza vaccine in combination with chitosan derivatives on dendritic cells

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The aim was to study the direct effect of inactivated influenza vaccine in combination with chitosan derivatives on maturation of dendritic cells (DCs) produced from mice bone marrow. Experiments used the following chitosan derivatives: 1% chitosan glutamate solution and 1% suspension of chitosan sulfate micro/nanoparticles. Preparation of chitosan was added in equal volumes to the vaccine. Inactivated splitted influenza vaccine "Vaxigrip" (Sanofi Pasteur, France) was used in this study. DCs were obtained from C57/B16 mice bone marrow precursors while cultivating in complete medium with mice recombinant GM-CSF and IL-4 (BioSource International Inc., Belgium).Medium was replaced on the 6th day of incubation and was supplemented with either "Vaxigrip" vaccine or 1% suspension of chitosan sulfate in micro/nanoparticles, or 1% chitosan glutamate solution, or vaccine combined with chitosan derivatives to induce DC maturation with subsequent cultivation for 3 days. When the inactivated vaccine "Vaxigrip" was added to immature DC culture the minimal CD34-bearing cell number was detected. Co-stimulating effect of chitosan sulfate micro/nanoparticles in combination with "Vaxigrip" vaccine was found to be modest, but when sulfate chitosan solution combined with vaccine was added to DCs there was observed more pronounced decrease in immature DCs. Addition of chitosan glutamate or chitosan sulfate micro/nanoparticles to immature DCs did not cause the increase in the number of CD83-positive cells. "Vaxigrip" rendered most marked action on DC maturation. This vaccine causes the increase in amount of CD14 macrophage marker-expressing cells. Micro/nanoparticles of chitosan sulfate are able alone to stimulate macrophage differentiation in contrast to chitosan glutamate. Combined introduction of vaccine and chitosan derivatives results in sharp weakening of macrophage differentiation stimulation. Following the introduction of chitosan sulfate micro/nanoparticles in culture of immature DCs the number of cells expressing the CD40 and CD80 co-stimulating molecules was elevated as actively as in combination with the vaccine. On introduction of chitosan glutamate the amount of these cells was increased and approached the level of TNF-a-induced increment but in combination with vaccine. Capabilities of chitosan sulfate micro/nanoparticles in augmentation of TLR2-expressing cells nearly by 2-fold exceeded the control values (immature DCs). On combined introduction with vaccine the number of TLR2-bearing cells was increased as compared with the control. Chitosan derivatives do not affect the content of TLR9-positive cells, but when combined with the vaccine these elevated their number. Thus, one of molecular mechanisms underlying the adjuvant action of chitosan is direct interaction of polysaccharide fragments with DC receptors (both in contact with antigen and without it).

Keywords: inactivated influenza vaccine; chitosan derivatives; dendritic cells

Intestinal response to β-glucan oral immunostimulation involves cathelicidin mediated pro-inflammatory activities in the rainbow trout

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The use of immunostimulants in fish diets in order to increase disease resistance has been considered as one of the major strategies of prevention over the last years. Among them, the protective effect of β-glucans has been demonstrated *in vivo* against infections in numerous fish species. However, little is known about the functional effects of β-glucan oral administration on fish mucosal immunity. Host defense peptides (HDPs) are a diverse group of effector molecules with documented roles in innate immunity. Fish HDPs are abundantly expressed in epithelia linings, suggesting an important role in the mucosal response. Thus, the aims of the present work were (i) to investigate the effects of the β-glucan zymosan on the intestinal response in the rainbow trout *Oncorhynchus mykiss* and (ii) to assess the immunomodulatory function of trout HDPs. For this, we first evaluated the zymosan effect *in vitro*, using the intestinal epithelial RTgutGC cell line. RTgutGC cells respond to zymosan with increased expression of the proinflammatory cytokine IL-1β and the HDP cathelicidin. Next, to evaluate whether trout cathelicidins display any immunomodulatory function, we produced the complete mature peptides of three cathelicidin variants (OmCATH-1, OmCATH-2A and OmCATH-2B) as recombinant and synthetic peptides. All three cathelicidins display strong bactericidal effects against Gram-positive and Gram-negative bacteria. Importantly, OmCATHs exert a proinflammatory effect on RTgutGC cells, increasing the gene expression of IL-1β in cells exposed to the peptides. The modulation of IL-1β expression by zymosan and OmCATHs in RTgutGC cells was also evidenced by immunofluorescence confocal microscopy. Finally, the expression of OmCATHs and IL-1β in the intestine of rainbow trout fed with 0.3% zymosan-supplemented diet was identified after three weeks on feed trial. From our experimental data, we propose that trout cathelicidins are expressed by intestinal epithelial cells and may act as proinflammatory mediators to improve the local mucosal immune response of the trout intestine triggered by immunostimulants.

Keywords: mucosal immunity, host defense peptides, trout cathelicidin, β-glucan, immunostimulation

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Novel Benzothiadiazole-7-Carboxylic Acid Derivative as the Inducer of systemic resistance against viruses in tobacco plants

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Plants are invaded by different pathogens but only a few succeed in causing diseases. The attack by other viruses is countered by a sophisticated immune system possessed by plants. Induced resistance has been recognized as an attractive tool for plant disease management in modern agriculture, especially viral ones. Traditional pesticides play a critical role in the control of plant diseases, however, they could cause negative effects for consumers and induce drug resistance. Comparatively, induced resistance acts against a broad spectrum of diseases (1) and do not induce any resistance of pathogens to this phenomenon. Resistance to pathogen infection can be induced in plants by a wide range of biotic and abiotic agents. Variety of plant systemic immunity activators have been reported, of which the most successful one is the commercial inducer S-methyl benzo(1,2,3) thiadiazole-7-carbothioate (BTH) (2-4) . In this study, we aim to discover new amine derivative (BTH-WA) of potential systemic resistance activator with high activity.

Antimicrobal activity screening in vitro showed that BTH-WA did not have direct effect on viruses, bacteria and fungi and acts against plant diseases only by activating the systemic resistance of plants. Compound BTH-WA as ecological product in the plant protection may keep important balance among microorganisms in the environment.

Tobacco plants were sprayed or watered once with compound solutions at the concentrations between 20 and 100 mg/L and 7 days after treatment were mechanically infected with purified Tobacco mosaic virus at the concentration of 3-4 µg/ml. Potato virus Y and Olive latent virus 1 also were used in the studies. On the treated leaves or plants the local or systemic viral infection was almost completely inhibited by BTH-WA. Under greenhouse conditions the similar results were obtained on the treated tomato plants infected with virus and powdery mildew. Greenhouse experiments on tobacco plants showed that modified BTH is more effective as resistance activator than commercial BTH product (Bion™) (Fig. 1). These promising results indicated that compound was a powerful plant immunity inducer for agriculture application but still more studies are needed.

a. b. c.

Figure 1. Comparison of induction of systemic resistance against viruses in tobacco plants; (a) control, (b) commercial BTH (Bion®) at 20 mg/L, (c) BTH derivative BTH-WA at 20 mg/L

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Keywords: Systemic Acquired Resistance, Induced Systemic Resistance, plant immune system, benzothiadiazole, resistance elicitors, viruses

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Topographic pharmacokinetics of Interleukin-1 beta, encapsulated into the autologous erythrocyte ghosts

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Introduction. Due to short elimination half-life of cytokines in the blood (5-7 minutes) the continuous intravenous infusions are required (over 5-8 hours) for treatment of various disorders. Targeted delivery of cytokines directly to the area of inflammation can solve this problem. Such strategy can promote the accumulation of cytokines in high concentrations and for a long period of time at the affected area of the body.

Purpose: To study the pharmacokinetics of Interleukin-1 beta (IL-1β) incorporated into the autologous erythrocyte ghosts.

Material and methods. The experiments were conducted on albino rats with mass of 200.0-220.0 g (n = 28). IL-1β was used in two dosage forms: encapsulated in autologous erythrocytes (pharmacocytes obtained via the authors' original method) and standard free form. The animals were randomly divided into two groups, where each group has received a different form of IL-1β via injection to the tail vein. Group A received 500 μg of free IL-1β, while group B received an injection of pharmacocytes loaded with 500 μg of IL-1β. For both groups samples of serum were collected at 15, 30, 60, 180, 540, 720, and 1440 minutes after intravenous injections. Homogenates of liver, spleen, lung, heart, kidney, adipose tissue were obtained 24 hours after injections. Concentration of the cytokine IL-1β in the collected organs and blood plasma was measured by using ELISA method (ELISA Kit Sigma – Aldrich). Modeling was performed using Borgia 1.03 software.

Results. The main rational of use of erythrocyte ghosts loaded with IL-1β is the increase of cytokines' half-life ($T_{1/2}$). The half-life period was one hour when cytokines were administrated in the free form. At the same time, the injection of pharmacocytes with incorporated cytokines resulted in an increase of the half-life period up to 15 fold (1043.40 ± 137.92 min). The level of IL-1β activity in the blood remained high during 24 hours. The increased time of IL-1β presence in the body, when administered in the form of pharmacocytes could be explained by the reduction of elimination constant (C_{el}) by 1.6 fold, and clearance (CL_{el}) by more than 100 fold.

When IL-1β was administered in the free form, the highest concentration of the drug was detected in the homogenate of kidney tissue (both, cortical and medullary layers), which indicates the final stage of elimination. The distribution of the IL-1β was also detected (in descending order) in the following organs: liver, heart, spleen and subcutaneous tissues.

Administration of IL-1β in the form of pharmacocytes led to the deposition of the drug mainly in the liver (increased concentrations more than 7-fold compared to free IL-1β), spleen (3.5 fold increase), and lungs (2.6 fold increase). Some increase of the activity of IL-1β was observed in the subcutaneous tissue. Concentration of IL-1β in homogenate of cardiac tissue was reduced down to 3.7 fold. Apart from that, more than 8 fold reductions in IL-1β concentration was detected in renal tissue, which can be interpreted as an indicator of delay in the drug elimination.

Conclusions. Investigation of the pharmacokinetics of pharmacocytes with incorporated IL-1β showed significant differences in bio-distribution compared to administration of the free form of the drug. Pharmacocytes provide prolonged activity of IL-1β in an organism by increasing the half-life and reduction of elimination and clearance. Moreover, they can stabilize the level of the drug in serum, and facilitate re-distribution with the maximum accumulation of IL-1β in liver, spleen and lungs. These findings can be utilized for enhancing of therapeutic effect and developing of new clinical strategies.

Keywords: pharmacokinetics; Interleukin-1 beta; targeted delivery; distribution; autologous erythrocyte ghosts

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Vaginal levels of lactic acid and NGAL in vulvovaginal candidiasis and bacterial vaginosis: Are they responsible for the different immune responses?

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Introduction: The pathophysiologic mechanisms of bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC) are still poorly understood despite their frequent occurrence in women. Lactic acid-producing bacteria in the vagina controls the growth of undesiable microbes [1]. NGAL (neutrophil gelatinase-associated lipocalin), a siderophore-binding protein, inhibits bacterial growth by sequestering iron [2]. In addition to acidifying the vagina, lactic acid stimulates the IL-23/IL-17 T lymphocyte pathway [3] and is capable of inducing the release of pro-inflammatory cytokines from vaginal epithelial cells [4]. Pro-inflammatory cytokines induce the transcription factor, NFκB, that is also responsible for up-regulation of NGAL expression [2]. Lactic acid also stimulates NFκB activation [5].

Objective: We evaluated associations between L-lactic acid and NGAL levels in vaginal fluid and the occurrence of BV and VVC.

Methods: Vaginal samples were collected from healthy women and women diagnosed with VVC and BV at the outpatient clinic in the Department of Obstetrics and Gynecology at the University of Campinas in Brazil between May and November 2013. The samples were assayed for L-lactic acid by colorimetric analysis (BioAssay Systems, Hayward, CA) and for NGAL by a commercial ELISA (R&D Systems, Minneapolis, MN). Exclusion criteria were women having menses, pregnancy, current use of antibiotics, anti-fungal medications, corticosteroids or any other immunosuppressive medication or any vaginal product and whose last vaginal sexual intercourse was less than 24 hours. The final analysis was performed on 77 healthy controls with a Lactobacilli-dominated vaginal microbiota, 52 women with VVC and 43 with BV.

Results: L-lactic acid levels were lower in vaginal fluid of BV patients (Median 0.02mM, Range <0.02-1.43, $p<0.001$) compared to healthy controls (Median 0.11mM, Range <0.02-1.64) and VVC (Median 0.13mM, Range <0.02-2.14). There was no difference between the L-lactic acid levels in healthy controls and women with VVC. NGAL levels were lower in BV patients (Median 402ng/ml, Range 45-944, $p=0.0014$) and higher in VVC patients (Median 741ng/ml, Range 280-1969, $p=0.0003$) when compared to healthy controls (Median 561ng/ml, Range 71-1228). The vaginal fluid concentrations of NGAL and lactic acid were highly correlated (Spearman $r = 0.5756$, $p < 0.0001$).

Conclusion: The lower levels of L-lactic acid and NGAL in BV samples and the correlation between L-lactate and NGAL suggest that lactic acid is an NGAL inducer and NGAL production is optimal in the presence of a Lactobacilli-dominated vaginal microbiota. The higher levels of NGAL observed in VVC indicate that NGAL is up-regulated as a compornent of the innate immune response against a *Candida* infection.

Keywords: neutrophil gelatinase-associated lipocalin (NGAL), bacterial vaginosis, vulvovaginal candidiasis, vaginal fluid, innate anti-microbial immunity

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Vitamin D: the foundation of Human Innate Immunity

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Introduction: Medical scientist way back in 17th century had observed that nutritional rickets the prototypical disorder of vitamin D deficiency had close association with infections, Howland and Holt, major scholars of rickets, coined the term "rachitic lung" suggesting alliance of frequent pulmonary infections with vitamin D deficiency. Manville suggested that fat-soluble vitamin deficiency ''leads to frequently pyogenic infections of the respiratory tract and. The research in last decade has unearthed many new facets of vitamin D; the most important among them is its role as immunomodulator. All the cells of immune system (Dendritic cells, macrophages, and T and B cells) express VDR and CYP27b1 enzyme. Calcitriol active form of vitamin D influences immune cells genes and thereby regulates their various functions.

Calcitriol the foundation of Innate Immunity: Evolutionarily innate immune system is one of the most crucial aspects of life protecting any organism from invaders. Recent studies have revealed potent effects of vitamin D on all the aspects of immune system, particularly on the ability of vitamin D to promote innate immune responses. Calcitriol influences plethora of human innate immune responses; some of these are described in following text.

Innate Immunity: Antimicrobial Peptides: Human body produces human antimicrobial peptides in response to pathogens. These AMPs include: defensins and cathelecidin (LL-37). Vitamin D acts as a potent stimulator of antimicrobial peptides particularly cathelicidin. Apart from its antimicrobial properties, cathelecidin also has other immune regulatory properties. The response time for cathelecidin production is very short; within few minutes of pathogen recognition this protein is manufactured as defence mechanism. Besides immune cells many of the non immune epithelial tissues also produce this important AMP.

Innate Immunity: Barrier function: The innate immune system begins with the epithelial barrier between the sterile hosts and infected outside environment; Vitamin D has a pivotal role in maintaining this physical barrier by upregulating genes which encode proteins required for tight junctions.

Innate Immunity: pathogen recognition: Microbes have pathogen associated molecular patterns (PAMP's). When PAMP gain entry in to the host, they are recognized by a class of receptors in the plasma membrane of macrophages & epithelial cells known as (TLRs) Toll-like receptors. TLR function is influenced by vitamin D levels.

Innate Immunity: Autophagy, superoxide anions: *Autophagy* and superoxide anions are a eukaryotic mechanism that involves intracellular microbial killing; these mechanisms are cathelicidin dependent;

Innate immunity: Adaptive Immune response Cytokines regulation: Dendritic cells (DC) and macrophages the two specialized cells in antigen presentation initiate the adaptive immune response; they activate T and B lymphocytes. Activation & proliferation of T & B cells; among the many of factors which influence this process vitamin D is the most vital one.

Conclusion: Vitamin D plays the pivotal role for the proper functioning of the body's immune system; strengthening human immune system would decrease infectious diseases, thereby curb antibiotic uses.

Key words: Innate immunity, Vitamin D, Calcitriol, cathelecidin, PAMPs, TLR.

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Antimicrobial resistance

Mechanisms of action of antimicrobial agents

A clinical resistant isolate of opportunistic fungal pathogen, *Candida albicans* revealed more rigid membrane than its isogenic sensitive isolate

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The opportunistic dimorphic fungal pathogen, *Candida albicans* is among the top five most common causes of global nosocomial infections leading to almost 40 % mortality and morbidity in immunocompromised patients. Increasing incidence of multidrug resistant strains has emerged as a significant threat to the treatment of *Candidiasis*.

The fundamental physical properties of the cell membrane are strongly linked with their biological functions. Membrane provide a permeability barrier to the entry of biomolecules across the cell. It is extremely interesting to investigate the key role played by membrane fluidity in regulating the drug resistance observed in the clinical microbial strains. This study has been carried out to characterize the changes in the membrane fluidity in different phases of growth which includes the lag phase, log phase and stationary phase of cells in an isogenic pair of clinical isolate (GU4-sensitive; GU5-resistant) of *Candida albicans*. Microviscosity of membrane has been measured in this study using fluorescence polarization and the probe, 1, 6-Diphenyl 1, 3, 5-hexatriene (DPH). The reciprocal of fluidity (Micro-viscosity) is the measure of fractional resistance to rotational and translational motion of molecule. With changes in growth phase from lag to log phase, the resistant isolate GU5 was found to attain a more rigid membrane than the sensitive isolate GU4 but after log phase it appeared that the equilibrium is achieved during stationary phase for membrane fluidity for the two isolates and they attain identical physical state of the membrane. Therefore, increased rigid behaviour of membrane may be responsible for reduction in passive drug diffusion for the resistant isolate in log phase and at the same time, it also appears that both the isolates appear to attain a steady state of equilibrium during the stationary phase adapting to the changes in physical state of the membrane. GU5 shows overexpression of the ABC pump membrane protein CDR1p which may be attributed to the observed differences in their membrane fluidity. Membrane fluidity is a parameter of the physical state of the membrane which largely affects the passive diffusion of the drugs and hence the observed resistance towards drugs. The resistance towards drugs also appears to be influenced by the host environment, prior exposure to drugs and overexpression to different drug efflux pump proteins. The *Candida* cells appear to adapt to changes in the environment through modulation of their membrane properties and lipid composition leading to altered membrane fluidity.

Keywords: Candida albicans; drug resistance; membrane fluidity; isogenic clinical isolates; ABC pump protein

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Analysis of quinolone and oxyiminocephalosoprin resistance mechanisms in *Salmonella* in Uruguay

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Introduction:
Fluoroquinolones and 3rd generation cephalosporins constitute the therapeutic option for extra intestinal infections caused by *Salmonella* spp.

Plasmid mediated quinolone-resistance (PMQR) has contributed to the swift dissemination of resistance to fluorquinolones, usually associated with resistance to oxyiminocephalosporins.

The aim of this work was to characterize mechanisms of fluorquinolones and oxyiminocephalosporins resistance in *Salmonella* isolates of human origin in Uruguay.

Materials and methods:
Salmonella enterica isolates were collected from 01/2011 to 11/2013, and selected based on resistance to nalidixic acid (NA) (extraintestinal criteria), and/or to oxyiminocephalosporins.

Antibiotic susceptibility by disk-diffusion assay and MIC to ciprofloxacin were determined following CLSI guidelines. Pulsed field gel electrophoresis (PFGE) was performed following PulseNet protocols; phylogenetic analyses were performed with GelCompar software. Extended-spectrum β-lactamases (ESBLs), AmpC alleles and PMQR were sought, in nalidixic acid and/or oxyiminocephalosporins-resistant strains, by PCR and sequencing. Mutations in the quinolone-resistance determining region (QRDR) of *gyrA* and *parC* were sought by PCR and sequencing for all strains with MICs to ciprofloxacin equal or higher than 1µg/mL.

Results:
We detected 108/583 NA-resistant, 9/583 oxyiminocephalosporin-resistant and 2/583 isolates resistant to both antibiotic families. Thirteen isolates carried *qnrB* alleles, mostly *qnrB19*, whereas one isolate carried *qnrB2*. No other PMQR genes were detected. ESBLs were detected in eight strains (CTX-M=4, SHV=2; unidentified=2), whereas three strains carried CMY-like plasmidic *ampC* genes.

Most of the resistant strains studied corresponded to serovars Typhimurium and Enteritidis, being 26/110 quinolone-resistant strains of extraintestinal source. PFGE assays identified the presence of several co-circulating clones of *S.* Typhimurium, whereas *S.* Enteritidis corresponded mainly to a single circulating clone. Nearly 25% of NA-resistant extraintestinal isolates shared the same pulsetype with intestinal isolates.

Mutations in the QRDR of gyrases corresponded mainly to S83Y in GyrA, whereas ParC alleles featured two changes, T57S and E84K.

Conclusions:
During the study period, oxyiminocephalosporin-resistance reached 2%, whereas resistance to NA neared 20%. PMQR genes were detected in only 12% of quinolone-resistant isolates. In this context, we witnessed an increase in the frequency of NA-resistant strains (2011=3.5% - 2013=16.2%), albeit displaying lower MIC values to ciprofloxacin. The detection of the same NA-resistant clones recovered from both, intestinal and extraintestinal samples, highlights the significance of epidemiological surveillance of antibiotic susceptibility for all *Salmonella* spp isolates of human origin.

Keywords: *Salmonella*; quinolones; oxyiminocephalosporins, resistance

Antibiotic resistance patterns in *Staphylococcus* spp. originated from companion animals in Lithuania

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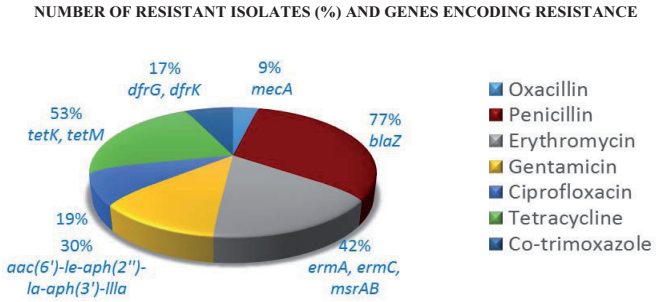
Staphylococcus spp. are a global human health problem causing infections in both hospitals and the community. Companion animals are also frequently colonized and can become infected. Transmission of resistance genes between staphylococci in animals and humans is possible, thus susceptibility data collection is very important. The aim of this study was to investigate the frequency of *Staphylococcus* species in companion animals in Lithuania and to determine their antibiotic resistance patterns.

Between 2012-2014, clinical samples were collected from diseased (dermatitis, otitis, wound infections, reproductive and respiratory tract infections) dogs (n=445) and cats (n=55) in Lithuania. Staphylococci isolation was performed on Blood Agar (E&O Laboratories UK), Mannitol Salt Agar (Oxoid, UK) and Brilliance MRSA2 Agar (Oxoid). Staphylococci species identification was performed using biochemical testing (Microgen, UK) and by sequencing of the 16S rRNA gene. Antimicrobial susceptibility testing was carried out by broth microdilution using "Sensititre" plates and ARIS 2X system (Thermo Scientific, UK) and interpreted using EUCAST epidemiological breakpoints. PCR amplification was used to detect antimicrobial resistance genes.

The rate of *Staphylococcus* spp. isolation from animals was 87%. The most prevalent species were *S. pseudintermedius* (55%), *S. haemolyticus* (16%), *S. lentus* (14%), *S. xylosus* (6%), *S. chromogenes* (6%) and *S. warneri* (5%) The antibiotic susceptibility data and resistance genes are presented in Figure 1. The *mecA* positive species included *S. haemolyticus*, *S. lentus* and *S. pseudintermedius*. None of the isolates was resistant to vancomycin or linezolid. According to the data obtained it could be outlined that clinical *Staphylococcus* isolates from companion animals are resistant to several antibiotics including those that are critically important for humans. This highlights the concern for potential zoonotic transfer of resistant staphylococci between animals and humans.

Keywords: antimicrobials; genes; dogs, cats

Figure 1. Antibiotic resistance profiles of the *Staphylococcus* spp. isolated from companion animals in Lithuania



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Antimalarial drug resistance: Monitoring artemisinin resistance in *Plasmodium falciparum* in Odisha state of India

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Antimalarial drug resistance is one of the major obstacle in combating malaria problem throughout the world. In Southeast Asia (SEA), India, Indonesia and Myanmar contribute to 96% of reported malaria cases and deaths. The two major species causing malaria are *Plasmodium falciparum* and *Plasmodium vivax* amongst which *P. falciparum* is responsible for causing more than 50% of malaria cases in India itself. WHO recommends artemisinin based combination therapy (ACT) for treatment of uncomplicated *P. falciparum* malaria throughout the world. ACT is recommended with the view that fast acting artemisinin component having short half life takes care of the initial high parasitaemia reducing the parasite biomass to 10^4 fold per 48 hour life cycle while the remaining parasitaemia is effectively taken care of by long acting partner drug. In India, artesunate+sulfadoxine-pyrimethamine (AS+SP) is recommended for the treatment of malaria, except for north-eastern (NE) states where artemether-lumefantrine (AL) is recommended.

Resistance to artemisinin has already been reported from Pailin, western Cambodia which is marked by delay in parasite clearance time (PCT) after administration of artesunate. In an effort to foresee decreasing artemisinin susceptibility in India, if any, this study was planned where *Plasmodium falciparum* infected patients were treated with ACT (AS+SP) in Bisra CHC, Sundergarh district, Odisha state and followed upto day 42 to monitor the clinical outcome. Thin and thick blood smears and filter paper blood spots were collected on day 0, 1, 2, 3, 7, and then weekly upto day 42 of follow-up. Parasite clearance time was monitored over a period of three days on day 0, 1, 2 & 3 by Microscopy and Real time PCR. Results obtained were compared and representative samples were analysed for important SNPs in *Pfatpase6* gene and copy number changes in *Pfmdr1* gene, the putative candidates related with artemisinin resistance.

Out of the total 72 patients enrolled, 64 patients could complete the study while others were withdrawn due to loss to follow up (LFU). All the patients were cured at the end of the follow up attaining 100% adequate clinical and parasitological response (ACPR). Forty three patients having parasitaemia >10,000/µl of blood were analysed for Real time measurement of PCT and results were compared with microscopic measure of PCT. Microscopy revealed most of the samples to be parasite negative by the end of day 1, except three samples which were parasite positive upto day 2, while none of the samples had detectable parasitaemia on day 3. On the contrary, real time PCR data analysis revealed 12 of the 43 analysed samples to be parasite positive on day 3. The representative samples (n=30, all having initial parasitaemia >10,000/µl of blood) including the 12 which were day 3 positive by Real time were analysed for L263E, E431K, A623E and S769N SNPs in *Pfatpase6* gene revealing two samples to have E431K mutation while one had mixed type genotype for E431K, all the other analysed SNPs showed wild type genotype. These two samples were the ones which had day 3 positivity as per Real time analysis. One sample showing mixed type genotype was amongst the other group (n=18) which was day 3 negative by both the techniques. *Pfmdr1* copy number assessment using Dd2 as a multicopy strain revealed no change in *Pfmdr1* gene copy number in the representative samples (n=12) which were day 3 positive as determined by Real time.

The study revealed that Real time PCR can assist microscopy in close monitoring of PCT to detect delayed PCT in surveillance studies. Day 3 positivity, an important determinant of suspected artemisinin resistance, is likely to be correlated with high initial parasitaemia. All the samples having day 3 positivity identified by Real time PCR after administration of AS+SP had higher initial parasitaemia ranging from 52800 to 98000 parasites/µl of blood authenticating the finding. Moreover, mutation in codon 431 of *Pfatpase6* gene is also revealing. However, no change was observed in *Pfmdr1* gene copy number in these samples. The study, hereby, provides evidence that although the efficacy of the combination AS+SP was 100% in the studied site at Odisha, chances of representative strains to develop artemisinin resistance in future is high. Therefore, artemisinin resistance in India needs continuous surveillance to monitor sensitivity of *P.falciparum* to artemisinins.

Keywords: Artemisinin resistance, delayed PCT, *Pfatpase6*, *Pfmdr1* copy number, ACT

Antimicrobial activity of rifampicine with pharmacocytes

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Introduction. One of the leading trends in modern pharmacology is the establishment of targeted drug delivery system.
Targeted drug concentration in the zone of the pathological process can reduce undesired reactions of the organism, lower therapeutic dose and frequency of administration. Separate direction is the development of delivery systems with using natural container, human blood elements, which is not coated by antibodies to respective target cells. "Extracorporeal pharmacotherapy" is called methods of using autologous blood cells to modify their properties and implement targeted transport. The thus-obtained cells are termed "pharmacocytes".

Objective. Determining the antimicrobial activity of rifampicin combined with erythrocyte ghosts by method of agar diffusion.

Material and methods. Method of hypotonic hemolysis in the original modification has been used for obtaining an erythrocyte ghosts with rifampicin (Zhumadilov ZH.SH., RV Makarenko, 1990). Basic solution of 1000 µg/ml rifampicin in the distilled water was taken for the control, the solutions with concentration of 0.5 µg/ml in the FSB buffer is a standard test concentration. It was used the spore suspension of Bacillus subtillis ATCC 6633. It were determined the antimicrobial activity by the method of agar diffusion. Rifampicin concentrations 0,5mkg / ml with erythrocytes ghosts were added per hole in agar with a volume of 0.1 ml. Samples were taken from the bottom of the hole and from the supernatant after centrifugation of the suspension of rifampicin with erythrocyte ghosts. 10mg/kg dose of the antibiotic. It was incubated on agar 16-18 hours at 37^0C. The diameters of crack growth retardation were measured in mm on all the investigated plates. The average values were found for each test dose of rifampicin and rifampicin with erythrocyte ghosts. Average value of each cup were corrected by a constant area equal to 17 mm, which corresponds to a reference standard concentration of 0.5 µg/ml, and then make a correction to the value of the zones of the test solutions. All figures were adjusted respectively.

Result. The concentration of rifampicin is in average of 94% at a dose of 10 mg / ml in erythrocyte ghosts, that is 9,49mg / ml. The concentration of rifampicin in the supernatant is 2,13mg / ml, which is significantly lower. The diameter of the zones of growth inhibition Bacillus subtillis is 27.6 mm in average.

Conclusion. The transport form of rifampicin as erythrocyte ghosts was created. In the erythrocyte ghosts is deposited more than 94% of antibiotic i.e. the concentration of rifampicin is 9,49mg / ml.

Keywords: pharmacocyte; rifampicin; antimicrobial activity.

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Antimicrobial resistance in *Salmonella* strains isolated from retail chicken meats in Korea

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Resistance of nontyphoidal *Salmonella* to both fluoroquinolones and extended-spectrum cephalosporins have been reported for couple of decades and such resistance imply a therapeutic problem in the near future. The first report of extended-spectrum β -lactamase in diseased chickens were made in Korea in 2011 and more reports on food-producing animals are expected as clinical reports are made worldwide. To investigate on antimicrobial resistance in *Salmonella* strains isolated from whole chicken rinse of different integrated broiler operation, a total of 100 retail chicken meats were collected from retail supermarkets in Seoul, South Korea from May to June 2014. Twenty individually packaged whole chicken carcasses of five different retail brands were rinsed and 43 isolates of *Salmonella* spp. were recovered. The antibiotic susceptibility testing was performed on Muller-Hinton agar by disc diffusion method according to the standard criteria of the National Committee for Clinical Laboratory Standards. All the isolates (100%) were resistant to erythromycin, followed by nalidixic acid (77%), streptomycin, ampicillin, cefazolin, cephalothin, and cefotaxime (72%). Cefotaxamine resistant isolates were all confirmed as extended-spectrum β -lactamase CTX-M type possessing $bla_{\text{CTX-M15}}$ gene. In addition to $bla_{\text{CTX-M15}}$ gene, and $bla_{\text{TEM-1}}$ gene was amplified in one isolate showing multiple drug resistance, including chloramphenicol, ciprofloxacin, norfloxacin, and sulfamethoxazole-trimethoprim. To our knowledge, this is the first report of extended-spectrum β -lactamase producing *Salmonella* strains isolated from chicken meat from different integrated broiler operation.

Keywords: *Salmonella* spp.; chicken meat; extended-spectrum β -lactamase; multidrug resistance

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Antimicrobial resistance of *Escherichia coli* and *Salmonella* spp. from pigeons in Brazil

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The aims of this study were to evaluate the presence of *Salmonella* spp. and potentially pathogenic *Escherichia coli* in pigeons (*Columba livia*) that may contaminate food stored in warehouses through contact with feces and secretions, and set a profile of antimicrobial resistance of isolates. A total of 31 strains of *E. coli* and one sample of *Salmonella* Typhimurium were isolated from 30 pigeons caught in the Esteio – RS, and 15 samples of *E. coli* and two strains of *Salmonella* Typhimurium from eight pigeons caught in Guaiba - RS. The antimicrobial susceptibilities of bacterial isolates were determinate using the disk diffusion method. Ciprofloxacin 5 µg (CIP), enrofloxacin 5µg (ENO), florfenicol 30µg (FLF), gentamicin 10µg (GEN), nalidixic acid 30µg (NAL), neomycin 30µg (NEO), nitrofurantoin 300µg (NIT), sulfonamides 300µg (SUL), sulfazotrim 25µg (SZT), tetracycline 30µg (TET), ampicillin 10µg (AMP), chloramphenicol 30µg (CLO), norfloxacin 10µg (NOR) and doxycycline 30µg (DOX) were the antibiotics tested. Our results showed that the isolated strains were susceptible to the majority of the antibiotics tested. In *E. coli* isolates (46) were observed lower percentages of resistance (4%). *Salmonella* Typhimurium isolates were susceptible to all antibiotics tested. Our results demonstrate the presence of *E. coli* and *Salmonella* Typhimurium in pigeons and that the isolates showed low antimicrobial resistance.

Keywords: antibiogram; antimicrobial susceptibility; *Escherichia coli*; *Salmonella*; avian

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Antimicrobial resistance of *Staphylococcus epidermidis* isolated from the trauma unit in the University Hospital of Tlemcen "Algeria"

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Nosocomial infections are a real public health problem. We are interested in finding a particular species *Staphylococcus epidermidis* in the trauma unit knowing that it is more resistant to antibiotics and met more and more in a variety of infections in hospitals. Therefore we conducted 342 samples in this unit at the University Hospital of Tlemcen, which consisted of nasal ports (48h before surgery) and surgical wounds (4 days after surgery) in operate patients.

Using the API STAPH system 85 strains of *S. Epidermidis* were identified.
The antibiotic susceptibility testing was performed on Muller-Hinton agar by disc diffusion method according to the standard criteria of the National Committee for Clinical Laboratory Standards, reveals a rate of 90% of multiresistant strains, mainly to ampicillin (100%), penicillin (100%), oxacillin (93.33%). We also found that 66.66% of strains were resistant to erythromycin and 33.33% resistant to vancomycin and only one strain was sensitive to oxacillin.

The study of the minimum inhibitory concentration for oxacillin showed a MIC <= 0.25 mg / l, which is consistent with the results of susceptibility testing.

Keywords: *Staphylococcus epidermidis*, nosocomial infections, drug resistance, MIC.

Antimicrobial resistance of uropathogens isolated from women in Tlemcen «west of Algeria»

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Objective
To determine the causative agents of urinary tract infection in Tlemcen city, and the antibiotic susceptibilities of these agents by standards methods.

Materials and Methods
A study was conducted to examine the prevalence of antibiotic resistance in the strains of bacteria isolated from patients with suspected urinary tract infection. A total of 198 bacterial isolates were grown from semi quantitative urine culture and were of significant bacteriuria.
The antibiotic susceptibility testing was performed on Muller-Hinton agar by disc diffusion method according to the standard criteria of the National Committee for Clinical Laboratory Standards,

Results
Enterobacteriaceae found in urinary tract infections are *Escherichia coli* 25. 26%, Klebsiella 6.66%, *Proteus* in a proportion of 3.33%, *Citrobacter* and *Enterobacter* 1.66% And *Staphylococcus aureus* with 20.83%
The results of antibiotic resistance in *Enterobacteriaceae* and *staphylococci* tested against 18 antibiotics revealed a diversity of antibiotypes and a remarkable rate of resistant strains. *Staphylococci* are resistant to several antibiotics, including penicillin, ampicillin and erythromycin 100% , followed by oxacillin 85% , cefazolin 81% and cefotaxim, 71%, 81.8% resistant to tetracyclin, doxycyclin followed with a rate of 72.1%, 78.1% resistance to gentamicin, 85.7% resistant to chloramphenicol, 85% resistant to sulfamethoxazol and 89.5% resistant to nalidixic acid.
The gram negative bacilli have a total resistance to penicillin G and céfazolin (100%), 96.8% resistant to oxacillin, 96.3% to ampicillin and 83.3% resistant to cefotaxim.

Conclusions
The predominant organisms were *Escherichia coli* 25.26%, followed by *Staphylococcus aureus* with 20.83%.
Resistance rates among common uropathogens continue to evolve and appear to be increasing to many commonly used agents. Continued surveillance of resistance rates among uropathogens is needed to ensure that appropriate recommendations can be made for treatment of infected patients.

Keywords: urinary tract infection, *Enterobacteriaceae, staphylococci,* antibiotic resistance.

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Antimicrobial susceptibility of *Escherichia coli* isolated from small animals in Lithuania

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Escherichia coli is a commensal organism in people and animals but is also a causative agent of diarrhoea and extra-intestinal infections [1]. The sepsis-associated human mortalities due to *E. coli* are estimated at 868,000 per year globally [2]. Small animals often are more resistant to *E. coli* infections however, from public health perspective this species may represent a reservoir of virulence and resistance genes [1]. The aim of this study was to isolate *E. coli* from small animals and to determine susceptibility to antimicrobial classes important for humans. Rectal (n=200), vaginal (n=100) as well as other (n=80) samples from different organs (skin, eyes, throat) were taken from 380 animals (272 dogs, 55 cats and 53 other species) on small animal clinics in Lithuania. Both diseased (n=280) and healthy (n=100) animals were included in this study. Clinical material was inoculated onto TBX Agar (Biolife, Italy). Single suspected colony of *E. coli* from one sample was randomly selected for testing. Bacterial identification was performed using Microgen GNA+B identification system. The initial susceptibility testing was performed by disk-diffusion method. Resistant isolates to at least two antimicrobials were further tested for Minimal inhibitory concentrations (MIC's) using SENSITITRE (Thermo Scientific) plates and ARIX 2X system. Results were interpreted according to EUCAST clinical breakpoints. PCR was used for detection of genes encoding antimicrobial resistance.

Two hundred and sixty isolates of *E. coli* were obtained (68%) from the animals tested. The rate of isolated *E. coli* from rectum was 90%, from vaginal samples - 50% and 38% from the other organs. The most common resistances were demonstrated to ampicillin (39%), sulfamethoxazole/trimethoprim (16%) and ciprofloxacin (10%). Resistance to gentamicin was detected in 5% of the isolates. Extended spectrum beta-lactamases were produced by 3% of the isolates with attribution to the TEM gene. The only one isolate harboured the CTX-M gene. Other genes encoding resistances included *sul1*, *sul2* and *sul3* (sulphonamides), *dfr1*, *dfr5* and *dfrA7* (trimethoprim), *aac(3)II*, *aphA1* and *aadA* (aminoglycosides). No statistically reliable results according to antimicrobial resistance were determined between diseased and healthy animals or between different animal species.

The situation on antimicrobial resistance in *E. coli* spread in Lithuanian small domestic animals is still favourable however, ESBL producing isolates are found in rare occasions. Resistance to fluoroquinolones is relatively high and that might be associated with frequent use of enrofloxacin on small animal clinics.

Keywords: antimicrobials; genes; resistance

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Assessment of commercial probiotic organisms for their antibiotic resistance

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The discovery of antibiotics initiated a period of drug innovation and implementation in human and animal health. These discoveries were tempered in all the cases by the emergence of resistant microbes. Recent studies revealed that modern environmental and human commensal microbial genomes have a large concentration of antibiotic resistance genes. The human gastrointestinal tract is a massive reservoir of bacteria, including probiotics with a potential for both receiving and transferring antibiotic resistance genes. Probiotics are live, single or mixed and concentrated form of bacterial cell threshold (1×10^9 cfu), which, when administered by the different ways in humans and animals, grant cogent, aspired and site targeted health benefit/ benefits of total safety. The increased use of fermented food products and probiotics, as food supplements and health promoting products containing massive amounts of bacteria acting as either donors and/or recipients of antibiotic resistance genes in the human GI tract, also contributes to the emergence of antibiotic resistant strains. A large part of all food production is estimated to involve microbial fermentation processes by using strains of lactic acid bacteria (LAB) strains. Probiotics have become available in the market, containing a single strain or a combination of strains. The proposed problem is that probiotic strains and starter cultures might contain naturally occurring antibiotic resistance genes. From a safety point of view, it is necessary to distinguish between intrinsic and acquired resistance genes and most importantly the transferability of these. The increased level of ingested probiotic bacteria has caused new speculations that these bacteria might also contribute to the reservoir of antibiotic resistance genes, and when the right circumstances are present; the antibiotic resistance genes could be transferred to a pathogenic bacterium which could then lead to treatment failure of an infection. Keeping these facts in mind, we designed this project to assess the presence of antibiotic resistant genes in probiotic organisms which are prevalent in the market and used on a large scale by both normal and diseased persons. Commercial products including both pharmaceutical and food preparations, were procured from the Indian market and probiotic organisms were isolated and characterized from them. All the 28 isolates, including *Lactobacillus rhamnosus*, *L. acidophilus*, *L. casei*, *L. reuteri*, *L. plantarum* and *L. fermentum* were then used for the assessment of their antibiotic resistant profile using disc diffusion assay against a total of 38 antibiotics. Most of the isolates were found to have resistance against multiple antibiotics, including some of the most commonly used antibiotics for treatment of various diseases. The isolates displaying resistance were then subjected to the determination of minimal inhibitory concentration (MIC) using two different approaches viz. E Test strip method and broth microdilution method. When the MIC's were compared with guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) it was found that many of the organisms are having MIC higher than that prescribed, which qualify them as antibiotic resistant organisms. Thereafter, we proceeded towards the isolation of plasmid from the resistant organisms in order to identify the location of genes providing resistance to the organism. All of the isolates were found to carry plasmids. Their presence on plasmid makes them highly susceptible to transfer to other organisms present in the gut which may be pathogenic. Screening of different genes responsible for resistance on both plasmid and genomic DNA is underway.

Keywords- Lactic acid bacteria, Antibiotic resistance, Probiotics

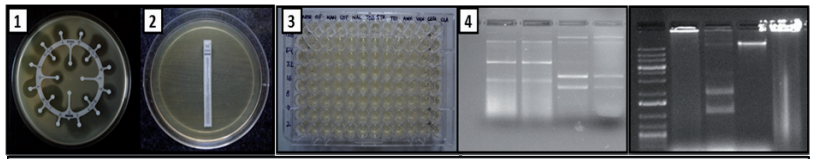

Fig1. Disc Diffusion Method Fig 2& .3. Determination of MIC by E-test strip and Broth Microdilution Method Fig4. Plasmid Profiling

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Association between use of biocides and resistance to antibiotics

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Introduction: The bacterial adaptation and resistance to biocides are not significantly a new phenomenon. For some decades several researchers have raised concerns about acquired antimicrobial resistance that occurs intrinsically in response to biocide exposure. Persistent exposure, particularly low levels of the biocides could cause the acquisition and / or expansion of bacterial resistance encoding reductions in susceptibility of the biocide, which would result in the persistence of these bacteria for longer periods in environmental tests and clinical studies. This paper addresses the bacterial resistance of nosocomial strains in relation to antibiotics and biocides, and the possible interaction of the genes involved.

Material and Methods: Review and analysis of literature.

Results and Discussion: In recent years, there is a significant increase in the frequency of isolation of multiple antibiotic resistant bacteria that were originally known as sensitive to drugs routinely used in clinical practice or almost all drugs on the market. Persistent exposure, particularly with low levels of biocides could cause the acquisition and / or expansion of bacterial resistance and reductions in susceptibility biocidal bacteria such would ultimately persist for longer periods in environmental tests and clinical studies. Studies conducted human clinical isolates of *Staphylococcus aureus* and *Staphylococcus* food-related suggest a link between the resistance to QACs (Quaternary Ammonium Compounds) and resistance to penicillin.In hospital environments has been shown that *Staphylococcus BC* resistant (Benzalkonium chloride) were more frequently resistant to certain antibiotics that were isolated from BC-sensitive, indicating that the presence of either resistance determinant.

Conclusion: If the increase in the use of antibacterial agents in household consumer products is likely to result in antibiotic resistance, similar effects should also appear on products and hospital environments. The deeper understanding of the relationship between resistance multiple to disinfectants and antibiotic resistance will bring greater technical foundation that can contribute to more responsible use of disinfectants and antiseptics.

Keywords: resistance; biocides; antibiotics.

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Bicarbonate enhances the in vitro antibiotic activity of kanamycin in *Escherichia coli*

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Changes in enteropathogenic *Escherichia coli* E2348/69 growth were followed in liquid cultures. Bicarbonate alone inhibited *E. coli* growth in a dose-dependent manner and at a 5 mM concentration it reduced growth by approximately 5%. Kanamycin at 3.12 µg/ml inhibited growth by 15%, yet a combination of these same concentrations of kanamycin and bicarbonate inhibited *E. coli* by approximately 80%. Unexpectedly, at bicarbonate concentrations >20 mM enhancement of the antibiotic activity virtually disappeared, i.e. there was a paradoxical Eagle-like effect. How bicarbonate acts is unclear, but neutral or alkaline pH also enhanced the activity of kanamycin. However, several differences indicated a separate effect of bicarbonate. First, bicarbonate alone was more effective at inhibiting growth than the corresponding increments in pH. Second, at low concentration, the enhancing effect of bicarbonate was stronger than the effect of pH alone. Third, 5 mM bicarbonate significantly enhanced the activity of kanamycin while the corresponding pH had no effect. Fourth, the Eagle-like effect was exclusive of bicarbonate because changes in pH did not induce an analogous behavior. Notwithstanding the mechanism, the enhancing effect of bicarbonate was indubitable. Furthermore, we have very recently confirmed a similar effect on the activity of gentamycin. Consequently, it seems worthwhile to explore further the potential of bicarbonate to improve the efficacy of aminoglycosides and maybe even other antibiotics.

Keywords: bicarbonate; aminoglycosides;paradoxical Eagle-like effect;antimicrobial;kanamycin;gentamycin

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Comparative survey of CDR1, CDR2 , MDR1 genes expression in resistance and sensitive *Candida albicans* to fluconazole by RT REAL-TIME PCR

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Background: Resistance to fluconazole resistance in *Candida albicans* isolated from patients that suffering from Candidiasis such as AIDS, cancers patient and organ-transporters are increasing. Several reasons are available for resistance to fluconazole in *Candida albicans* such as: alter in sterol biosynthesis pathway, mutation or over-expression in ERG11gene and expression in CDR1,CDR2 and MDR1 genes. The aim of this search was compare of CDR1, CDR2, MDR1 genes expression in resistance and sensitive *Candida albicans* to fluconazole **Method**: The Susceptibility test against fluconazole in *Candida albicans* isolated from vaginal and oral Candidiasis patients to were determined using the broth microdilution method. Expression of CDR1,CDR2, MDR1's genes in resistance and sensitive isolated was measured using RT REAL-TIME PCR reaction and compared together. **Results**: From 46 isolated *Candida albicans*:20sensetive,12 sensitive dose dependent and 14resistance to fluconazole by MIC method be identified . After The result of genes expression by RT REAL-TIME PCR'S reactions showed, the three genes expression: CDR1,CDR2,MDR1 in sensitive isolated to fluconazole showed that this genes had a middle expression as resistance isolates that this genes expression were so few or zero. **Conclusion**: It seem that, another mechanism reasons of resistant such as alter in sterol biosynthesis pathway, mutation or over-expression in ERG11gene, have more important role in resistance to fluconazole in *Candida albicans* isolated which collected in Iranian patients. Expression of CDR1, CDR2, MDR1 genes in have a non-significant role. probably this pumps mutants kind show the resistance phenotype.

Keyword: *Candida albicans*, fluconazole resistance,CDR1,CDR2,MDR1 genes expression ,RT REAL_TIME PCR Technique

Controlling resistant bacteria with a novel class of β-lactamase inhibitor peptides: from rational design to in vivo analyses

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Peptide rational design was used here to guide the creation of two novel short β-lactamase inhibitors, here named dBLIP-1 and -2, with length of five amino acid residues. Molecular modeling associated with peptide synthesis improved bactericidal efficacy in addition to amoxicillin, ampicillin and cefotaxime. Docked structures were consistent with calorimetric analyses against bacterial β-lactamases.

These two compounds were further tested in mice. Whereas commercial antibiotics alone failed to cure mice infected with Staphylococcus aureus and Escherichia coli expressing β-lactamases, infection was cleared when treated with antibiotics in combination with dBLIPs, clearly suggesting that peptides were able to neutralize bacterial resistance. Moreover, immunological assays were also performed showing that dBLIPs were unable to modify mammalian immune response in both models, reducing the risks of collateral effects.

In summary, the unusual peptides here described provide leads to overcome β-lactamase-based resistance, a remarkable clinical challenge.

Keywords: β-lactamase inhibitors, bacterial resistance.

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Design, Synthesis and mode of action of some 2-(4'-aminophenyl)benzothiazole derivatives as potent antimicrobial agents

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In the present study some 2-(4'-aminophenyl) benzothiazole derivatives were designed, synthesized and evaluated for their antibacterial activity and possible mode of action as described previously[1]. Structures of the synthesized compounds were elucidated by spectral data. These compounds were screened against four different gram-negative and two different gram-positive bacterial strains by agar disc diffusion method[2]. Among all the synthesised compounds, compound **A07a** and **A07b** displayed most potent inhibitory activity with minimum inhibitory concentration (MIC) values in the range of 3.91-31.2 µg/ml against *S.aureus, S.typhi, P.aeruginosa* and *E.coli.* Structure–activity relationship (SAR) studies revealed that electronic and lipophillic factors had a significant effect on the antimicrobial activity of the designed compounds. The obtained data suggested that better antibacterial activity was linked with introduction of hydrazone moiety in 2-(4'-aminophenyl) benzothiazole ring system. Outcome of the study also revealed that incorporation of hydrazone group exhibited different modes of action based on aryl group substitution as revealed by studies on intact bacterial cells and plasmid DNA. The present study provides us two active compounds (**A07a** and **A07b**) with membrane perturbing mode of action elucidated by membrane depolarization, and Fluorescent assisted cell cytometry (FACS); intracellular mode of action due to binding with DNA along with potent activity against clinically relevant pathogen *E.coli* and *S.aureus*.

Keywords: antibacterial;MIC

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Detection of metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa* in various hospitals in capital of Iran-Tehran

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Background and aims:
Metallo-beta-lactamases (MBL) producing *Pseudomonas aeruginosa* strains are responsible for several nosocomial outbreaks in hospital care centers across the world. It is well known that poor outcome occurs when patients with serious infections due to MBL producing organisms are treated with antibiotics to which the organism is completely resistant. Current study was undertaken with the aim of optimizing the choice, dose and duration in MBL producing *P. aeruginosa* infections in various hospitals in Tehran.

Material and methods:
Various clinical samples of *Pseudomonas aeruginosa* were obtained from patients admitted in hospitals or attending the OPD between January 2013 to January 2014. Antimicrobial sensitivity was performed by Kirby-Bauer disk diffusion method. Minimum inhibitory concentration (MIC) of Imipenem resistant isolates was done by agar dilution method. Metallo-beta-lactamase production was detected by combined disk method, MIC reduction of imipenem in presence of EDTA and by Epsilometer test (E-test).

Results:
Out of 212 *P. aeruginosa* isolates, 35 (16.5%) were resistant to Imipenem. 33 (15.56%) were found to be MBL producers by combined disk test and all of them showed reduction in MIC in the presence of imipenem-EDTA in E-test. The number of MBL positive isolates from ICU was statistically significant (p=0.027). The hospital stay was significantly longer (p=0.000) among patients infected with MBL producers than MBL non producers. Statistically significant association of antineoplastic chemotherapy, urinary catherization with MBL production was found. All MBL producers were resistant to commonly used antibiotics. However, they were sensitive to polymyxin B(100%), piperacillin/tazobactum (18.2%), amikacin and ciprofloxacin (9.1%).

Conclusion:
MIC reduction is a cumbersome, laborious method and given the cost constraints of E-test a simple screening test like combined disk test may be used. In absence of therapeutic MBL inhibitors, polymyxins, aminoglycoside or fluoroquinolone molecule that may have retained some activity against the isolate may be used for the treatment of MDR P. aeruginosa infections.

Keywords: metallo-beta-lactamase (MBL), *Pseudomonas aeruginosa,* Minimum inhibitory concentration (MIC)

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Determination of Antibiotic Resistance of Some Pathogen and *Lactobacillus* Species in Fermented and Heat Treated Sucuks*

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The study was performed physico-chemical analysis and the isolation of the some *Lactobacillus* bacteria species, *S. aureus*, *E. coli* and *E. faecium* and in order to identify the antibiotic resistance of isolates and *mecA*, *tetM* and *vanA* genes in fermented and heat-treated 100 sucuk samples offered in Konya city center. The average ($\bar{x}$±SE) total mesophilic aerobic microorganism (TMAM), *S. aureus*, coliform and *Enterococcus* bacteria counts of fermented sausage samples were found as ($\log_{10}$ cfu/g) 10.84±0.68, 1.84±0.34, 1.07±0.31, 2.88±0.42 respectively. In heat treated samples, the average ($\bar{x}$±SE) ($\log_{10}$ cfu/g) of TMAM, coliform and *Enterococcus* ssp. counts ($\log_{10}$ cfu/g) were determined as 5.72±0.41, 0.26±0.11 and 0.70±0.23 respectively. The average lactic acid bacteria counts ($\log_{10}$ cfu/g) of fermented samples were found 10.59±0.81 and 2.75±0.76 respectively. Most common species among the lactic acid bacteria (n=86) was detected *L. brevis* (22.0%), *L. plantarum* (19.7%) ve *L. curvatus* (12.7%) respectively, and other isolates (45.6%) were generated by *L. salivarius*, *L. delbrueckii*, *L. lactis*, *L. paracasei* ve *Weissella confusa* species.

The *S. aureus* isolates resistant to oxacillin, penicillin G, cefazolin and erythromycin were 100%, 100%, 55.6%, 44.4% respectively, the resistance of *E. faecium* isolates to oxacillin, kanamycin, trimethoprim/sulfamethoxazole, ciprofloxacin, gentamicin were determined as 100%, 91.7%, 75.0%, 58.4%, 58.4%, respectively. The oxacillin MIC values in *S. aureus* isolates were between 4-64 µg/ml, the tetracycline MIC values in *E. coli* isolates were between 1-4 µg/ml, the vancomycin MIC values in *E. faecium* were found between 1-4 µg/ml ranges.

L. plantarum and *L. curvatus* isolates were found to have 46.5 % resistance to kanamycin, 42.9% to ciprofloxacin, 64.3% to trimethoprim/sulfamethoxazole and oxacillin, and 53.6% to vancomycin. The oxacillin MIC values of *L. plantarum were* ≤0.12-32 µg/ml, tetracycline MIC values were ≤0.12-32 µg/ml, vancomycin MIC values were 0.25-≥256 µg/ml, and the oxacillin MIC values of *L. curvatus* were ≤0.12-1 µg/ml, tetracycline MIC values were ≤0.12-0.5 µg/ml, and vancomycin MIC values were found as 0.25-1 µg/ml. *mecA*, *tetM* ve *vanA* genes could not be detected in all isolates with PCR analysis.

In conclusion, the isolation of pathogenic bacteria in sausages is crucial in terms of microbiological quality. Along with these isolated pathogenic bacteria, the high antibiotic resistance levels of *L. plantarum* and *L. curvatus* isolates are also of great importance as regards the public health. It is essential for sanitation that necessary measures be taken as well as advanced molecular studies conducted into the issue.

Keywords: Antibiotic Resistance; *Lactobacillus;* Pathogen; PCR; Sucuk.

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Distribution of Antibiotic Resistant Bacteria in tropical aquatic systems

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Antimicrobial agents are widely distributed in freshwater and marine environments introduced by wastewater effluent, and runoffs from agricultural and fish farming activities. Aquatic systems contaminated with anthropogenic compounds (*e.g.* heavy metals, antibiotics) are hotspots for the selection of antibiotic resistant bacteria (ARB) and dissemination of antibiotic resistant genes (ARG). To understand environmental antibiotic-resistance risks, we acquired local surveillance data on the distribution of ARB and ARG in marine and freshwater systems in Singapore. We sampled biomass from surface waters of a marine (a habour) and two freshwater sites (a reservoir and urban river), quantifying selected ARG gene targets and ARB counts. To determine levels of ARB, we used a culturing method to detect bacterial growth on minimal media supplemented with 7 antibiotic belonging different classes (tetracycline, sulphanilamide, norfloaxacin, kanamycin, erythromycin, lincomycin, trimethoprim). We observed a low abundance (<2 %) of bacteria resistant to norfloaxin, erythromycin and kanamycin and higher resistance (>11 %) to lincomycin and trimethoprim across the three sampled sites. Quantitative PCR results targeting macrolide resistance genes (*ermA*, *ermB*, *ermC*) showed levels below the detection limit. From a public health stance, and for the purpose of developing human health risk assessments (HHRA), it would be worthwhile to continue to monitor the occurrence of ARB and a larger array of associated ARG in these environments. Further phylogenic characterization using 16S rRNA gene sequencing of resistant bacterial isolates provides information on microbial composition to determine if antibiotic resistance observed is largely attributed to pathogens or environmental bacteria.

Keywords: antibiotic resistance; antimicrobial resistance genes; antibiotic resistant genes; pathogens; water quality; surface waters

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Effect of different antibiotic doses on antimicrobial resistance in *E. coli* strains from broilers

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The inappropriate use of antibiotics is the main cause of antimicrobial resistance. This practice could carry out a problem in the clinic treatment of the illnesses[1]. The objective of this work was to determine the susceptibility of *E. coli,* isolated in faeces of broilers, to different antimicrobial agents. This susceptibility was analysed after each one of the three treatments made with amoxicillin throughout their lifecycle. With this aim, four groups with 6 broilers were made: 1) C=Control group where broilers did not received any treatment; 2) ND= broilers which received a normal dose (24 mg of amoxicillin/kg); 3) LD= the dose received was lower (16 mg of amoxicillin/kg) and 4) VLD= the dose was very low (8 mg of amoxicillin/kg). In addition, each group was divided into two subgroups according to the route of administration (oral or diluated in the drinking water). A total of 158 strains of *E. coli* were analysed and the resistance was evaluated according to CLSI standards, on 12 antimicrobials chose by importance in clinic use amikacin (AK: 30µg), ampicillin (AMP: 10µg), amoxicillin-clavulanate (AMC: 20/10µg), ceftriaxone (CRO: 30µg), ciprofloxacin (CIP: 5µg), chloramphenicol (C: 30µg), gentamicin (CN: 10µg), kanamycin (K: 30µg), nalidixic acid (NAL: 30µg), tetracycline (TE: 30µg), cephalothin (KF: 30µg), streptomycin (S: 10 µg). The results showed that the control group had the highest percentage of sensitivities (70.49%) followed by VLD (65.44%), LD (59.26%) and ND (56.25%). Antibiotics with the highest frequency of resistance, in all dose groups, were AMC, AMP and NA. Regarding the type of administration, no significant differences between them were observed (p value-0,9940). The results of resistance and sensitivity throughout the study showed that in the control group was kept stable. In the groups of LD and VLD, the resistance decreased with the different treatments. However for the DN group, an increase was observed at the end of the study. Taking into account the type of antibiotic, *E. coli* strains were more resistant to AMC, AMP, KF and TE. The amount of resistant strains to NA were similar throughout all the study. This fact confirms the appearance of resistant strains to antibiotics normally used in clinic; which means a public health problem. A high percentage of resistance and multi-resistance was obtained for the antimicrobials classified as being of "high" and "medium importance" in relation to human health, which can jeopardize the effectiveness of clinical treatments and increase the severity of diseases[2]. This highlights the importance of continued surveillance and the usefulness of this information to take measures in the primary sector.

Keywords: antimicrobial resistance; *E. coli*; bacterial resistance; broilers

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Effect of thioridazine on the elimination of plasmids coding for drug resistances and other properties in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a highly invasive and toxigenic aerobic Gram-negative bacterium. This is non-sporing, non-capsulate and usually motile with the help of one or two flagella. Moreover, it is resistant to most of the antibiotics at a very high level [1]. Removal of antibiotic resistances by known pharmacological compounds can be beneficial for successful remedial control of this deadly pathogen. Phenothiazines may be used for determining their anti-pseudomonal potentiality along with their action on elimination of plasmids conferring multidrug resistance in the bacterium [2].

The level of resistances against various antibiotics and the antipsychotic phenothiazine thioridazine (Tz) was determined in 12 clinical isolates of *P.aeruginosa* including ATCC 27853 following international standard procedures. All of the 12 strains were found to be highly resistant to antibiotics like most β-lactams, cephalosporins, aminoglyocosides, flouroquinolones, but showed lower resistances against pipericillin, carbenicillin, amikacin and ciprofloxacin. After determining the level of resistance to Tz in the *P.aeruginosa* strains, plasmid curing test was performed by growing each strain in broth culture containing Tz. The culture was plated out on nutrient agar containing Tz so as to produce numerous isolated colonies. Then at least 100 colonies of each strain were stabbed onto plates containing different antibiotics; a plain nutrient agar plate containing no antibacterial drug was also stabbed in the same manner; this served as the control. Plates were incubated overnight to detect presence or absence of growth which suggested possibility of elimination of a plasmid responsible for a certain antibiotic resistance. Tz treated strains showed curing of many antibiotic resistances. With the application of Qiagen kit combined with a manual procedure, plasmid DNA was isolated from both the wild type and cured strains of *P.aeruginosa*. Agaraose gel electrophoresis revealed the presence of plasmid bands in wild type strains, whereas, such bands were absent in few Tz treated cured clones. Thus this study suggests that the simultaneous application of Tz with other antibiotics in patients suffering from infections due to MDR *P.aeruginosa* would help to eliminate the drug resistant plasmids from the infectious bacterial cells. This may open up a new arena of therapy which would facilitate an orchestrated response against this pathogen.

Keywords : *Pseudomonas aeruginosa,* thioridazine, multidrug resistance, plasmid curing

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Efflux Pumps mediating rifampicin resistance in Brazilian clinical isolates of *Mycobacterium tuberculosis*

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Introduction: Antimicrobial resistance occurs normally by 4 mechanisms: (1) enzymes that inactivate antibiotics, (2) target alterations (genetic mutations), (3) destabilization of enzymes involved in prodrug activation and (4) efflux pumps activity. Drug efflux mediated by a transporter has been one important mechanism purposed to explain drug resistance in *M. tuberculosis* strains, especially in circumstances in which genetic alterations are not found. In this study, we selected 45 *M. tuberculosis* clinical isolates resistants to rifampicin and sequenced their RRDR (RIF resistance-determining region) of the *rpoβ* gene, seeking mutations that could explain the resistance emergence. After that, we chose randomly 4 resistant clinical isolates and the standard strain H_{37}Rv ATCC 27294 without amino acid alterations in RRDR and evaluated their expression profiles of five putative multidrug efflux pump genes (*Rv1258c*, *Rv1410c*, *Rv2459, Rv1217c* and *Rv1218c*) by RT-qPCR. **Methods: MIC determination:** The rifampicin MIC was determined by REMA technique. The assays were performed in triplicate, read in SPECTRAFluor Plus and results interpreted according to the variations of fluorescence emitted. **RT-qPCR:** To evaluate the efflux pumps expression profiles the isolates were grown in 7H9 medium (supplemented with 10% OADC) until the mid-log phase. Reaching OD_{600nm}=0.6 rifampicin was added in the concentration 1/4 of the MIC for each isolate. Samples for RNA extraction were collected in 4 different times, considering the treatment: 0h, 24h, 48h and 72h. RNA was extracted using RNeasy Mini Kit (QIAGEN) and cDNA was synthesized with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems®). The reactions were run in Applied Biosystems 7500 Real-Time PCR System and the results interpreted in Biogazelle qbase+ software by the ΔΔCt method, using *groEL2* and *sigA* as reference genes. **Sequencing:** It was sequenced the RIF resistance-determining region (RRDR) of the *rpoβ* gene for each isolate using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®) and the runs were performed in 3130 Genetic Analyzer (Applied Biosystems®). **Results:** It was found alterations in RRDR amino acid sequence in 37 of 45 clinical isolates (82.22%). The most frequent mutations were Ser531Leu (25/37 – 67.56%) followed by mutations in the position 526, in which the amino acid Histidine was changed by Tyrosine (4/37 – 10.81%), Asparagine (1/37 – 2.70%), Arginine (1/37 – 2.70%), Cysteine (1/37 – 2.70%) and Proline (1/37 – 2.70%). The mutations Asp516Val, Asp516Phe, Lys527Gln were also found. Regarding the expression profiles of efflux pump genes by RT-qPCR it was observed most commonly an overexpression of *Rv1258c* gene after treatment in the strains CF93 (1.57±0.11), CF104 (2.58±0.17) and CF102 (5.24±0.48). In a general way CF104 was the isolate with efflux pumps genes more expressed, presenting besides *Rv1258c* mentioned above, an overexpression of *Rv1218c* (2,65±0.13) and more significantly *Rv1217c* (6.30±0.46), which may explain its high resistance profile. **Conclusion:** The results strengthen the role of efflux pumps in determining antimicrobial resistance for Brazilian clinical isolates of *Mycobacterium tuberculosis* and corroborate the literature worldwide, reinforcing the idea that the development of molecules able to inhibit efflux pumps (E.P.I.´s) are a powerful strategy for the treatment of TB, preventing the spread of resistance. Furthermore, the mutations present in RRDR of Brazilian Clinical Isolates have already been registered in other countries.

Keywords: *Mycobacterium tuberculosis*; Minimal Inhibitory Concentration (MIC); sequencing; efflux pumps; RT-qPCR.

Evaluation of Multidrug Resistance and Antimicrobial Sensitivity Pattern at Kaduna Tertiary Care Hospital, Kaduna State, Nigeria

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Introduction: Challenge associated with health care associated infection of multidrug organism in particular Extended spectrum beta-lactamase producing organisms (ESBLs) and methicillin resistant Staphylococcus aureus (MRSA) have been increasing world-wide and pose significant challenges to infection control. Antimicrobial evaluation is necessary step for monitoring and development of policies to limit its overuse/misuse.

Objective: The aim was to evaluate isolates of ESBLs and MRSA, and to determine their antimicrobial agents' sensitivity pattern

Methodology: A hospital based and descriptive cross sectional study conducted and isolates were recovered from various sample sites by culturing on standard media and identified by gram staining reaction and standard biochemical test. Antibiotic sensitivity tests were performed by the disc diffusion technique and results were read and interpreted.

Results: Ninety four (29%) of 324 total isolated gram-negative bacilli were positive in terms of ESBL production with *Klebsiella pneumoniae* comprising 43 cases (13%) as the dominant and most ESBL producing organisms were found in urine samples (45 cases; 48%). Majority of the isolates were susceptible to Ciprofloxacin (76%). 256 isolates of *S. aureus* were examined of which 120 (47%) were resistant to methicillin. 78% of the MRSA were from in-patients while 12% were from out-patients with significant difference ($p < 0.05$) and highest rate of 68% in surgical wound. 66% of the MRSA were sensitive to Ofloxacilin.

Conclusion: Generally effective control measures aimed at reducing the pool of antibiotic-resistant organisms are inadequate and all effort must be made for an establishment of antimicrobial policy for successful implementation infection control practices

Keywords: Antibiotic; resistance; methicillin; *staphylococcus aureus;* beta-lactamase

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Evaluation of the antimicrobial activity of colloidal silver-hydrogen peroxide against model cooling tower biofilm

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Biofouling is a major problem in cooling tower systems which are an integral component of many industrial processes. The chemical and physical conditions of cooling water systems provide an ideal environment for microbial growth and biofilm formation. In addition to the accumulation and dissemination of pathogenic organisms, especially *Legionella pneumophila*; biofilms also lead to many undesired conditions such as equipment damage through corrosion, decreased energy efficiency, local blocking of cooling towers and increased heat transfer resistance. The conduction of accurate/effective biocide usage in parallel with the microbiological analysis will be beneficial for diminishing economic losses, operational and public health problems.

Recommended biocides/dosages for decontamination of industrial systems by manufacturers may not be suitable for each system and target microorganisms. To establish a successful biocide programme, the antimicrobial activity of biocides should be evaluated against both planktonic and sessile bacteria under *in vitro* and *in situ* conditions. Although colloidal silver-hydrogen peroxide is recommended for disinfecting cooling towers, there is a lack of published data about the efficacy of this compound against both planktonic and sessile populations of cooling tower. Effectiveness of the recommended dosages (100 mg/L and 200 mg/L) of biocide at different contact times were investigated against both planktonic and biofilm bacteria (on stainless steel (SS), glass (G), polyvinyl chloride (PVC) slides) monthly during the 6 months experiment period.

Model cooling tower system was experimentally infected by *L. pneumophila* ATCC 33152 and has been run to the nearest operating conditions of full-scale system for six months. Each month, samples were analyzed in terms of the heterotrophic plate count (HPC), the presence of *Legionella* spp., the epifluorescence microscopy, the total and free ATP concentration.

HPC counts were reduced to zero with the both dosages after 24 h of contact time, at the 6th month on SS and at the 5th month on G and PVC slides. On the other hand, the significant increase in the respiration activity (values are > %98) was determined. This indicates that fluorescent staining is superior to the conventional plate count for the biocide efficacy evaluation.

The number of planktonic *L. pneumophila* was reduced to zero after 24 hours contact time with 200 mg/L biocide from the 1st month and 100 mg/L biocide from the 2nd month. The same reduction has been achieved at time point zero with 200 mg/L from the 3rd month and 100 mg/L biocide from the 5th month.

The number of sessile *L. pneumophila* with the both tested biocide dosages was reduced to zero after 24 hours contact time from the 2nd month; after 1 hour contact time from the 5th month, on all slides.

In total and free ATP values, significant reduction ($p < 0.05$) was detected compared to control in both planktonic and biofilm samples and values were varied according to months.

A considerable variation was found in the response of biofilm to the biocide when biofilm reached the mature phase (from the 4th month). The reduction to zero in HPC values, from the 2nd month in planktonic phase and from the 5th month in biofilm phase, has been interpreted as induction of the viable but non-culturable form (VBNC) state in bacteria because of the increased resistance mechanisms of biofilm and cell exchange between planktonic and biofilm phases. The results of the study emphasized that biocide should be applied on a regular basis prior to the settlement of pollution in the system and microbial burden monitoring/data evaluation should be done periodically by using different microbiological techniques for the proper evaluation of the system.

Key words: Biofilm; cooling tower; heterotrophic plate count; *Legionella pneumophila*

Genome-wide discovery of leishmanial drug-resistance genes by Cos-seq

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Leishmaniasis is a significant health problem that continues to have a major impact on much of the world's population. An estimated 12 million people worldwide suffer from leishmaniasis and 1.5 to 2 million new cases of this disease arise each year. Increasing drug resistance towards first line antimony-derived compounds forced the introduction of novel therapies in leishmaniasis endemic areas including amphotericin B, paromomycin, pentamidine and miltefosine. However, their use is threatened by the emergence and spread of drug-resistant strains. Thus, in-depth knowledge of resistance mechanisms will help to design new therapeutic strategies and to overcome treatment failure. We have developed a novel functional-genomics approach for the deciphering of underlying resistance mechanisms, which relies on cosmid functional complementation coupled to next-generation sequencing (Cos-seq).

To this aim, we generated a *L. infantum* cosmid library by partial digestion of gDNA by SAU3AI and later ligation into the cLHYG vector, followed by transfection in wild-type *L. infantum* parasites. The whole cosmid-transfected population was submitted to increasing selection pressure to the model drug methotrexate and independently to the main five anti-leishmanial drugs (antimonials, miltefosine, paromomycin, amphotericin B and pentamidine). For each selection step, cosmids were isolated and subsequently subjected to next-generation sequencing. Resulting-data analyses were performed using the Trinity software package, leading to the identification of genes that are selected for low- and high-resistant parasites.

As expected, genes encoding DHFR-TS and PTR1 were identified for model-drug methotrexate. In addition, a novel candidate-cosmid encoding for a phosphatase 2C-like protein was found to produce resistance to this drug. Antimony Cos-Seq study revealed one cosmid encoding the ABC protein MRPA as well as two Sb-resistance cosmids covering novel resistance genes located on chromosomes 12 and 34, respectively. Moreover, paromomycin selection led to the identification and isolation of seven resistance-related cosmids, one of which contains several ATP-binding cassette proteins and was highly enriched during the selection. Amphotericin B treatment showed the importance of genes involved in ergosterol metabolism, phosphorylation and methylation reactions during the selection of resistance against this drug. Pentamidine Cos-Seq study revealed the importance of a small cluster of genes that includes cosmids encoding a putative cell cycle-associated protein MOB1. Moreover, we identified the already-known pentamidine-resistance protein 1, but this demonstrated to be enriched only at the first steps of selection. Lastly, analyses after miltefosine selection showed the importance of several cosmids including genes related to the metabolism of lipids, as a putative phospholipid-translocating P-type ATPase flippase or a C-8 sterol isomerase-like protein.

Whole-genome resistance studies are now achievable in *Leishmania* and have huge potential use to decipher novel resistance genes as well as to develop new therapeutic strategies for preventing the evolution of anti-leishmanial resistance. Here we present the Cos-seq as a powerful strategy able to explore in an extensive way the whole genome of *Leishmania* and identify (both qualitatively and quantitatively) those genes responsible for the survival of the resistant population during drug selection. Furthermore, our study regarding the five main leishmanicidal drugs shows how this strategy could be used for any novel leishmanicidal compound or extended to other infectious agents.

Keywords: Drug resistance; Next-generation sequencing; Functional genomics; Leishmaniasis; Antileishmanial drugs

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Identification of Thioridazine resistance inducing mutations in *Staphylococcus aureus*

Lund CL, Thorsing M, Lauritzen SP, Kallipolitis BH, Kolmos HJ, Klitgaard JK

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a growing problem in the healthcare setting, resulting in an urgent demand for new and effective treatments. Thioridazine (TDZ) is a potential candidate drug, with a bactericidal effect at high concentrations and a synergistic effect in combination with β-lactam antibiotics. It is postulated that TDZ causes it's effect by intercalating in the cytoplasmic membrane, thereby disturbing membrane- and cell wall related processes. The precise target of TDZ though, is still unknown.

Unpublished work from our research group has shown that *S. aureus* is able to develop resistance towards TDZ. In this study we wanted to investigate, whether resistance towards TDZ leads to a loss of synergy between TDZ and β-lactam antibiotics and what genetic changes occur when *S. aureus* becomes TDZ resistant.

Viability assays and whole-genome sequencing (WGS) were conducted on a set of TDZ-resistant strains of *S. aureus* USA300. Mutations identified through WGS were reproduced by gene knockout in the wildtype strain and further viability assays were conducted with the deletion mutants. Our results indicate that TDZ-resistance leads to a loss of synergy between TDZ and β-lactam antibiotics. Through WGS 11 mutations in nine different genes were identified, where of most were either cell-wall or cytoplasmic membrane related. A knockout mutant of the cardiolipin synthase gene exhibited reduced susceptibility towards TDZ, indicating that cardiolipin may play a major role in TDZ's bactericidal effect.

Increasing resistance to β-lactams associated to hyperproduction TEM-1 β-lactamase in *Haemophilus influenzae*

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Background: Ampicillin resistance by TEM-1 β-lactamase production is common in *H. influenzae* and is associated with different bla_{TEM} genes that differ in the regulatory region. Currently, three different promoters are known; Pa/Pb and Pdel promoters have been associated with ampicillin MIC higher than P3 promoter, however has not been demonstrated if these promoters induce at different levels bla_{TEM} expression yet. To demonstrate this, quantitative Real-Time PCR was performed to evaluate if the expression of bla_{TEM} is related to the presence of any of three promoters and its relation to ampicillin resistance.

Methods: Four strains of *H. influenzae* were studied: ATCC33930, BUAPNan (CSF isolated), BUAP28 and BUAP172178 (carriers isolated). The resistance profile to β-lactams was determined by MIC (CLSI 2013). PCR analysis and sequencing was performed to detect bla_{TEM} gene and its promoter region. The bla_{TEM} expression was measured by qPCR from strains grown in media with and without ampicillin. The *16S* gene was used to normalize the relative expression of bla_{TEM}; and the efficiencies of amplification of both genes, were used for the accurate quantification method.

Results: All strains were resistant to ampicillin and two to amoxicillin-clavulanic acid (table 1). The $bla_{TEM\text{-}1}$ complete gene was identified and none mutations were detected. P3, Pdel and Pa/Pb promoters were found (table 1). Isolates with high ampicillin MIC and Pdel or Pa/Pb promoters had significantly higher bla_{TEM} expression than the strain with low ampicillin MIC and P3 promoter.

Table 1. Features of *H. influenza* strains

Strain	Serotype	Phenotype type	Promoter	MIC (µg/ml) Ampicillin	MIC (µg/ml) Amoxicillin-Clavulanic Acid
33930	Hib	BLPAR	P3	8	-
BUAP28	Hib	BLPAR	Pdel	128	-
BUAPNan	Hib	BLPACR	Pdel	64	32/16
BUAP172178	Hib	BLPACR	Pa/Pb	128	16/8

Conclusions: The high-level bla_{TEM} expression in *H. influenzae* could be the cause of the increased ampicillin MIC values, and to other antibiotics (CTX, CAZ, FEP, data no shown), and these increases are mainly due to the expression from Pa/Pb or Pdel promoters. The ampicillin stimulates the overexpression of the bla_{TEM} gene in this isolates.

Keywords: *Haemophilus influenzae*; bla_{TEM}; promoter

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Large-scale differential selection analysis on influenza A and B neuraminidase gene: a new approach for studying antiviral drug resistance and reduced susceptibility

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Antivirals play an important role in the prevention and treatment of influenza infections, particularly in high-risk or severely ill patients. The potential emergence of resistant viruses is continually threatening their use and the currently limited repertoire of influenza antivirals makes resistance an even more serious public health threat. Neuraminidase inhibitors (NAIs) oseltamivir and zanamivir are presently the only effective antivirals available at worldwide level for influenza management, targeting neuraminidase (NA) protein that is under both antiviral and antibody selective pressure (SP).

This study aims to investigate the SP acting on the NA of seasonal (A(H3N2), A(H1N1), B-Yamagata, B-Victoria) and pandemic (A(H1N1)pdm09) influenza viruses, comprising two main objectives: (1) to evaluate the contribution of positive SP (PSP) for the emergence of NAIs resistance (R) or reduced susceptibility (RS); and (2) to determine the impact of NAIs introduction into clinical practice and of NAIs wide use during A(H1N1) 2009 pandemic on the SP acting on influenza NA.

Large datasets of NA sequences were constructed and treated for each influenza (sub)type/lineage, using sequences retrieved from GISAID and NCBI Databases and from viruses circulating in Portugal. Seasonal datasets comprised a total dataset and 3 temporal sub-datasets: (1) before NAIs clinic introduction (1999); (2) before pandemic oseltamivir wide use (2009); and (3) from 2009 to date. Regarding A(H1N1)pdm09 lineage, a total dataset and 2 sub-datasets defined by the end of the pandemic period (10th August 2010) were studied. Maximum-likelihood phylogenetic trees were inferred using PhyML 3.0 in SeaView, after determining the best-fit model in jModelTest. SP analyses were performed in HyPhy, including estimation of global dN/dS, estimation of site-specific dN/dS and identification of positively selected sites by SLAC and FEL methods, and differential selection analyses according time, using a significance level of ≤ 0.05.

Preliminary analysis of the results already obtained revealed PSP on position 275 (R oseltamivir) of A(H1N1) seasonal NA after 1999 but not from 2009 onwards. The widespread emergence of H275Y viruses since 2007 may be interfering in these results. The site 151 (RS oseltamivir and zanamivir) of A(H3N2) NA showed to be under PSP since 1999. A(H1N1)pdm09 post-pandemic period results cause some concern since PSP was found on positions 247 (RS oseltamivir) and 275. The remaining SP results are now being obtained and a more detailed and complete analysis will then be conducted.

Keywords: selective pressure; antiviral drug resistance

MAS NMR study of interaction of antimicrobial peptide dendrimers with phospholipids

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Crucial for the activity of many antimicrobial peptides is their interaction with the lipid membrane of the target cell. To characterize the interaction between antimicrobial peptide dendrimers and model membranes we used solid state NMR spectroscopy. Dendrimers with a lysine core were rich in Trp residues and carried 6 to 8 positive charges situated at the primary and ternary amine groups. These chemical properties give rise to a very low minimal inhibitory concentration (MIC) against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. Moreover, several dendrimers were characterized by a low hemotoxicity.

We were working with three phospholipids: the zwitterionic DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) and the anionic DMPG (1,2-dimyristoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol). Using 2D ^{1}H-^{1}H NOESY and ^{1}H-^{19}F HOESY MAS experiments we determined the location of the dendrimers in the membrane. Our results indicate that dendrimers interact with both hydrophobic and hydrophilic part of the bilayer. We observed strong cross-peaks between the aromatic protons of the rings and the acyl chain protons of the phospholipids. The cross-peaks between the lipid tails and the aromatic rings of dendrimers are more intense than those of between the lipid head group and the dendrimers. This indicates that the dendrimers are located closer to the interior of the bilayer than to the glycerol backbone.

We acknowledge financial support from the National Science Centre in Poland, project FUGA-2 2013/08/S/ST4/00558.

Keywords: NMR spectroscopy; peptide-phospholipid interaction; antimicrobial peptide dendrimers

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Mechanisms of *Brevibacillus laterosporus* B4 induced plant growth promotion and systematic resistance to bacterial brown strip of rice

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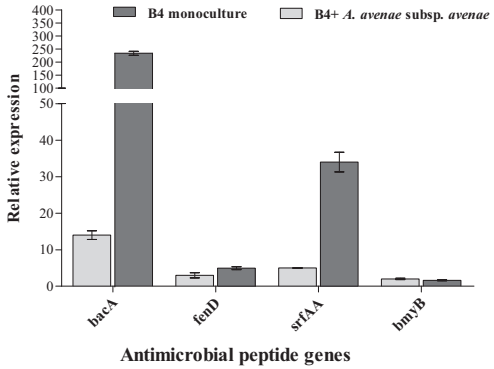
Brevibacillus laterosporus B4, a first biocontrol agent for *Acidovorax avenae* subsp. *avenae*, isolated from rice rhizosphere soil in Baluchistan, was tested for its ability to induce resistance against bacterial brown stripe of rice and determine its mechanisms. Laboratory assays indicated that B4 and its culture filtrates (70 %; v/v) exhibited similar inhibitory effects compared to chitosan (5 mg/ml), a biochemical bactericide (chitosan) used as positive control. However, the culture suspension of B4 induced systemic resistance as expressed by greater response (72%) for controlling brown stripe in vivo than chitosan (56 %). Bacterization of rice seeds for 24 h significantly promoted the rice plant growth by increasing plant heights and fresh weights by 28.31% and 52% (GPE) respectively. We also observed that the expression of genes for biofilm formation, motility, niche adaptation, membrane functionality and virulence of *A. avenae* subsp. *avenae* were significantly down-regulated, when recovered from B4-treated seedlings under greenhouse. *In vitro* search of mechanisms revealed that culture filtrates (70 %; v/v) of B4 suppressed the biofilm and severely disrupted cell membrane integrity of *A. avenae* subsp. *avenae*, which resulted into the leakage of intracellular substances. These culture-free supernatants of B4 were found to be of proteinious nature, as shown by ammonium sulfate precipitation and subsequent treatment with protease. Multiplex PCR assay confirmed that B4 is harbouring four antimicrobial peptide biosynthetic gene markers *srfAA*, *fenD*, *bmyB* and *bacA* in its genome. During co-culture with *A. avenae* subsp. *avenae,* the expression of antimicrobial peptide genes for surfactin and bacylisin were highly up-regulated in B4, quantified by qRT-PCR. Further characterization of strain B4 revealed its strong capability for biofilm formation, inorganic phosphate solubilization and production of high amounts of Indole-3 acetic acid, siderophores and ammonia *in vitro.* A positive correlation was observed among the phosphate solubilization, IAA contents of B4, and subsequent plant growth. Likewise, the results obtained from this study also indicated that *Brev. laterosporus* B4 may produce antimicrobial peptides inculding iturin and bacylisin, which destroyed the biofilm and cell membrane integrity of the pathogen, which helped in the control of bacterial brown stripe of rice *in vivo*.

Keywords: antimicrobial peptides; bacterial brown stripe; biofilm formation; gene expression

A

B



A. Amplification products obtained with PCR specific primers designed to detect antimicrobial peptide biosynthetic genes in *Brev. Laterosporus* B4. Lane M: 2,000 bp marker (TaKaRa).

B. Result of qRT-PCR showing differential expression of four antimicrobial biosynthetic genes in *Brev.laterosporus* B4 during monoculture and interaction with *A. avenae* subsp. *avenae.*

Multidrug-resistance transference in *Escherichia coli* isolates of food samples in Mexico

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Background: Transmission of ESBL-producing *Enterobacteriaceae* from person to person has been demonstrated in hospital and community. However, there is an increasing prevalence of ESBL/pAmpC producing *E. coli* isolated from food-producing animals providing evidence to recognize farm animals as carriers of these microorganisms and their possible role as reservoirs in the spread of strains harboring transmissible resistance genes by plasmids. To obtain a partial molecular characterization of plasmid-mediated antibiotic resistance genes, *Escherichia coli* isolates from foods collected in México, were analyzed.

Methods: 46 *E. coli* strains were obtained from foods (10 from pigs/36 from chickens) in different markets in Puebla, Mex. Resistance profile was made by disk diffusion method. ESBL/pAmpC genes (CTX-M, OXA, TEM, CMY); *int1, int2, qac, sul1,* as well as *clmA, sul3, tetA* and *tetB* were investigated by PCR and sequencing. Phylogenetic group and ST were also determined. Seven isolates were chosen in basis to their resistotype to describe the mechanism of genetic transference. Plasmid profile and conjugation assays were made. Transconjugants analysis included antibiotic resistance and plasmid profile. Plasmid incompatibility groups were detected by PBRT and sequencing.

Results: Isolates chosen belonged to the phylogenetic groups B1 or B2 (except one strain) and were typed as ST1266, ST155 (ST155C), ST10 (ST10C), ST359, ST617, ST501 and ST12 (ST12C), and presented multidrug resistant phenotype. They also harbored ESBL/pAmpC genes (CTX-M-1, CTX-M-2 and CMY-2) and *int1, qac, sul, cmlA, sul3, tetA* and *tetB* were detected in six of seven isolates and transconjugants. The multidrug resistance transference was mainly driven by IncF plasmids, but IncI1, IncN, InccolE plasmids were also detected in six isolates and five transconjugants.

Conclusion: IncF conjugative plasmids are dominant in *E. coli* isolates from foods and transfer multiple antimicrobial resistance genes generally contained in class 1 integrons. However, IncN plasmids were also detected in CTX-M-1 and CTX-M-2 producing strains. Currently, this study represents the first analysis of plasmids in *E. coli* isolated from foods in Mexico.

Keywords: Food of animal origin; plasmid; incompatibility group

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Multiple mechanisms of carbapenem resistance in Enterobacteriaceae bloodstream isolates: a molecular study in an Indian hospital

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Background & Objectives: Recent reports of emerging resistance to carbapenems (ertapenem, meropenem, imipenem) and widespread outbreaks of carbapenem resistant Enterobacteriaceae (CRE) is a cause of concern. Mechanisms of resistance have been attributed to porin loss in combination with an AmpC type enzyme or extended-spectrum beta-lactamase (ESBL), increaed efflux activity or acquisition of carbapenemases, both metallo-beta-lactamases (MBLs) such as NDM, IMP or VIM, and serine non-metallo-carbapenemases (NMCs) of the IMI/NMC, SME, OXA or KPC families [1]. The objective of the present study was to elucidate the frequency of carbapenemase production, to determine the role of ESBL and AmpC, and to characterize the molecular profile of carbapenemases in CRE bloodstream isolates in an Indian tertiary care hospital.

Material & Methods: The study was conducted on consecutive non-duplicate isolates of *Escherichia coli* and *Klebsiella pneumoniae* from January 2011 to December 2013. Antimicrobial susceptibility testing and phenotypic characteization of ESBL and AmpC was performed by standard CLSI and AmpC disk test respectively. Isolates with ertapenem minimum inhibitory concentration (MIC) > 0.5µg/ml (Etest) were defined as CRE and were screened for serine-carbapenemase production by the modified Hodge test (MHT) as per CLSI and MBL- production by imipenem/ imipenem-EDTA Etest. Further, all CRE isolates were subjected to PCR for the following carbapenemase genes $bla_{\text{NDM-1}}$, bla_{IMP}, bla_{VIM}, bla_{KPC}, $bla_{\text{OXA-48}}$, and $bla_{\text{OXA-181}}$ using previously published primers and cycling conditions [2,3]. Data was analysed using WHO Net software.

Results: During the study period, 387 isolates were recovered (214 *K. pneumoniae* and 173 *E.coli*). One hundred eighty one were from the paediatric, 97 from intensive care unit, 60 from medical, 36 from surgical and 12 were from burn units respectively. ESBL and AmpC production was observed in 191 (49.3%) and 210 (54.2%) isolates respectively. A total of 92 (23.7%) isolates were CRE, i.e., ertapenem resistant. Of these, 66 (70.9%) and 52 (55.9%) were resistant to meropenem and imipenem respectively. None of the isolates were positive for the MHT; 62 (67.3%) isolates were MBL-positive. PCR analysis confirmed the presence of carbapenemase genes in 71 (77.1%; 45 *K. pneumoniae* & 26 *E.coli*) isolates. Twenty seven isolates (29.3%) showed presence of multiple carbapenemase genes. The highest frequency was that of $bla_{\text{NDM-1}}$ (59, 64.1%), followed by $bla_{\text{OXA-48}}$ (23, 25.0%), $bla_{\text{OXA-181}}$ (21, 22.8%), bla_{VIM} (6, 0.06%) and bla_{KPC} (2, 0.02%). Of the 21 carbapenemase- negative CRE isolates, 16 were found to be ESBL and /or AmpC producers which may explain the observed resistance to carbapenems in association with porin deficiency.

Conclusion: A considerable proportion of carbapenem resistance in CRE is mediated by carbapenem-hydrolyzing enzymes, especially NDM in our set-up. Non-carbapenemse mediated resistance may also contribute to the same. Since phenotypic tests may be inadequate to detect these mechanisms, molecular testing should be performed on all CRE to determine the mechanism of resistance for optimum therapeutic benefit and controlling further dissemination of these isolates.

Keywords: Antibiotic resistance; Carbapenemase; Enterobacteriaceae; Metallo-beta-lactamse

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New insights into the mechanistic function of the antifungal protein PAF: the link between cAMP/PKA signalling, lipid biosynthesis and calcium homeostasis

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By now only a very limited number of licensed drugs are available to prevent and combat fungal infection. The generation of new antifungal compounds encounters major obstacles because host and invading fungal organisms show high cellular, physiological and metabolic similarities. Therefore, new cost effective strategies and novel antifungal drugs that attack unique cellular factors of fungi but lack severe side effects for the infected host are urgently needed. Antifungal proteins from filamentous Ascomycetes are promising candidates in this respect.

We have been focussing our research on the antifungal protein PAF, which is produced by the easy-to-handle filamentous Ascomycete *Penicillium chrysogenum*. PAF is certainly one of the best-studied antifungal proteins within this group: the cysteine-rich, cationic protein (i) exhibits a narrow and defined antifungal activity against opportunistic human-, animal-, and plant-pathogenic fungi [1], (ii) shows no toxic effects on mammalian cells *in vivo* [2], (iii) interacts synergistically with other antifungal drugs [3], (iv) is highly stabile [4], and (v) has low production costs. Undoubtedly, these features render PAF a highly interesting compound for biotechnological production and open the possibility to develop new antifungal strategies in the field of medical treatment, pest control and preservation.

Our specific aim is the identification of fungal determinants for PAF susceptibility to gain a better insight into the mode of action. We reported previously that PAF toxicity is mediated by the ionic strength of the medium (e.g. Ca^{2+} ion concentration) and by a sustained increase of the intracellular Ca^{2+} concentration [5], as well as by the activation of the cAMP/protein kinase A (PKA) signalling pathway [6] in sensitive target fungi. The cAMP-dependent PKA, consisting of a catalytic PkaC and a regulatory PkaR subunit, is a central player in the response to environmental stimuli and regulates for example hyphal tip growth, lipid metabolism and programmed cell death [6,7].

In the present study we investigated in more detail the role of this specific signalling pathway in the toxicity of PAF and monitored the fungal growth and the intracellular Ca^{2+} ion concentration in response to PAF exposure. We selected *Aspergillus niger* mutants with deregulated PKA activity that express the recombinant Ca^{2+}-sensitive photoprotein aequorin [8]. The *A. niger* PKA mutants were previously characterized to have defects in the lipid biosynthesis: PKA mutants that lack the catalytic subunit exhibited an increased content of neutral lipids (+100%) and a decreased content of phospholipids (-30%) compared to the wild-type strain. In contrast, PKA mutants over-expressing the catalytic subunit had a similar lipid composition as the wild-type strain [7]. Our experiments showed that over-expression of the catalytic subunit of PKA in the mutant mc*pkaRC* resulted in slightly reduced PAF susceptibility and no/only minor effects on the intracellular Ca^{2+} response compared to the wild-type strain. In contrast, the mutant lacking the catalytic subunit Δ*pkaC* was resistant towards PAF and suffered from a disturbed Ca^{2+} signalling response compared to the control. We report here for the first time that cAMP/PKA signalling and lipid biosynthesis are closely linked with Ca^{2+} homeostasis in the PAF-sensitive model fungus *A. niger.* Furthermore, we conclude that the lipid composition of the plasma membrane and/or the signalling function of phospholipids play a central role in PAF toxicity and determine the susceptibility of target fungi to this antifungal protein.

Keywords: antifungal protein PAF; cAMP/PKA signalling; lipid biosynthesis; Ca^{2+} homeostasis

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Non-antibiotic drugs and their potentiality in reversal of multidrug resistance in microorganisms

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Non-antibiotics are classed as therapeutic agents not originally designed for antibacterial or chemotherapeutic purposes, but subsequently exhibited such properties quite distinctly.

Subinhibitory combination of non-antibiotics e.g. the appropriate isomers of neurotropes the depressant (DPR), phenothiazines and related thioxanthenes , the antidepressants (ADPR) dibenzepin, phenylpiperidines, and antihistamines with low tropism and toxicity alone and in combination with natural and synthetic antimicrobials have the promises for early, quite new clinical therapeutic possibilities in severe infections and cancer diseases. We have especially investigated these antimicrobial possibilities in, in vitro and in vivo on clinical sensitive and resistant strains of *S. aureus*, *S. epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Enterococcus faecium* and *E. faecalis*, typical and atypical mycobacteria, pseudomonas and salmonella strains since the late 1970`s. Of the non-antibiotics the antihistamine promazine, and the (DPR), trans(E) - chlorprothixen, trans (E) - clopenthixol, trans (E)-flupenthixol, L-thioridazine (JEK47), D- thioridazine, L,D-thioridazine, and isomers of phenylpiperidines NNC 20-4962 (isomer of femoxitine) and NNC 20-7052 (isomer of paroxetine) were potent antimicrobials with the least neurotropic activity, pointing to a possible general isomeric structure-activity relationship. Moreover, these isomers have considerably reduced, and in some cases absent, neurotropism and reduced mammalian toxicity.

Combinations with selected non-antibiotics and classical antibiotics may alleviate the concerns about adverse effects and therapeutic safety especially in the treatment of MDR and XMDR infected patients in life-threatening situations such as MRSA and XMDR Mycobacterium tuberculosis where the non-antibiotic dosage would be in the lower, non-toxic dosage ranges and in general could be given in a shorter period than when used for treatments of individuals with mental health diseases.

Novel bis-benzimidazole exhibits selective inhibition of *E. coli* topoisomerase IA through metal chelation based mechanism: A way to overcome multi-resistant strains

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DNA topoisomerases are vital ubiquitous enzymes that control the torsional state of DNA via the concerted cleavage and religation of DNA strands. The ever increasing antibiotic resistance in pathological bacteria is a major clinical challenge now days. Target mediated mutations, alteration in metabolic pathway and reduced drug accumulation due to decreased drug permeability or activated efflux pump cumulated antibiotic resistance crisis. Type II topoisomerase is a well-known drug target and there are increasing evidences of resistance against these drugs due to mutations in gyrase and/or topoisomerase IV. Whereas, topoisomerase IA though a very good target but is less explored and drugs targeting this enzyme are not known till date. Thus, there is a need to exploit the know-how of existing targets to unveil novel mechanism and to search for new drug targets based antibiotic/drug discovery. In the present study, we tried to develop antibacterial agent that targets topoisomerase IA. Our study demonstrates, a novel bisbenzimidazole, PPEF, as preferential *E. coli* topoisomerase IA inhibitor. PPEF had most significant inhibitory effect against *E. coli* topoisomerase IA among the series of compounds used in the study, with IC_{50} = 2± 0.05 μM. PPEF has also shown lowest MIC against most of the clinical, pathogenic, and resistant *E. coli* strains among the 24 compounds evaluated. Our studies exhibits that these compounds act as poison inhibitor and Mg^{2+} chelation as a probable mechanism of inhibition. Further, these compounds did not show inhibition of *E. coli* DNA gyrase and human topoisomerase IB up to 100 μM. Binding affinity constant of PPEF with *E. coli* topoisomerase IA and human topoisomerase IB was determined to be 6.8 x 10^6 M^{-1} and 1 x 10^5 M^{-1} respectively from isothermal titration calorimetry which clearly exhibit better interaction of PPEF with *E. coli* topoisomerase IA. Docking and modeling study showed PPEF interacts better particularly at the catalytic residues near the acidic triad of the enzyme in *E. coli* topoisomerase IA-ds DNA complex over human topoisomerase IB-ds DNA. *In vivo* mouse systemic infection and neutropenic thigh model experimental results confirmed the therapeutic efficacy of PPEF.

In conclusion, PPEF is positively charged molecule, significantly increased positive electrostatic potential at the active site in *E. coli* topoisomerase IA enzyme affecting the Mg^{2+} affinity of the ternary complex required for complete activity. This study illuminates new properties of bis-benzimidazole to develop it as an efficient anti-bacterial agent targeting topoisomerase IA and giving us a hope that this mechanistic based development of new chemical entities as drug may overcome antibiotic resistance.

Key words: Bisbenzimidazoles, *E. coli* topoisomerase, Antibacterial agent.

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Novel $bla_{CTX\text{-}M\text{-}2}$-type gene coding extended spectrum beta-lactamase CTX-M-115 discovered in nosocomial *Acinetobacter baumannii* isolates in Russia

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Nosocomial infections are one of the most important problems of modern healthcare worldwide, one of the leading agents of these infections in the intensive care unit (ICU) is *Acinetobacter baumannii*. This pathogen is often characterized by multi- (MDR), extreme- (XDR) or pan-resistance (PDR) to antimicrobials [1]. Major mechanism of the resistance to beta-lactams is producing of OXA-type beta-lactamases and extended spectrum beta-lactamases (ESBLs) including CTX-M-type ESBLs [2].

This paper describes a new variant of $bla_{CTX\text{-}M\text{-}2}$-like gene detected in two clinical isolates of nosocomial *A. baumannii* (n=59) collected from the patients with mechanical ventilation in neurosurgical ICU in Moscow in 2012-2014. Isolates of the collection have been collected from the respiratory system (75%), nervous system (10%), urine (8%), and blood (7%). These isolates were resistant to amoxicillin/clavulanate, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin and nitrofurantoin (100%); to cefepime and chloramphenicol (98%); to meropenem (96%); to imipenem (93%); to amikacin (86%); to gentamicin, trimethoprim and co-trimoxazole (82%); to ampicillin/sulbactam (73%); to tobramycin (67%); to tetracycline (57%); to cefoperazone/sulbactam (34%); to tigecycline (10%).

The presence of genetic markers of resistance in the genomes of isolates has been shown by PCR: class 1 integrons (54% of isolates) carrying gene cassettes *dfrA17-aadA5* (GenBank: KJ579283) and *aacC1-orfX-orfY-aadA1* (GenBank: KM009103; KM009104; KM009105); class 2 integrons (one isolate), carrying gene cassette *dfrA1-sat2-aadA1* (GenBank: KM009107). Genes of bla_{OXA}-type beta-lactamases have been detected in all isolates: 100% of isolates carry $bla_{OXA\text{-}51}$-like genes (GenBank: KJ187467; KJ187469; KJ187471; KJ187473), 73% - $bla_{OXA\text{-}40}$- like genes (GenBank: KJ187468; KJ187470), and 14% - $bla_{OXA\text{-}23}$- like genes (GenBank: KJ187472; KJ187474); 7% of isolates have genes of TEM-type ESBLs, and 18% of isolates – genes of CTX-M-type ESBLs. Three alleles of $bla_{CTX\text{-}M}$-type genes have been identified by sequencing analysis: $bla_{CTX\text{-}M\text{-}2}$ (GenBank: KJ187478; KM085434), $bla_{CTX\text{-}M\text{-}15}$ (GenBank: KF971880), and novel allele $bla_{CTX\text{-}M\text{-}115}$ submitted to Lahey Clinic database (http://www.lahey.org/Studies/other.asp#table1) and to GenBank (KJ911020; KJ911021).

Sequencing of $bla_{CTX\text{-}M\text{-}115}$ and gene environments has been done using the set of specific primers: ISEcp1U1 [3], CTX-MR1 [4], CTX-M2-K-F1 (5'-tgaaaaatctgacgctgggt-3') и CTX-M2-K-R (5'-gcaagacaagactgaagttcagg-3'). Comparison of the $bla_{CTX\text{-}M\text{-}115}$ gene primary structure with the $bla_{CTX\text{-}M\text{-}2}$ gene primary structure (GenBank: X92507) revealed six nucleotide substitutions: G336T, G357T, T441G, G751A, A835G, and G868A, resulting the three amino acid substitutions in enzyme molecule: Val251Ile, Ile279Val, and Gly290Ser. Similarity search using BLAST software (http://blast.ncbi.nlm.nih.gov) determined that the most similar sequence is *Kluyvera ascorbata* $bla_{kluA\text{-}1}$ gene (GenBank: AJ272538.2), differ from which is one nucleotide substitution G868A, resulting in an amino acid substitution Gly290Ser. It was shown that $bla_{CTX\text{-}M\text{-}115}$ gene is located on the plasmid belonged to IncA/C incompatibility group; his nearest genetic environment includes an upstream the 3'-end of the mobile genetic element ISEcp1 and the 43 bp-intergenic spacer; and a downstream the 59 bp-nucleotide sequence which has 100% identity to the sequences of enterobacterial plasmids adjacent to the $bla_{CTX\text{-}M\text{-}2}$ gene.

So, this study identified a novel $bla_{CTX\text{-}M\text{-}115}$ gene encoding CTX-M-2-type beta-lactamase in two MDR nosocomial *A. baumannii* isolates collected in the neurosurgical ICU in Moscow. This fact is important for the research of molecular mechanisms of antibacterial resistance, evaluation of the epidemiological situation, and the choice of optimal therapy strategies in the future.

Keywords: *Acinetobacter baumannii*; ESBLs; CTX-M-2-type beta-lactamase; $bla_{CTX\text{-}M\text{-}115}$ gene; IncA/C plasmid

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Occurrence of carbapenem resistance bacteria in the East Sea

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Carbapenems are considered drugs of last resort. But, carbapenem resistance (CR) in pathogenic bacteria is becoming rapidly globalized. Emerging resistance genes in pathogens have been shown to be originated likely from environmental bacteria. Thus, to better understand the diversity and evolution of CR genes and predict the emergence of new resistance mechanisms in marine pathogens, it is necessary to investigate CR genes in marine samples. Information on CR in marine environments is limited. A few marine bacterial species including opportunistic pathogens have shown CR. Recently, meropenem-resistant bacteria are reported from coastal water and marine invertebrates. Thus, CR bacteria seem to occur in marine environments. However, systematic investigation has not been made. Here, we isolated carbapenem resistant bacteria in depth-profiles, and concentrated marine bacteria on filters from coastal and offshore stations. The isolates were characterized through determination of 16S rRNA gene. The fraction of carbapenem resistant bacteria in total bacteria ranged from 1.4x to $0.2x10^{-5}$ and tended to decrease with depth. Isolates belonged mostly to families *Vibrionaceae* and *Flavobacteriaceae.* Isolates and extracted DNA will be characterized by molecular detection of carbapenem resistant genes based on multiplex-PCRs and single PCRs. Sequence diversity of carbapenem resistant genes will be presented and discussed.

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Proteomics and functional analysis of outer membrane vesicles from *E. coli*

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Introduction and objectives: Outer membrane vesicles (OMVs) are proteolipids released from bacteria during growth. They are implicated in several biological functions such as cell to cell communication, horizontal gene transfer, nutrient acquisition and defensive functions. The main objective of the study is to characterize the outer membrane vesicles, determine their activity against different antibacterial molecules.

Methods: The role of OMVs from *E .coli* was studied in the presence of different antibacterial molecules using growth curve experiments. Proteomic studies were carried out to identify different functionally significant proteins.

Results and discussion: OMVs to protect both producer bacteria and other bacterial species form the growth inhibitory effects of membrane active antibiotics colistin, melittin. The OMVs of Escherichia coli MG1655 could also protect *Pseudomonas aeruginosa* NCTC6751 and *Acinetobacter radioresistens* MMC5 against these membrane active antibiotics. However, OMVs could not protect any of these bacteria against the other antibiotics such as ciprofloxacin, streptomycin and trimethoprim. Hence, OMVs appears to protect the bacterial community from membrane active antibiotics but not against other antibiotics, which have a different mechanism of actions. E. coli OMVs sequestered the antibiotic colistin, and degraded the antibacterial peptide melittin to protect the bacteria. Thus, the protection of bacteria by OMVs against antibiotics is situation dependent and the protective mechanism differs from each other. The proteomics of OMVs from *E. coli* helped in understanding the functions in detail. The presence of proteases and peptidases in the OMVs are degrading melittin, whereas colistin is sequestered by OMVs as revealed by the uptake studies of a fluorescent probe NPN. These results show that different mechanisms operate to protect the bacteria in different situations.

Conclusions: These studies show that the OMVs are significant players in the innate defense of bacteria. They can protect a mixed population of bacteria from some antibiotics. These results may open up ways to devise sensitization of the bacteria targeting the functioning of OMVs.

Key words: OMVs, antibiotic resistance, bacteria, peptide antibiotics

Rapid Detection of Beta-lactam antibiotic resistance using Liquid Chromatography tandem Mass Spectrometry

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Beta-lactam antibiotics are most widely used but resistance is one of the most serious health threats. Beta-lactamses are major source of resistance. The resistance mostly involves the chemical modification of an antibiotic to an inactive form by the enzyme expressed of the bacterium. The modification causes structure change and characteristic mass shift of the chemical. Using the mechanism, we developed new liquid chromatography-mass spectrometry based quantitation method for Beta-lactams antibiotic susceptibility testing. Penicillin, ampicillin, amoxicillin and its penicillonic acids susceptibility were analyzed by the method for *Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium.* This developed method may contribute to accelerated and reliable susceptibility for antibiotic resistance.

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Resistome of a multiresistant clinical isolate of *Salmonella enterica* ser. Typhimurium (*S.* Typhimurium) from Uruguay

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Background:
Multiresistant *S.* Typhimurium isolates are an increasing problem in South America; nevertheless, the mechanisms accounting for such resistance are rarely well defined.

The aim of this work was to characterize the mechanisms of antibiotic resistance in an intestinal isolate of *S.* Typhimurium obtained in the year 2012, from an elderly outpatient in Uruguay.

Methods:
Bacterial identification and antibiotic susceptibility were performed with the VITEK2® system. The susceptibility profile was widened by disk diffusion assay; results were interpreted according to CLSI guidelines. Serotyping was conducted at Centro Nacional de *Salmonella* (CNS). Complete genomes were sequenced as 50-76 bp paired end runs on the Illumina. High quality reads were selected and de novo assembly was performed with SPAdes Genome Assembler (http://bioinf.spbau.ru/spades). Contigs were analyzed using bioinformatic platforms available at the Center for Genomic Epidemiology http://www.genomicepidemiology.org/, the Wellcome Trust Sanger institute (http://www.sanger.ac.uk/resources/), and the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

Results:
Four plasmids belonging to different incompatibility groups were detected in *S.* Typhimurium strain ST827, namely IncA/C, IncI1, IncX and ColE1. Strain ST827 showed resistance to third generation cephalosporins, conferred by $bla_{\text{CTX-M-8}}$. Resistance to kanamycin, tobramycin and streptomycin was explained by the presence of *aac(6')Iaa*, and *strAB* respectively. The occurrence of *sul2* accounted for resistance to sulfonamides, and resistance to tetracycline was conferred by *tetAR*. Additionally, we detected the presence of *floR* which mediates resistance to chloramphenicol.

Conclusions:
The accumulation of resistance genes encoded in conjugative plasmids in *Salmonella* strains circulating in the community setting calls for active surveillance policies aimed at controlling and reverting this tendency.

Acknowledgements: Dr. Gordon Dougan, the Wellcome Trust Sanger Institute.

Keywords: *Salmonella*; multiresistance; genomics.

Response of *Escherichia coli* O157:H7 against various antimicrobials under the spaceflight analogue

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Background On-board infections have been concerned with the increase of manned space exploration [1]. Since antimicrobials are the primary countermeasure of infectious disease, it is important to determine how bacteria respond against antimicrobials during spaceflight [2].

Objectives The aim of this study was to investigate antimicrobial susceptibility and survival characteristics of *Escherichia coli* O157:H7 ATCC 43889 (human feces isolate) cultured under Low-Shear Modeled Microgravity (LSMMG: spaceflight analogue) and Normal Gravity (NG: Earth condition).

Materials and methods High-Aspect-Ratio Vessel (HARV) and Rotary Cell Culture System (RCCS) were used to generate LSMMG and NG (Figure 1). Antimicrobial susceptibility was tested by disc-diffusion method [3] and survival characteristics were determined by combined treatment of gravity condition with resistant breakpoint of antimicrobials [4] at 37°C up to 96 hr. Tested antimicrobials (n=9) classified by 3 biological effects are as follows: cell wall synthesis inhibitors (ampicillin, cephalothin, and ceftriaxone), protein synthesis inhibitors (chloramphenicol, tetracycline, and gentamycin), and DNA synthesis inhibitors (trimethoprim/sulfamethoxazole, ciprofloxacin, and nalidixic acid).

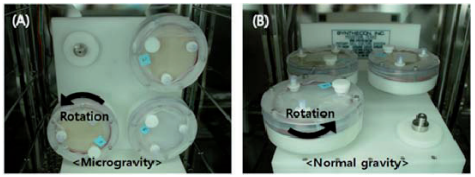

Figure 1. HARV-RCCS apparatus: (A) LSMMG and (B) NG .

Results and discussion Antimicrobial susceptibility of LSMMG cultures showed no difference compared to that of NG cultures to all antimicrobials; however, survival characteristics under the simultaneous conditions of LSMMG or NG with antimicrobials showed the different trends according to the antimicrobial classifications. When treated by cell wall synthesis inhibitors and DNA synthesis inhibitors, survival populations of LSMMG cultures were significantly higher than those of NG cultures ($P < 0.05$). Effects of cephalothin were especially reduced under LSMMG after 96 hr-cultivation that *E. coli* O157:H7 reduced by 1.56 and 4.34 log CFU/ml under LSMMG and NG, respectively. Otherwise, when treated by protein synthesis inhibitors, survival of LSMMG cultures were not significantly different compared to those of NG cultures ($P > 0.05$) except gentamycin treatment for 24 hr. These results represented that LSMMG could adversly affect the efficacy of cell wall and DNA synthesis inhibitors against *E. coli* O157:H7.

Significance and impact This study intends to investigate how *E. coli* O157:H7 responds to the various antimicrobials with different biological effects under the spaceflight analogue and provides new scientific knowledge of antimicrobial effects under the microgravity condition.

Keywords: *Escherichia coli* O157:H7; Spaceflight analogue; Microgravity; Antimicrobial efficacy

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ß-lactamases in *Escherichia coli* isolated from broilers

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Objectives: The aim of present study was to characterize ß-lactamases producing *E. coli* strains isolated from broilers in East Slovakia, particularly the occurrence of their virulent factors, mobile gene elements presence and the plasmid replicon typing.

Methods: Antibiotic susceptibility testing was performed according to Gattringer et al. [1] using the modified microdilution method, towards 20 antimicrobial agents using the VetMIC panel (Bel-MIDITECH, Slovakia) according to M31-A3 CLSI [2]. The PCR method was used for screening of resistance genes such as *blacmy-2, tetA, tetB, qnrS, blaCTX-M, dfrA, dfrB, aadA, sul1, sul2* and integrase genes *Int1, Int2*. The plasmid replicon typing was performed according Carattoli et al. [3] and method described by Delicato et al. [4] was applied for virulent factors detection (*tsh, kpsII, cvaC, iutA, papC, iss* and *ibeA*).

Results: Among fifteen ß-lactamases producing *E. coli* strains, two fluoroquinolone non-susceptible *qnrS*-positive were detected with the following properties: tetracycline resistance with *tetA* gene, cotrimoxazol-streptomycin-resistance and Integrase 1 with gene cassettes *dfrA, aadA, sul1, sul2* and virulence factor *iss*. Second one was tetracycline-resistant, *tetA, tetB* genes with cotrimoxazol-streptomycin-resistance and Integrase 1, the same gene cassettes and virulence factors *tsh, iss, papC, iutA*. Other 13 CMY-2 producing strains were fluoroquinolone- and tetracycline-resistant with *tetA, tetB* genes.

The most dominant plasmid type was *IncY* (15 strains) and *IncI1* (11 strains), which is associated with *blaCMY* genes occurrence (73%).

The most often detected virulence genes were *iss* (13 strains), *iutA* (6 strains), but also *papC, kpsII, tsh*, which is not so alarming, however it is known, that quinolone-resistant *E. coli* are less virulent.

Conclusions: All 15 ß-lactamases producing *E. coli* strains exhibited multiresistance, which was associated with Integron 1 (12 isolates) and gene cassettes carrying. Two of them carried *qnrS* gene with another resistant and virulent attributes, which represent a potential zoonotic health risk caused by horizontal gene transfer. Eleven ß-lactamases producing strains contained plasmid type *IncI1*, which is commonly associated with mentioned gene.

Acknowledgment This study was supported by the project APVV 0009-10 and VEGA project No. 2/0014/13.

Keywords: ß-lactamases; *Escherichia coli;* plasmid replicon typing; virulence

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Study of the antibacterial activity of silver nanoparticles (AGNPS) on *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus* is well known for its resistance to many antibiotics, so that the number of effective antibiotics against it is limiting. Therefore, in order to control and prevent infections, other antimicrobial agents are surveyed. Due to its known antibacterial activity, silver nanoparticles are considered to be used in various applications against bacteria that are resistant to common antibiotics or even multiresistant bacteria as the *S. aureus*. This work had as objective the evaluation of the antimicrobial activity of silver nanoparticles in strains of *Staphylococcus aureus* resistant to a large number of antibiotics.

Materials and Methods: Materials: Strain of *S. aureus* ATCC 27853 and two strains of *S. aureus* obtained from hospital-acquired infections and called S.a1 and S.a2.; Silver nanoparticles: NanoAG 20µm/mL solution (SIGMA) , tested at dilutions : 10.0; 5.0 ; 2.5; 1.25; 0.625; 0.312 and 0.156 µm/ mL; Antibiotics: ceftazidime, meropenem, amikacin, ampicillin + sulbactam, levofloxacin, chloramphenicol, vancomycin , penicillin, oxacillin and cefoxitin .

Methods: The susceptibility tests were performed by the agar diffusion method and logarithmic serial dilution and in base 2 (log 2) of the nanoparticles to determine the minimum inhibitory concentration.

Results and Discussion: The results showed that *S. aureus* is a strong producer of biofilm. Regarding antimicrobial susceptibility tests, the reference strain and the clinical strain S.a1 showed sensitivity to 50% of the evaluated antibiotics, while S.a2 strain showed resistance approximately 75%.

Conclusions: Silver is known for its antibacterial activity. This activity depends on the contact surface, wherein the silver can inhibit the respiratory chain enzyme systems of some bacteria and alter their DNA synthesis. The suspension of silver nanoparticles showed antimicrobial activity on the reference strain and on one of the hospital strains. Further studies should focus on the *in vitro* toxicity of nanoparticles in order to use them as new materials and substances in medical application.

Keywords: Silver nanoparticles, antimicrobial activity, multiresistant, antibiotics.

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Sub-lethal antibiotic concentrations of rifampicin and isoniazid lead to drug synergy via time-kill kinetic studies

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The rise of antibiotic resistance especially multidrug resistant (MDR) bacteria is an immense public health problem. Tuberculosis (TB) alone accounted for 450,000 cases of MDR-TB infections in 2012 with 170,000 deaths globally (1). Though the need for novel antimicrobials is great, the use of combination therapy with synergistic and antagonistic effect is an important strategy to overcoming resistance. Synergistic drug combinations are predominantly employed clinically, but the underlying cause of most drug synergies are not clearly understood. Thus there is an urgent need to understand the mechanisms underlying drug synergy. Using a wide range of antibiotic concentrations, we investigated the *in vitro* synergistic potential of rifampicin (RIF) and isoniazid (INH) against *Mycobacterium smegmatis* mc^2155. Bactericidal activity of the antibiotics alone and in combination was examined using time-kill curves. Minimum inhibitory concentration (MIC) of the antibiotics was determined by log_2 serial dilutions. Bacterial growth rate was measured with or without antibiotics. From the time kill-curves rifampicin on its own was bacteriostatic at low concentrations (<8 µg $ml^{-1)}$ but exerted a more bactericidal effect at higher concentrations (>32 µg ml^{-1}). *M. smegmatis* was however refractory to INH even at a high concentration of 128 µg ml^{-1}. The observed synergistic effect of combining RIF and INH at sub-lethal concentration is encouraging. These results could have important implications for the design of novel antibiotics effective in multi-drug therapy.

Keywords: drug synergy, multi-drug resistance, time-kill curves, Tuberculosis

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Susceptibility of *Aspergillus* species isolated from cutaneous and visceral lesions to antifungal drugs in Iran

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Background: Different studies have shown, despite the expanding antifungal agents, opportunistic fungal infections death incidence rate caused by species of *Aspergillus* have increased during recent decades due to the growth of potential factors and immunosuppressed individuals.

Susceptibility decrease, drug-resistance occurrence, MIC (Minimum Inhibitory Concentration) increase, and cross resistance among the isolated *Aspergillus* SPP., lack of effective response to conventional treatments, inaccessibility of the antifungal susceptibility patterns of the most common Iranian isolated *Aspergillus* SPP. have become an excuse to design and carry out the present study. *It is important to remember that the rude mortality from invasive Aspergillosis is around 85% and falls to around 50% if treated more precisely.*

Methodology: During 13 months 50 clinically isolated *Aspergillus*, which have been isolated from viseral and cutaneous samples, based on Klich 2002 method and the morphological features were devided into 40 strains of A. flavus, 9 strains of A. niger, and one strain of A. fumigatus. Then their susceptibility test was carried out according to the standard method of NCCLS – M38A Broth Microdilution.

Results: Through this study we found out that 7.5% of the isolated *A. flavus* with MIC > 2µg/ml in relation to AMB medicine, according to CSLI Guideline are probably considered to be as clinically resistant isolated types or treatment failures, and 25% of them in relation to ITR medicine with MIC = 1 µg/ml and by MIC < 8 µg/ml are considered to be as less sensitive isolated species. On the whole, the domestic isolated *A. flavus* species were less sensitive than those which have been under studies overseas.

The MIC range of 9 strain *A. niger* in relation to AMB, ITR, VRC medicines respectively came out 0.5 – 1 µg/ml, 0.5 – 2µg/ml, and 0.25 – 2µg/ml, that in comparison with similar foreign studies had less sensitivity in spite of being in the standard strain of MIC range and protocol.

The MIC range of 1 strain *A. niger* in relation to AMB, ITR, VRC medicines respectively came out 1, 2, and 0.25 MIC µg/ml, that according to CLSI protocol are considered as high. In comparison with similar foreign studies had less sensitivity.

Conclusion: Through this study we found out that the MIC range Iranian *Aspergillus* isolated from clinical specimens in the majority of the cases go into the reference standard strains of MIC range and the MIC range of some foreign studies. But in some important cases go out of this range that shows lower sensitivity of Iranian isolated *Aspergillus* and their MIC increase.

Keywords: *Aapergillus*, Drug resistance, MIC, Amphotericin B, Itracunazole,Voricunazole

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The Evaluation of Cross Resistance between Chloramine T Biocide and Rifampicin Antibiotic in Cooling System Biofilm Including *Legionella pneumophila*

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Cooling towers provide an ideal environment for microbial growth and have been attributed as a source for accumulation and dissemination of pathogenic organisms, especially Legionella pneumophila. Since Legionella infections have frequently been traced to contaminated aerosols generated at distances over 6 km, cooling systems have a potential risk for public health. Inhalation of Legionella-contaminated aerosols may cause Legionnaires' Disease or Pontiac fever in humans. The most common approach to eliminate or reduce the biofouling problem in contaminated systems is chemical treatment primarily by using biocides. Owing to the fact that biofilm bacteria are more resistant than their planktonic counterparts to the biocides, increasing biocide concentrations can lead to microbial resistance build-up first to the biocide and then consequently to the antibiotics. In the current study, the co-evaluation of Chloramine T trihydrate biocide and rifampicin antibiotic resistance in Legionella pneumophila was investigated.

For this purpose, 4 months-old mature biofilm samples which had been grown on glass surfaces were divided into 4 groups: 1st group was exposed to the recommended dosages of biocide for the water systems (2000 mg l^{-1}), 2nd group to 4,16 mg/ml rifampicin antibiotic for 24 hours, 3rd group slides to the same antibiotic concentration for 24 hours after the same biocide concentration and contact time exposure, and 4th group was used as control.

After the contact times, the samples were analyzed in view of heterotrophic plate count (HPC), the presence of Legionella spp., the epifluorescence microscopy, the total and free ATP concentration.

According to our results, Chloramine T, rifampicin and CT + rifampicin reduced cultivable heterotopic plate account by > 4 log, and L. pneumophila by > 3 log in the samples where they are applied together. It has been detected that all the antimicrobial applications ensured a significant rate of reduction in the total and free ATP values compared to the control ($p < 0.05$). It has also been detected that apart from biocide that was exclusively applied in the plate count, the application of antibiotic and biocide + antibiotic reduced the count of the cultivable bacteria in the biofilm to 0, but the rate of vitality did not decrease in the samples dyed with fluorescent staining.

On the other hand, Chloramine T trihydrate as the oxidizing biocide preferred in the study is purported not to induce resistance build-up; according to our results, it correspondingly did not build up a cross resistance against the rifampicin preferred in the treatment of Legionnaires' Disease.

The Relationship among Lytic Transglycosylases, β-lactamase Expression, and β-lactam Resistance in Stenotrophomonas maltophilia

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Peptidoglycan (PG) is an important component for the bacterial shape and structural integrity. In the *ampR*-β-lactamase cluster-bearing gram-negative bacteria, PG recycling is linked to the chromosomal β-lactamases expression. Lytic transglycosylases (LTs) are important enzymes involved in the PG recycling. *Stenotrophomonas maltophilia* is an important opportunistic pathogen with high resistance to β-lactam antibiotics because of the inducibly expressed L1 and L2 β-lactamases. In this study, the relationship among LT, β-lactamase expression, and β-lactam resistance in *S. maltophilia* was elucidated. According to the sequenced *S. maltophilia* K279a genome, *S. maltophilia* harbors six putative LTs, that is Smlt0155 (*mltA*), Smlt4052 (*mltB1*), Smlt4650 (*mltB2*), Smlt0994 (*mltD1*), Smlt3434 (*mltD2*), and Smlt4007 (*slt*). The individually LT isogenic deletion mutant of *S. maltophilia* KJ was constructed, yielding KJΔMltA, KJΔMltB1, KJΔMltB2, KJΔMltD1, KJΔMltD2, and KJΔSlt. In the meanwhile, the LT overexpression constructs were prepared by introducing the LT-containing plasmid into strain KJ, respectively, generating KJ(pMltA), KJ(pMltB1), KJ(pMltB2), KJ(pMltD1), KJ(pMltD2), and KJ(pSlt). The β-lactamase activities and β-lactam susceptibility of the LT deletion mutants and LT overexpression constructs were determined. The results indicated that *mltD1* inactivation and *mltB1 overexpression* caused a notable phenotype of derepressed basal-level β-lactamase activity. Inactivation of *mltB2* or *slt* increased the susceptibility to ampicillin, piperacillin and carbencillin, as well as inactivation of *mltB1* or *mltD1* decreased the resistance to piperacillin. These observations concluded that the imbalance in the LTs activities is associated with β-lactamase expression and β-lactam susceptibility in *S. maltophilia*.

Keywords: *Stenotrophomonas maltophilia*; lytic transglycosylases; β-lactam resistance

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Attenuation of virulence as antimicrobial strategy

A Drug Repositioning Screen Identified Pentetic Acid as a Potential Therapeutic Agent to Suppress Elastase-mediated Virulence of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa, a Gram-negative bacterium of clinical significance, produces elastase as a predominant exotoxin. Here, we screened a library of chemical compounds currently used for human medication and identified diethylene triamine pentaacetic acid (DTPA, pentetic acid) as an agent that suppresses the production of elastase. Elastase activity found in the prototype *P. aeruginosa* strain PAO1 was significantly decreased when grown with as low as 20 μM DTPA. Supplementation with Zn^{2+} or Ca^{2+} ions restored the suppressive effect of DTPA suggesting that the DTPA-mediated decrease in elastase activity is associated with ion-chelating activity. In DTPA-treated PAO1 cells, transcription of the elastase-encoding *lasB* gene and the level of pseudomonas quinolone signal (PQS), a molecule that mediates *P. aeruginosa* quorum sensing (QS), were significantly downregulated, proposing a potential involvement of the PQS QS system in DTPA-mediated elastase suppression. Biofilm formation was also decreased by DTPA treatment. When A549 airway epithelial cells were infected with PAO1 cells in the presence of DTPA, A549 cell viability was substantially increased. Furthermore, intranasal delivery of DTPA to PAO1-infected mice alleviated the pathogenic effects of PAO1 cells in animals. Together, our results uncovered a novel function for a known molecule that may help treat *P. aeruginosa* airway infection.

Keywords: *Pseudomonas aeruginosa*, Elastase, Pentetic acid, Virulence, Drug repositioning

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Characterization and use of Aii20J, a wide spectrum *N*-acylhomoserine lactonase, as a promising control method for Gram-negative pathogens

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Numerous human, animal and plant pathogenic bacteria coordinate important biological functions, including the expression of virulence factors, through a cell-density-dependent gene regulation system known as quorum sensing (QS). Since in the absence of QS the pathogenic capacity of the bacteria is highly impaired, the inhibition of quorum-sensing, also known as Quorum Quenching (QQ) constitutes a promising strategy for the control of bacterial pathogens. Recently, the marine bacterium *Tenacibaculum* sp. strain 20J [1] was identified, presenting a very wide spectrum activity against AHLs, the most common QS signals in Gram-negative pathogens.

The gene coding the enzyme responsible of the wide spectrum QQ activity from the marine bacterium *Tenacibaculum* sp. strain 20J was identified, cloned and overexpressed [2]. A functional screening in a fosmid genomic library was performed to obtain the DNA sequence of the aii20J gene. A putative lactonase gene was identified by pyrosequencing the 40 kb insert in the only positive clone obtained in the library. The sequence codifies a novel metallo-β-lactamase of 286 amino acidic residues with less than 31% identity to AiiA-type AHL lactonases. The gene is present in several other strains of the genus *Tenacibaculum*. The purified enzyme has a broad substrate spectrum of QQ activity, degrading most of the AHL known, with or without oxo- substitutions, with a certain preference against long-chain AHLs.

Besides being much more active and having a wider spectrum activity than the lactonase AiiA from *Bacillus*, Aii20J is highly thermo-resistant, and is not affected by protease K and α-chymotrypsin, being active at a wide range of pH, which makes it suitable as feed additive. Metal ions improve the hydrolytic activity of Aii20J due to its metallo-enzyme condition. In addition, purified enzyme does not interference with the action of any of the β-lactam antibiotics and β-lactamases inhibitors tested.

Although *E. coli* does not produce AHLs, *E. coli* cells respond to the AHLs released by other bacterial species, activating, among other genes, the glutamate-dependent acid resistance system [3], which contribute to the survival of pathogenic *E. coli* in different acidic environments as specific foods and in the gastrointestinal tract. The addition of Aii20J to *E. coli* K-12, in which the acid-resistance system had been activated by exogenous addition of AHLs, resulted in a significant reduction of survival (50%) after exposure to acid environment. The strong and non-specific QQ activity of Aii20J, with clear advantages in comparison with the enzymes from *Bacillus* species and other known AHL-lactonases, confirm this enzyme as novel promising candidate anti-pathogenic agent with diverse applications.

Keywords: Quorum sensing; quorum quenching; lactonases; acid resistence*; Escherichia coli*

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Genetic characterization and virulence control by calcineurin in the dimorphic fungus *Paracoccidioides brasiliensis*

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Paracoccidioides brasiliensis is a dimorphic pathogenic fungus that causes human paracoccidioidomycosis. We have previously shown that calcineurin, a calcium/calmodulin dependent protein phosphatase, is essential for the thermal dimorphic transition from mycelium to yeast (M-Y) forms. Here, we characterize the structure and expression of genes coding the A (*PbCNA*) and B (*PbCNB*) subunits of *P. brasiliensis* calcineurin and show that calcineurin full activity is required for fungus virulence. *PbCNA* comprises 1802 and *PbCNB* 862 nucleotides in length, which are divided into 4 exons, which are conserved among calcineurin genes. Analysis of regulatory regions indicates that *PbCNA* is a TATA-less gene, whereas *PbCNB* presented all the elements of the basal transcriptional machinery. These genes also present motifs for nutrient-regulated transcription factors. Transcript levels of both genes were low in mycelia forms. *PbCNA* expression is increased at early stages of the M-Y transition and *PbCNB* showed a later expression pattern. Finally, calcineurin inhibition with the immunosupressive drug cyclosporin A attenuated virulence of *P. brasiliensis* in a murine model.

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Glycoproteins of *Mycobacterium tuberculosis* as virulence determinants-deglycosylated attenuated vaccines

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A Statens Serum Institute *Mycobacterium bovis* BCG (BCG-SSI) recombinant expressing MTB Rv1860 (BCG-TB1860) showed loss of protective ability compared to the parent BCG strain expressing the control GFP protein (BCG-GFP). Since Rv1860 is a secreted mannosylated protein of MTB and BCG, we investigated the effect of BCG-TB1860 on innate immunity. Relative to BCG-GFP, BCG-TB1860 effected a significant near total reduction both in secretion of cytokines IL-2, IL-12p40, IL-12p70, TNF-α, IL-6 and IL-10, and up regulation of co-stimulatory molecules MHC-II, CD40, CD54, CD80 and CD86 by infected bone marrow derived dendritic cells (BMDC), while leaving secreted levels of TGF-β unchanged. These effects were mimicked by BCG-TB1860His which carried a 6-Histidine tag at the C-terminus of Rv1860, killed sonicated preparations of BCG-TB1860 and purified H37Rv-derived Rv1860 glycoprotein added to BCG-GFP, but not by *E. coli*-expressed recombinant Rv1860. Most importantly, BMDC exposed to BCG-TB1860 failed to polarize allogeneic as well as syngeneic T cells to secrete IFN-γ and IL-17 relative to BCG-GFP. Splenocytes from mice infected with BCG-SSI showed significantly less proliferation and secretion of IL-2, IFN-γ and IL-17, but secreted higher levels of IL-10 in response to *in vitro* restimulation with BCG-TB1860 compared to BCG-GFP. Spleens from mice infected with BCG-TB1860 also harboured significantly fewer DC expressing MHC-II, IL-12, IL-2 and TNF-α compared to mice infected with BCG-GFP. Glycoproteins of MTB, through their deleterious effects on DC may thus contribute to suppress the generation of a TH1- and TH17-dominated adaptive immune response that is vital for protection against tuberculosis. We propose removal of glycosylation on proteins of BCG to improve protective efficacy.

Phenotypic analysis of *ygdP* mutant from *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic human pathogen that frequently causes hospital infections. The observed difficulties in eradicating infection caused by this microorganism is due to its high intrinsic and acquired resistance against a wide range of antibacterial agents. Thus, there is a need for a search of novel factors involved in *P. aeruginosa* pathogenesis that could serve as possible targets for new anti-bacterial strategies.

The YgdP protein belongs to Nudix pyrophosphatases which are widely distributed among all classes of organisms. These enzymes catalyze the hydrolysis of a variety of nucleoside diphosphate derivatives. It was shown that YgdP homologs from pathogenic bacteria *E. coli K1, Legionella pneumophila* and *Pasteurella multocida* are involved in pathogenesis and may act as virulence factors.

In order to elucidate the biological function of the YgdP protein from *P.aeruginosa*, the *ygdP* mutant strain was constructed. RT-PCR analysis confirmed the lack of *ygdP* transcript in *P. aeruginosa* mutant strain. The mutant strain is characterized by reduced number of cells in logarithmic and stationary growth phase as compared to the wild type. The mutated cells formed smaller, morphologically changed colonies and exhibited weakened motility as compared to the wild type.

The biological significance of the YgdP protein in pathogenesis is currently investigated with a help of *P. aeruginosa-C. elegans* model.

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Quorum quenching enzymes as a novel antipathogenic strategy

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The expression of virulence factors in many pathogenic bacteria is controlled by a cell density-dependent communication mechanism known as quorum sensing (QS), in which small diffusible molecules are released to the medium, allowing bacteria to coordinate their behaviour once a minimal effective quorum has been reached. The interference with these signalling systems, also known as quorum sensing-inhibition or Quorum Quenching (QQ), represents a promising strategy to tackle bacterial infections. In the past decade, several enzymes capable to degrade or modify the Gram-negative QS signal molecules N-acyl-homoserine lactones (AHLs) have been described. Bacterial enzymatic QQ has been explored as a novel anti-pathogenic therapy to control bacterial infections with positive results in plants, the nematode *Caenorhabditis elegans* infection model and in the field of aquaculture.

Recently, the marine bacterium *Tenacibaculum* sp. strain 20J [1] was identified, presenting a very wide spectrum activity against AHLs [1]. The gene responsible for the QQ activity in this strain, *aii*20J, has been cloned and overexpressed. Aii20J is a lactonse belonging to the metallo-β-lactamase family that presents a low homology (31% identity) with the well characterized AiiA-type lactonases that are present in several species of the genus *Bacillus*. As for live cells and cell extracts, the purified enzyme has a broad substrate spectrum, degrading all the AHLs tested, with or without oxo- substitutions. Aii20J is between 25 and 50 times more active than AiiA, presenting a specific activity of 280 U/mg towards C6-HSL and 230 U/mg towards C10-HSL. Aii20J presents a high thermo-stability, with less than 10% activity loss after being exposed to 60ºC for 10 minutes. The purified enzyme has no interference with β-lactam antibiotics and β-lactamases inhibitors. The addition of Aii20J to *Psuedomonas aeruginosa* PAO1 cultures in which biofilm formation is controlled by C4-HSL and 3-oxo-C12-HSL signals among others, strongly inhibits biofilm formation (Figure 1). Due to its strong and non-specific AHL-QQ activity, with clear advantages in comparison with *Bacillus* species, Aii20J constitutes a novel promising anti-pathogenic tool with diverse applications in the field of animal or human health.

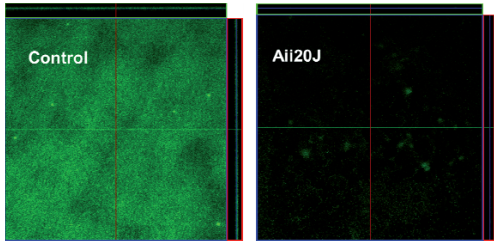

Figure 1. Effect of the Quorum-quenching enzyme Aii20 on biofilm formation by *Pseudomonas aeruginosa* PAO1 (Lausanne strain) constitutively expressing GFP. Confocal analysis of the biofilm attached to glass bottom plates was carried out after 4 days of growth in absence (Control) or in the presence of the QQ enzyme Aii20J. Green colour is produced by the GFP-expressing bacterial cells attached to the surface.

Keywords: Quorum sensing; quorum quenching; Acyl-homoserine-lactones; lactonases; biofilm; *Pseudomonas aeruginosa*

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The Use of Tetraspanins as Potential Barriers to Infection

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Tetraspanins are a superfamily of eukaryotic membrane-bound proteins involved in a wide array of processes such cell adhesion and migration. They do this by forming large extended networks known as tetraspanin-enriched microdomains (TEMs) on the surface of the cell where they act as membrane organisers to partner proteins, such as integrins, ensuring confirmations that aid in their function. Many of these partner proteins are also the receptors to which bacteria can adhere onto using adhesins. TEMs may cluster together these receptors to allow for multiple interactions from a bacterial pathogen with a cell resulting in firm adhesion to a host.

It is believed that by potentially interrupting the TEMs through pre-treating epithelial cells with a variety of recombinant tetraspanin extracellular domains (EC2s) it can reduce the ability of the bacterium to attach to host receptors. This is by disrupting these adhesion platforms. This has been demonstrated using the bacterial pathogens *Neisseria meningitidis* and *Staphylococcus aureus.* Chimeric EC2 proteins highlight important regions of the domain for achieving this phenomenon. This effect has been successfully replicated using synthetic peptides based on regions of a particular tetraspanin EC2 peptide sequence, in a dose response-like fashion. This does not appear to be cell or bacterial specific. The antimicrobial protection is slowly lost from the cell over time, possibly due to recycling of the tetraspanins from the cell surface. The effect is likely due to disruption of the TEMs, as cholesterol depletion, which similarly disrupts TEMs, removes the effect of subsequent treatment with synthetic peptides.

EC2s of some tetraspanin molecules (particularly CD9 and CD63) and synthetic peptide derivatives show signs of promise for possible use as anti-adhesion therapeutics for bacterial infections. This could possibly be in conjunction with current antibiotics, providing further protection to the patient during periods of sub-MIC levels of drugs between dosing.

Keywords: tetraspanins, tetraspanin-enriched microdomains, bacterial adherence, anti-adhesion therapy

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Techniques and Methods

A protocol for screening protein-protein interaction inhibitors with the "Two phages" Two Hybrid Assay

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The study of bacterial division interaction network shows an important applicative fallout since protein–protein interactions have emerged as promising drug targets. Inhibitors of these interactions could account for the purpose of the modern pharmaceutical research focused on the identification of novel antibacterial agents able to play down the rising of bacterial resistance that, still represents the main problem of antibiotic therapy.

Small molecules that bind to specific residues on the protein contact surfaces [1] could constitute a novel class of antimicrobial agents with a very low risk of resistance. As a matter of fact, impairing the interaction between two proteins A and B forming a heterodimer A-B, essential for the bacterial survival, will be lethal. Bacterial mutants, antiA-B resistant, need a mutation at the interface between antiA-B and A or B protein. These mutants will be also lethal, since the protein, mutated in the interaction site, will be not able to interact with its wild type partner. Only the double mutants, simultaneously mutated in both the two partner proteins, will be selected. This kind of mutants is much less frequent compared to the mutants in the A or B domains, recognized by the old style antibiotics, resulting in a very low rate of bacterial resistance.

The "Two phages" two hybrid assay (THA) already used to depict the *E. coli* and *S. pneumoniae* cell division interactome [2], could constitute a useful tool to select small molecules interfering with the interactions among the division proteins. These molecules, binding to residues involved in protein interactions, on the proteins contact surfaces, could provide both a complementary and more flexible approach for studies on molecular mechanism of cell division and, as important applicative consequence, they potentially constitute a novel class of antimicrobial agents with a very low risk of resistance.

At this regard, we set the assay, for the screening of small molecules (or peptides/peptidomimetics) to identify protein-protein interaction inhibitors. This assay, based on the two phages THA, was validated using 3'-(2-phenyl-1H-indol-3-yl)-[1,1'-biphenyl]-3-carboxylic acid, that is known to interfere with the interaction between the *E. coli* division proteins FtsZ and ZipA [3].

This screening assay is characterized by feasibility and low cost and can be used to test ligands with a wide range of size (from 15 to about 400 residues, in the reported case) and proved to be selective and reproducible.

Due to its feasibility and operating speed we easily used this assay for high-throughput drug discovery efforts screening a library of small molecules inhibitors of division protein interactions. Preliminary data will be presented on the poster.

Keywords: protein-protein interaction inhibitors; antibacterial agents

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Antifungal activity of *Thymus vulgaris* essential oil: Disc diffusion versus vapour diffusion methods

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Objective: *Thymus vulgaris* (Lamiaceae family) is an aromatic herb used as a traditional therapy because of their pharmacological activities. However, to the best of our knowledge, no systematic studies comparing antifungal potential (in liquid and vapor phase) of the essential oil (EO) are available.

Methods: The composition of *Thymus vulgaris* essential oil (TVEO) and its antifungal activity against yeast strains and filamentous fungi were investigated. The extraction of TVEO was obtained by steam distillation. Chemical composition of the EO from thyme grown in Algeria was determined by Gas Chromatography-Mass Spectrometry (GC-MS). A total of thirteen compounds were identified. Carvacrol (83.8%) was the major component, followed by cymene (8.15%), terpinene (4.96%) and linalool (1.44%).

Results: Antifungal action of the TVEO against nine clinically isolated molds and eight yeast strains was determined by using standard agar disc diffusion and vapour diffusion methods at three different doses (20, 40 and 60 µl per disc). By disc diffusion method, TVEO showed potent antifungal activity against *Candida* strains more than antifungal drugs (Amphotericin B). The Diameter of Inhibition Zone (DIZ) varied from 34 to 60 mm for *Candida* yeasts.

However, the results obtained by both agar diffusion and vapour diffusion methods were different. Significantly higher antifungal activity was observed in the vapour phase at lower concentrations. *Candida albicans*, *C. tropicalis* and *C. parapsilosis* were the most susceptible strains to the oil vapour with DIZ varied from 35 to 90 mm. Therefore, smaller doses of EO in the vapour phase can be inhibitory to pathogenic yeasts. Else, the DIZ increased with increase in concentration of the oil.

Conclusion: There is growing evidence that TVEO in vapour phase are effective antifungal systems and appears worthy to be considered for practical uses in the prevention or treatment of candidiasis and fungal infections. The present study indicates that TVEO has considerable antifungal activity, deserving further investigation for clinical applications. Also whilst the mode of action remains mainly undetermined, this experimental approach will need to continue.

Keywords: *Thymus vulgaris*; essential oil; antifungal activity; *Candida albicans*; vapour diffusion.

Antimicrobial efficacy gaseous ozone on berries and baby leaf vegetables

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Ozone, the triatomic form of oxygen,is a strong broad-spectrum antimicrobial agent widely used for improving food safety; it rapidly auto-decomposes to oxygen and does not leaveresidues.

However, its antimicrobial efficacy against microorganisms contaminating foods is greatly reduced as it promptly reacts with food organic matter, and consequently, its concentration decreases.

In the last years, following the consumers'demand for RTE foods, fruits and vegetables are usually manipulated, processed and cold stored for some days before being ready for consumption. Thus, in comparison with fresh fruits and vegetables, RTE produces lead to a new matter of safety concerns with a greater frequency of foodborne illnesses.

Aim of this work was to evaluate the impact of gaseous ozone treatments on microorganisms contaminating berries and baby leaf vegetables.

In the case of berries, ozone was applied at 2000 nL L^{-1} for 5 min, or with continuous fumigation at 300 nL L^{-1} evaluating the effect on yeast and mold population, the microflora mainly responsible for fruit decay, during seven days of cold storage. As concerns baby leaf vegetables, ozoneation was constant (at 500nL L^{-1}) for seven days but maintaining leaves at 4°C and 10°C, evaluating its effect on *Pseudomonadaceae*, bacterial population responsible for browning of leaves, as well as on total mesophilyc bacteria.

Ozone caused a significant reduction of fungal contaminants on treated berries, during the conservation as compared with untreated fruits, in both application mode 2000 nL L^{-1} for 5min or in continuous fumigation at 300 nL L^{-1}. The storage of baby leaves under 500 nL $L^{-1}$of ozone did not significantly affect the concentration of bacterial cells in any of the storage times evaluated at both 4°C and 10°C.

In conclusion, this study underlines as gaseous ozone can improve microbial quality of fruits and vegetables but the impact of its efficacy depends on both target microflora and treated vegetable. In the light of these results the control of undesired microorganisms contaminating fruits and vegetables needs to be evaluated case by case

Keywords: food safety, RTE vegetables, shelf-life, ozone sanification, risk management

Application of synthetic adsorbents to antimicrobials separation processes

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Synthetic adsorbents, such as shown in Fig. 1, are spherical and highly porous crosslinked polymers. Synthetic adsorbents show hydrophobic interaction with various organic compounds: they are used to separate various kinds of pharmaceutical compounds including antimicrobials. However, most of the separation processes are limited to aqueous solvent systems, and most of the target compounds are limited to natural, fermented and cultivated ones. But, nowadays, applications of separation processes that use synthetic adsorbents in non-aqueous systems are emerged. Therefore, applicability of synthetic adsorbents to separation processes could be expanded to semi-synthetic and synthetic pharmaceutical compounds.

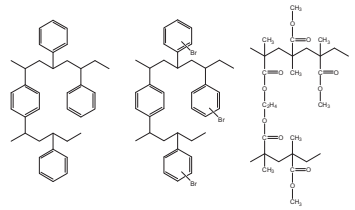

Fig. 1. Chemical structures of styrenic, brominated styrenic and methacrylic synthetic adsorbents.

When highly polar methacrylic adsorbents are used in the non-aqueous solvent systems, retention behaviors of the target compounds comply with normal phase mechanism. On the other hand, retention of the target compounds behave in accordance to reversed phase mechanism when less polar styrenic synthetic adsorbents are used in polar non-aqueous solvent systems.

In addition to the applicability to the non-aqueous solvent systems, synthetic adsorbents have various advantages in industrial processes. For instance, synthetic adsorbents of the same chemical and pore structure have become available in many particle sizes. This promotes easy scale-up and optimization: once optimum separation conditions has been established by use of HPLC column packed with small size adsorbent, scale-up can be easily achieved using larger particle size adsorbent under similar elution conditions. In addition, optimum particle size adsorbent can be selected to match the separation process scale.

Moreover, synthetic adsorbents possess high chemical stability: caustic solution can be directly loaded to them. This is far different characteristics from that of silica-based materials.

Those favorable characteristics of synthetic adsorbents would be suitable for industrial separation processes of pharmaceuticals including antimicrobials. Applicability of synthetic adsorbents in non-aqueous solvent systems as well as aqueous solvent systems will be discussed in detail.

Keywords: synthetic adsorbent; separation process; aqueous solvent system; non- aqueous solvent system

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Approach to target searching for *β*-amino fatty acid containing lipopeptides in cyanobacteria using LC-HRMS technique

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A group of cyanobacterial lipopeptides puwainphycins, which have structural similarities to members of the antibiotic of iturin family (bacillomycin, mycosubtilin, lichenysin), is investigated in this contribution. Puwainaphycins, ranging in the molecular weight from 1000–1250 $g \cdot mol^{-1}$, comprise of 9 amino acid residues and unusual functionalized *β*-amino fatty acid. They are cytotoxic and show antifungal activity. Because of these bioactivities it is important to develop LC-MS method for monitoring and screening of cyanobacterial lipopeptides in cyanobacteria. It is generally difficult to recognize *β*-amino fatty acid containing lipopeptide from other metabolites within the complex extract. In the MS/MS experiments where the collision energy used is suitable to see losses of amino acids, no loss of the fatty acid chain observable thus we tried to optimize the collision energy to obtain fragments specific for *β*-amino fatty acid chain. Three *Cylindrospermum* strains were analyzed for the presence of puwainaphycins using 60 eV and 100 eV collision energy. It was found that using high collision energy allows creation of fragments which are not only characteristic for each type of incorporated *β*-amino fatty acid, but they are also very abundant and thus recognizable for the first sight. Moreover length of the aliphatic chain of each lipopeptide variant has linear response of retention time when linear gradient is used for the LC separation. Performing this method more than 30 different variants of lipopeptides containing *β*-amino fatty acid chain were detected in each strain. So this is a good approach for screening of unknown lipopeptides, where *β*-amino fatty acid chain is incorporated, in crude extracts.

Keywords: cyclic lipopeptides; LC-HRMS

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Challenges in antimicrobial activity testing of dry surfaces

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The development of efficient bactericidal surfaces requires concomitant relevant testing methods to optimize the surface functionalization process. However, the analysis of bactericidal surfaces in dry environments presents a challenge since typical antimicrobial efficacy testing procedures largely rely on wet procedures for both the bacterial surface contamination and the release of the microorganisms from the surface or their staining with fluorescent dyes. For example can the presence of liquid during the inoculation of the test surfaces be expected to influence the release of the antimicrobial agents as well as their contact with the microbial cells. Additionally the manual inoculation process might also introduce spatial heterogeneities, which is the focus of the research presented here.

The “Test Method for Efficacy of Copper Alloy Surfaces as a Sanitizer” of the United States Environmental Protection Agency (EPA), which is currently in the process of getting adopted by the ASTM International [1], describes the application and spreading of a bacterial inoculum of 0.02 ml per square inch of the copper surface (which equals a liquid layer of 30 - 50 µm) followed by air drying. The drying of such inocula on surfaces proceeds from the edges towards the center and, when done on glass samples in our laboratory environment, took approximately 20 minutes. To characterize the spatial distribution of the bacteria and their survival during the drying process on such contaminated surfaces we applied fluorescence staining procedures (dead/live stainings) followed by confocal fluorescence microscopical analysis. When co-applying propidium iodide/SYTO 9 dead/live stain together with 10^7 bacteria (*S.aureus* or *E.coli*) on unmodified glass surfaces, followed by drying of the inoculum, we observed a heterogenous staining pattern of dead and live bacteria with distinct regions corresponding to the edges of the inoculum, the fast drying outer area and the slow drying center region. Such patterned regions were also observed when the bacteria were first applied to the test surface and dried and afterwards stained with a small amount of staining solution. While these protocols work well for the characterization of the survival patterns of large amount of bacteria upon drying on surfaces for the analysis of smaller number of bacteria (10^3-10^4) we employed contact slides.

The results from these experiments will be presented and discussed with respect to their implications for the meaningful efficacy testing of antimicrobial surfaces in dry environments.

Keywords: antimicrobial surfaces; assay; dead/live staining

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Comparison of different methods for detection of methicillin susceptibility in Coagulase-Negative Staphylococci

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Background: Coagulase-Negative Staphylococci (*CoNS*) are a major cause of sepsis in neonatal intensive care units (NICU) worldwide and in our Perinatal Center. Consequently tests for Methicillin resistance in *CoNS* are frequently used and accuracy of those tests is very important. We noted that tests for Methicillin resistance in *CoNS* by Cefoxitin disk diffusion (DD) are often inadequate compared to other methods. The object of the study was to compare and determination Methicillin resistance in *CoNS* by different phenotypic susceptibility tests and molecular methods (RT-PCR as the 'gold standard') and to evaluate the sensitivity and specificity for those tests.

Methods: Were tested 50 isolates of *CoNS* from newborns in NICU (pharyngeal and rectal swabs, blood and others): 33 - *S.epidermidis*, 6 - *S.haemolyticus*, 8- *S.hominis*, 2 – *S.warneri*, 1- *S.lugdunensis*. MALDI-TOF MS (Bruker Daltonics, Germany) was used for microorganism identification. RT-PCR was used to detect *mecA* gene in isoletes. 1- µg Oxacillin DD using Mueller-Hinton agar (MHA) supplementing with 2% NaCl (BioRad), 1- µg Oxacillin DD using MHA no 2% NaCl supplementation, 30-µg Cefoxitin (BioRad) DD using MHA (BioRad) and MIC oxaclillin by SENSITITRE (Trek Diagnostics, Cleveland, OH, USA) was used. Oxacillin zone diameters were read as recommended by CLSI [1]; Cefoxitin DD breakpoints (by ADAGIO System, BioRad) and MIC Oxacillin were read as recommended by CLSI 2013 [2]. Russian standards make provisions Oxacillin DD. A strain was designated as Oxacillin resistant when either an intermediate (where appropriate) or a resistant category was obtained. Sensitivity (Se) was defined as the percentage of mecA-positive strains determined to be resistant by phenotypic testing, and specificity (Sp) was defined as the percentage of mecA-negative strains determined to be susceptible by phenotypic testing. One strain S.aureus ATCC 25923 Oxacillin susceptible was used as a control one.

Results: 34 (68%) of isolates showed the presence of *mecA* by RT- PCR. MIC Oxaclillin by SENSITITRE showed the highest sensitivity among phenotypic susceptibility tests. But it had the lowest specificity, because three *mec*A-negative strains (2 - *S.warneri* and 1- *S.epidermidis*) had MIC 0,5 µg /ml although Oxacillin and Cefoxitin DD were susceptible. Sensitivity Cefoxitin DD was the lowest (67,6%): 11 from 34 *mecA* positive isolates had zone diameters more 25 mm (mean 28 mm), while methicillin resistance was confirmed by other methods. Oxacillin DD tested on the MHA without 2% NaCl was the highest sensitivity (97%) and specificity (100%) test.

	*mec*A	MIC (SENSITITRE)	Cefoxitin DD	Oxacillin DD	Oxacillin DD + 2% NaCl
Se	positive n=34	34 (100%)	23 (67, 6%)	33 (97%)	30(88%)
Sp	negative n=16	13 (81%)	16 (100%)	16 (100%)	16(100%)

Conclusion: Cefoxitin DD often lead to inadequate detection methicillin resistance in *CoNS* in our Perinatal Center. We think that we need to continue our study using more isolates.

Keywords: Coagulase-Negative Staphylococci (*CoNS*); methicillin resistance; disk diffusion (DD).

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Complementary biophysical tools to investigate lipid specificity in the interaction between antimicrobial molecules and the plasma membrane

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Plasma membranes are complex entities common to all living cells. The basic principle of their organization appears very simple, but they are actually of high complexity and represent very dynamic structures. The interactions between bioactive molecules, notably antimicrobial molecules and lipids are important for numerous processes, from drug bioavailability to viral fusion. The cell membrane is a carefully balanced environment and any changes inflicted upon its structure by an antimicrobial molecule must be considered in conjunction with the overall effect that this may have on the function and integrity of the membrane. As a general concept, understanding the mechanism at the molecular level by which bioactive molecules interact with cell membranes is of fundamental importance.

Lipid specificity is a key factor for the detailed understanding of the penetration and/or activity of lipid-interacting molecules and of mechanisms of some diseases. Further investigation in that way should improve drug discovery and development of membrane-active molecules in many domains such as health, plant protection or microbiology.

In this talk, we propose to overview some complementary "*in vitro*" and "*in silico*" biophysical approaches that can give information about lipid specificity at a molecular point of view. We will illustrate our strategy on antimicrobial cyclic lipopeptides, such as surfactin, mycosubtilin or fengycin.

Keywords: lipid bilayer, biobased molecule, lipid-specific interaction, antimicrobial lipopeptide, molecular biophysics, molecular modelling

Reference:

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Diffusion, Bioavailability and Reactivity of Antibiotics against *Staphylococcus aureus* Biofilms: a New Approach by Dynamic Fluorescence Imaging

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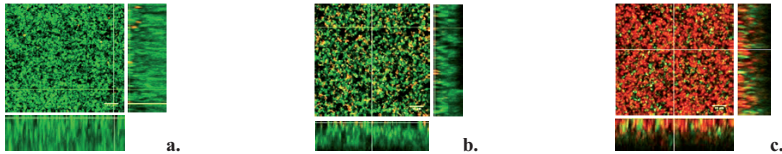
Background
Daptomycin, vancomycin and their combination with rifampicin exhibits activity against circulating infections but seem to be less efficient on biofilms. Our work focused on the study of the diffusion, bioavailability and activity of these antibiotics through methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus* biofilms using dynamic fluorescence imaging.

Methods
Staphylococcus aureus ATCC 27217 (MSSA) and ATCC 33591 (MRSA) biofilms were grown *in-vitro* in Tryptic Soy Broth medium enriched with proteins and divalent ions for 24 h at 37°C and then exposed to daptomycin 20 µg/mL, vancomycin 40 µg/mL or their combination with rifampicin 20 µg/mL. Colony-forming units (CFU) were quantified from detached biofilms after different times of antibiotics exposure: 3h, 6h, 24h and 48h.

Results
Dynamic fluorescence imaging ascertains both the free diffusion and the bioavailability of the antibiotics through the biofilm depth and their interaction with the cellular targets.[1] In spite of that, as revealed by CFU measurements and fluorescence intensity imaging, vancomycin and daptomycin as monotherapies are totally ineffective in biofilm clearance; on the other hand, both drug combinations are more efficient on MSSA and MRSA biofilms even if not able to eradicate them. Moreover, this lack of antibiotics efficacy is already perceptible for a monolayer of bacteria attached to the substrate.



Fluorescence intensity images of *S. aureus* **ATCC 33591** biofilms recorded 48h after antibiotic injection. **a.** Without antibiotic. **b.** In the presence of daptomycin (20µg/mL). **c.** In the presence of daptomycin (20µg/mL) associated with rifampicin (20µg/mL). Dead bacteria appear in red and alive bacteria in green. Images stacks acquired on the whole biofilm thickness by 1µm axial step and projected according two planes perpendicular to the observation plane. Image dimension: 120×120 µm².

Conclusions
These *in vitro Staphylococcus aureus* biofilms constitute promising high throughput systems to visualize and quantify antibiotics tolerance within biofilms and decipher the associated molecular mechanisms. They are currently being compared with a mouse model of *Staphylococcus aureus* infection on a medical implant to assess their *in vivo* relevance.

Keywords: *Staphylococcus aureus* biofilms; dynamic fluorescence imaging; daptomycin, vancomycin, rifampicin

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Discrimination of *Escherichia (E.) coli* outer membrane mimetic systems by ATR-FTIR spectroscopy

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Bacterial resistance to antibiotics is a global problem that requires fundamental understanding on bacterial structure and molecular mechanisms of reaction to the drugs, as well as new analysis techniques to overcome the time constraints posed by complex microbiological and biochemical analysis that delay the choice of a therapeutic strategy. A rapid isolation and identification of pathogenic microorganisms from clinical specimens is paramount to a timely and targeted antibiotherapy that can more effectively control infection avoiding the use of broad-spectrum antibiotics and so, further preventing phenomena of antibiotic resistance [1].

Vibrational spectroscopy has long been used in bacterial identification with different levels of taxonomic discrimination but its true potential for intra-species differentiation remains poorly explored. Both transmission Fourier-transform infrared (FTIR) and attenuated total reflectance (ATR)-FTIR spectroscopy have been used to analyse *Escherichia* (*E.*) *coli* strains that differ solely in their porin expression profile. The applicability of both FTIR-spectroscopy techniques was compared with the same collection of unique strains. ATR-FTIR spectroscopy proved to reliably distinguish between several *E. coli* porin mutants with an accuracy not replicated by FTIR in transmission mode (using previously optimized procedures) [2].

In order to further explore the discriminatory abilities of ATR-FTIR, *E. coli* outer membrane mimetic systems were constructed using both a binary mixture of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) (POPE:POPG 60:40) and total *E. coli* lipid extract. Proteoliposomes of the later with OmpC, OmpF (the two major porins in this strain) and two OmpF mutants (W61F and W214F) were also prepared and studied. The effects of the lipid and buffer compositions, vesicle concentration, lipid organization and protein presence were investigated.

Obtained results clearly showed that this technique is capable of molecular discrimination between lipossomes and proteolipossomes and also of discrimination between single-point protein mutants.

Further studies should allow the identification of the individual contribution of the single porin channel to the overall bacterial infrared spectrum and mixed population studies could lead to molecular predictive patterns of porin alterations. ATR-FTIR spectroscopy followed by cluster analysis could become a useful tool in the discrimination of antibiotic-susceptible and non-susceptible isolates speeding up the choice of a therapeutic strategy.

Keywords: infrared spectroscopy; FTIR; ATR; bacterial discrimination; *Escherichia coli*; liposomes, porins

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ELISA for detection of immunoglobulin IgA and IgG against HPV

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The interest in HPV seropositivity has increased considerably since HPV vaccines have become available worldwide.

Objective: To assess the performance of ELISA in analyzing serum samples provided from women with and without genital DNA-HPV infection confirmed by PCR, for detection of specific antibodies of the isotypes IgG and IgA recognizing HPV-16, and 18 as well as virus-like particles.

Subjects and Methods: Fifty women sexually active female patients, between 18-35 years from the outpatient clinic at university hospital from August 2013 to December 2013, were enrolled. In order to test them, positive controls were obtained from patients with HPV induced lesions and DNA-HPV positive confirmed by PCR while negative controls were obtained from DNA-HPV negative women confirmed by PCR. A specific assay was used to identify antibodies to HPV virus-like particles by ELISA. The samples were divided into HPV positive and negative, and an ELISA detecting IgA and IgG anti-HPV-VLP was carried out. The ROC curve was constructed using the values obtained from the first 25 positive and 25 negative samples evaluated.

Results: The effectiveness of ELISA and the Kappa index were obtained from the values entered in the ROC curves for IgG and IgA. IgG-VLP-HPV16 showed a good correlation between ELISA and PCR [weighted kappa coefficient = 0.75 95% CI (0.546 to 0.955)] and IgG-VLP-HPV18 showed a very good correlation between ELISA and PCR [weighted kappa coefficient = 0.84 95% CI (0.546 to 0.955)] while the IgA antibody correlation was also positive, although weaker: IgA-VLP-HPV16 was moderate [weighted kappa coefficient 0.45 95% CI (0.176 to 0.727)] and IgA-VLP-HPV18 was good [weighted kappa coefficient 0.66 95% CI (0.401 to 0.918)] . The efficacy of the assay concerning IgG was the following: IgG-VLP-HPV16: Sensitivity: 82.3% (56.6 - 96.2). Specificity: 92% (73.9 - 99). PPV: 87.5% (61.6 - 98.4). NPV: 88.5% (69.8 - 97.5). Accuracy: 88%. IgG-VLP-HPV18 was: Sensitivity: 100% (63.1 - 100). Specificity: 92% (74 - 99). PPV: 80% (44.4 - 97.5). NPV: 100% (85.2 - 100). Accuracy: 94%.The efficacy of the assay concerning IgA was the following: IgA-VLP-HPV16: Sensitivity: 64.7% (38.3 - 85.8). Specificity: 80% (59.3 - 93.2). PPV: 68.7% (41.3 - 89). NPV: 76.9% (56.3 - 91). Accuracy: 73.8%. IgA-VLP-HPV18: Sensitivity: 100% (63.1 - 100). Specificity: 80% (59.3 - 93.2). PPV: 61.5 (31.6 - 86.1). NPV: 100% (83.2 - 100). Accuracy: 84.8%.

Conclusions: IgG and IgA antibodies against HPV-16 and 18 can be detected in unvaccinated individuals by using the VLP that serve as the basis for bivalent HPV vaccine. The values for ELISA assays and the values found for IgG correlate good/very good with HPV16 /18 detected by PCR.

Key words: Immunity; Humoral; Immunoglobulin G; Immunoglobulin A; HPV

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Expression, Purification and Characterization of Antimicrobial Peptides using engineered Green Fluorescent Protein's Scaffold

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Increasing number of multidrug antibiotic resistant pathogenic microorganism is considered as a grave concern in the health industry. Recently, antimicrobial peptides (AMP) received substantial interest due to their high activity against the board range of pathogenic microorganism. However, due to their extreme toxicity and proteolytic degradation, the mass production of AMP by conventional heterologuos expression system is still facing challenges to be addressed. Although, few heterologuos expression systems such as the insoluble fusion partner system were employed for the production of few AMPs, none of the expression systems showed promising mass scale production due to lower expression in our hands and fails to completely abolish toxicity.

Hence, we challenged this question and developed the most efficient and simple expression system for AMPs or toxic proteins to meet the demand for mass production at industrial scales. We inserted the mature peptide coding sequences of AMPs into the scaffold of Green fluorescent protein (GFP) to abolish the toxicity and proteolytic degradation. In the first step, highly expressible internal methionine (Met) free-GFP was generated. Subsequently, AMPs flanked with Met followed by flexible loops sequences on the both termini, respectively, were inserted into the engineered GFP loop 172 position. Secondly, the engineered construct was successfully expressed in Escherichia coli as insoluble fraction without inducing any toxicity, purified and cleaved by cyanogen bromide to release the AMP at considerable yield.

As results, we successfully expressed and purified Protegrin-1 (PG1), PMAP36, and BuforinII (Bfu) in Escherichia coli with much higher yields. PG1, Bfu, and PMAP36 were considered as candidates for potential pharmaceutical agents due to their broad range of activity and efficacy. In this study, we were able to produce PG1, PMAP36 and Bfu about 12 to 14 mg per liter of culture which is much higher comparing to the results of previous studies. The expression of AMP inside the scaffold of GFP showed >7 fold compared to the fusion partner system (KSI-AMP). We presume that the lower expression of KSI-AMP is due to the accumulation of unfavorable structurally constraint insoluble proteins, which in turn altered the E. coli metabolism, and slowed growth rate.

Finally, we also characterized these AMP against pathogenic microorganisms to evaluate their functional efficacy. In summary, we were able to demonstrate the successful development of as method for large scale-production of biologically active toxic proteins including antimicrobial peptides such as PG1, Bfu, and PMAP36. Our new method could be useful for large scale production with low cost of any types of toxic peptides, which is extremely important in the stand point of bioindustry.

Keywords: Anitmicrobial Peptides; Green fluorescent protein

Identification and characterization of halophilic actinomycetes by sequence analysis of 16S rRNA gene and antibiotic susceptibility testing

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Halophilic actinomycetes represent a small group of microorganisms with great biotechnological and biomedical interest, because of their metabolites-producing ability and resistance to extreme salinity conditions. Besides, antibiotic resistance may involve novel mechanisms[1].

Water and sediment samples were taken from solar salterns in the coast of Oaxaca State, southern Mexico. Samples were processed and inoculated in MH medium. The strains were characterized morphologically and those corresponding to actinomycetous morphology were further assesed for optimal growth at 3, 5, 7.5, 10, 12.5 and 15% NaCl concentration. Antibiotic susceptibility was determined to disk diffusion method according to the NCCLS Standards[2]. Resistance or susceptibility to any of tested antibiotics was determined from the inhibition halo diameter. The strains were identified by sequence analysis of the 16S rRNA gene.

Six branched filamentous strains were obtained, all them grew optimally with 10% NaCl. Four strains (LRS4.210, LRS4.171, JAG.001 and LRS4.058) showed >99% sequence similarity to members of genus *Sacharomonospora,* and two strains (COSE-1A, COSE-1B) were identified as *Actinopolyspora mortivallis*. The latter strains showed identical antibiotic sensitivity patterns; while strains identified as *Saccharomonospora* resulted in more variable patterns.

Antibiotic	LRS4.210	LRS4.171	COSE1A	COSE1B	JAG001	LRS4.058
Ampicillin	R	I	S	S	R	S
Cefalotine	R	R	S	S	S	R
Cefotaxime	R	R	S	S	S	S
Ceftazidime	I	I	S	S	I	R
Ceforoxime	R	R	S	S	I	I
Dicloxacilline	R	R	S	S	R	R
Erytomycine	S	S	S	S	S	S
Gentamycin	I	I	S	S	I	R
Pefloxacime	R	S	S	S	I	S
Penicilline	R	R	S	S	I	S
Tetracycline	I	S	S	S	I	S
Trim-Sulfametoxasol	R	S	S	S	R	R

Keywords: halophile actinomyces; antibiotic susceptibility

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Impact on pharmaceutical expenditure in the detection of multiresistant pathogens by using PCR technique in patients with severe pneumonia admitted to ICU

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Introduction and Objective: PCR is a technique used to monitor the progress of a PCR reaction in real time; a relatively small amount of PCR product (DNA, cDNA or RNA) can be quantified. The detection of bacterial nosocomial pathogens to assist clinicians and clinical pathologists to initiate infection control measures and appropriate treatment in intensive care unit (ICU) patients in the hospital. The objective of our study was to evaluate the usefulness of the PCR technique in reducing economic costs due to the unnecessary use of antibiotics in cases of severe pneumonia.

Methods: Cost minimization study in patients admitted in the ICU, in one year diagnosed of severe pneumonia, who presented risk factors for multiresistant organisms (MDROs). We compare three packages of expenditure, one using the PCR technique and two other packages without using it, having done or not antibiotic adequacy in base of the microbiological culture, knowing the expense PCR. We analized 41 patients. The 95,12% with severe sepsis and 58,53% with septic shock. PCR was requested in 100% for Methicillin-resistant Staphylococcus (MARSA), 95.12% for Pseudomona Aeruginosa (PsA) and Acinetobacter Baumanii (AB).

Results: Delayed results of 3 ± 1 hours for PCR, being positive in 9 patients (21,95%) for PsA, AB 22 (53,65%), 3 MARSA (7,31%); and 72 ± 8 hours for the microbiological culture, being positive in 9 patients (21,95%) for PsA, 20 AB (48,78%), 2 MARSA (4,87%), with good association between microbiological culture results. PsA sensitivity(S) and specificity (E) of the technique was 100%, in AB was S 100% and E 91%. Antibiotic expense using PCR was 28.119€. However without PCR was 71.528€ without antibiotic adequacy in base of the bacterial culture, and was 37.768€ with antibiotic adequacy in base of the bacterial culture. The cost of the PCR technique was 4.130€.

Conclusions: The use of the PCR technique for MDRO is cost-effective, saving 43.409 € cost of antibiotics (60,68%) compared to the exclusive use of microbiological culture without antibiotic adequacy, and 9.649 € (25,55%) with antibiotic adequacy. PCR has good diagnostic capacity -Sensitivity 100% and Specificity 100-90% - and is earlier: diagnosis and treatment ahead in 3 days compared to microbiological culture.

Keywords: PCR1, Severe Pneumonia2; Multiresistant Pathogens3

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Insight into mechanistic aspect of photodynamic inactivation of *Candida albicans*

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In recent years, photodynamic inactivation (PDI) has been considered as an attractive antimicrobial treatment of infectious diseases. PDI involves the concerted action of three components: photosensitizer (PS), light and oxygen. There are many examples in the literature that describe efficient antimicrobial effects of PDI, showing the progress of the technique and growing interest in this field. Importantly, an in depth knowledge of the principles of PDI and its mechanism of action is necessary to understand and take the full advantage of this method.

The Quality Control strain of *Candida albicans* (ATCC 10231) was used in these studies. Planktonic cultures were incubated with imidazoacridinone derivatives (IA) as PS and irradiated with blue light. The light source used was custom made LED illuminator, emitting incoherent blue light at 405 nm with total power of 630 mW.

To determine the type of IA-based photodynamic reaction two biophysical methods were used: time resolved near infrared luminescence (TRL) to measure singlet oxygen quantum yield and electron paramagnetic resonance (EPR) for determination of free radicals generation. Fluorescent probes were used to determine the death mechanism of *C. albicans* cells.

In PDI, mediated by IA, a significant antimicrobial effect (up to 6 $\log_{10}$ units reduction in survival) against the planktonic form of *C. albicans* was observed. When the PDI effects were compared at different irradiance , the results showed that low to medium irradiation power was more effective, compared to high irradiation power.

Our data suggest that the photosensitized oxidation reaction, responsible for photodynamic inactivation of the yeast studied, was mostly Type I process with the involvement of superoxide anion. The data also indicate that the photodynamic killing of *C. albicans* occurs via apoptosis.

We believe that the observations reported here will be useful for understanding the mechanism through which the IA-mediated PDI operates, and might help in designing protocols for improved efficiency of the treatment.

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Morphological and pathological characterization of the *Agrobacterium tumefaciens* from almond nurseries in Chlef region in western Algeria

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PCrown gall is one of the destructive diseases and occurs worldwide. It is considered to be a disease of great economic importance in almond and other stone fruit tree nurseries due to the extensive losses. Based on their morphological characteristics on PDA and YMA, 10 isolates were selected on colonies of these isolates after 48 h at 28°C were circular, convex with smooth, transluscent and easily suspended in water. The bacterial cells were rod shaped with rounded ends and were either single or in pairs. The isolates were Gram negative, the optimum growth was between 25 and 27°C.

All strains are negative for L-tyrosine, and positive for mobility, catalase, oxydase and production of H2S. On the other hand, these isolates had all oxidized the lactose to 3-ketolactose. On the other hand, all Agrobacterium strains oxidized D-mannitol, inositol, indol, D-sorbitol, inositol, sucrose, melibioze, D-galactose, L arabinose, rhamnose and amygdalin. Furthermore, the isolates transform also the arginin, lysin, ornithin, citrate, gelatin and starch. The pathogenic nature of the organism was confirmed by a bioassay on carrot disks. Additionally, Koch's postulates for all isolates were also fulfilled.

Keywords: *Agrobacterium tumefaciens,* crown gall, almond, biochemical tests, pathogenicity test, Algeria.

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Phototherapy - A possibility in daily oral care?

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Reducing dental plaque, gingival inflammation and inhibit the biofilm formation in the mouth has during the years been regarded as one of the major treatment strategies for oral diseases. Periodontitis is an inflammatory and infectious disease where periodontopathogens such as *Aggregatibacter actinomycetemcomitans (A.a),* associated with aggressive periodontitis, have been proposed to be able to invade soft periodontal tissues and the underlying vascular endothelium. In light of the increased risk for bacterial resistance to antibiotics and the side effects of systemic antibiotic use, it would be of great importance if the use of antibiotics could be kept to a minimum.

A number of studies have indicated that bacteria that are able to emit red fluorescence can be detected by fluorescence techniques and also be killed by photodynamic therapy. Bacterial red fluorescence emission has been suggested to originate from porphyrins synthesized by and present in various microorganisms. Obligate anaerobic bacteria have been suggested to be responsible for this red fluorescence due to their increasing numbers in mature biofilm. Recently our research group has shown that the gram negative capnophilic bacterium *A.a* is able to produce red fluorescence on its own.

We are investigating the content of endogenous porphyrins as well as the influence of phototherapy on selected periodontopathogens such as *A.a* and *Porphyromonas gingivalis (P.g), in-vitro.* In phototherapy there is no addition of exogenous chemicals as is the case in photodynamic therapy (PDT) treatments where different chemicals are used as photosensitizers. In phototherapy treatment the porphyrins present in oral bacteria are used as endogenous photosensitizers.

In a pilot study performed by our research group we have shown that the bacterium *A. a* contains porphyrins and that this bacterium can be eradicated and its growth inhibited by the irradiation of light in the wavelength region of 405 nm, *i.e.* in the blue end of the visible electromagnetic spectrum. Our results suggest that *A.a* probably are able to synthesize porphyrins on its own, but that *P. g* probably not can synthesize porphyrins but uses an uptake of heme for that purpose.

The acquired knowledge will be put into practical phototherapy treatment in a Randomized clinical trial (RCT). The overall goal is to obtain a scientific basis for the implementation of the concept of phototherapy as a novel tool in everyday oral care. This would be a way to improve oral hygiene by decreasing harmful oral bacteria without the use of antibiotics and in an extension decrease the contribution to systemic diseases originating from infectious diseases in the oral cavity.

Keywords: gingival inflammation; periodontitis; porphyrin; phototherapy; blue light

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Potassium Clavulanate Supplemented Modified Charcoal-Cefoperazone-Deoxycholate Agar for Quantitative detection of Campylobacter in Chicken Carcass Rinse

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Potassium-clavulanate-supplemented modified charcoal-cefoperazone-deoxycholate agar (C-mCCDA) was compared with original mCCDA for the enumeration of Campylobacter in pure culture and chicken carcass rinse. The quantitative detection of viable Campylobacter cells from a pure culture, plated on C-mCCDA, is statistically similar ($P > 0.05$) to mCCDA. In total, 120 chickens were rinsed using 400 mL buffered peptone water. The rinses were inoculated onto C-mCCDA and mCCDA followed by incubation at 42 °C for 48 h. There was no statistical difference between C-mCCDA (45 of 120 plates; mean count, 145.5 CFU/mL) and normal mCCDA (46 of 120 plates; mean count, 160.8 CFU/mL) in the isolation rate and recovery of Campylobacter ($P > 0.05$) from chicken carcass rinse. The Pearson correlation coefficient value for the number of Campylobacter cells recovered in the 2 media was 0.942. However, the selectivity was much better on C-mCCDA than on mCCDA plates ($P < 0.05$). Significantly fewer C-mCCDA plates (33 out of 120 plates; mean count, 1.9 CFU/mL) were contaminated with non- Campylobacter cells than the normal mCCDA plates (67 out of 120 plates; mean count, 27.1 CFU/mL). The C-mCCDA may provide improved results for enumeration of Campylobacter in chicken meat alternative to mCCDA with its increased selectivity the modified agar possess.

Keywords: Campylobacter, cefoperazone, clavulanate, chicken, enumeration

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Separation, identification of methicillin-resistant from methicillin-susceptible *Staphylococcus aureus* in blood and their antimicrobial susceptibility by electrophoretic methods in fused silica capillaries etched with supercritical water

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Treatment of nosocomial infections has become more difficult and more expensive because of the increasing prevalence of multiresistant strains, especially methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA affects morbidity and costs of treatment of patients compared with infections caused by methicillin-susceptible *S. aureus* (MSSA). Rapid detection of low number of *S. aureus* cells ($10^1 – 10^2$ cells mL^{-1}) in blood is necessary to detect bloodstream infection. A fast detection and identification of MRSA and MSSA also enables to select an appropriate antimicrobial therapy.

Currently, three groups of techniques, phenotyping, genotyping, and mass spectrometry, are used for MRSA and MSSA strains differentiation. Most of phenotyping techniques are time-consuming. PCR and other molecular techniques are rapid. They have good reproducibility and repeatability and allow detection and differentiation between MSSA and MRSA directly from blood cultures. Potential disadvantages of genotyping methods lie in their discrimination ability, technical complexity, financial costs, and difficult interpretation of the results.

We tested capillary zone electrophoresis (CZE) and capillary isoelectric focusing (CIEF) techniques as fast and low-cost methods to detect bloodstream infection caused by *S. aureus*. CZE in fused silica capillaries etched with supercritical water and modified with (3-glycidyloxypropyl)trimethoxysilane can not only separate the methicillin-resistant from methicillin-susceptible *S. aureus* strains but it can also distinguish between the agar-cultivated and blood-incubated staphylococci of either MRSA or MSSA. The isoelectric point of MSSA and MRSA strains was determined as 3.4 by CIEF. The antimicrobial susceptibility of MSSA and MRSA strains was also tested by both techniques and the changes in their electromigration properties were monitored. The sensitivity and reproducibility characteristics of the CZE and CIEF separation appear to be sufficient for an initial rapid screening of clinical samples.

Keywords: capillary electrophoretic techniques; supercritical water; fused silica capillary; methicillin-resistant *Staphylococcus aureus* - MRSA; methicillin-susceptible *Staphylococcus aureus* - MSSA; whole human blood; antimicrobial susceptibility

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Surveillance for community outbreaks of human adenoviruses in Southern Taiwan, January to June 2014: Use of virus isolation and anti-adenovirus ELISA (IgM) test

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Background: Human adenoviruses (HAdVs) are major pathogens that can cause low respiratory tract infections, especially in young children and immunocompromised people. Statistics obtained from virus isolation experiments conducted at the virus laboratory of a medical center in Southern Taiwan show that the number of people infected by HAdVs between January 2014 to June 2014 substantially increased compared with the number of infected people during the same period in 2013. This phenomenon indicated that the community outbreak of HAdVs has reached a peak. Hence, the purpose of this study was to monitor the outbreak by employing virus isolation techniques and conducting serological tests and to analyze the characteristics and epidemic trends of viral infections.

Methods: This study was conducted using 130 sets of data obtained by using virus isolation and identification techniques and by conducting anti-adenovirus ELISA IgM tests between January 2013 to June 2014. SPSS Statistics was employed to analyze relevant factors, including the patient characteristics, specimen types, virus-positive rates, and test methods.

Results: An analysis of the results show that among the 39 people who were HAdV positive, 64.1% (25/39) were male and 35.9% (14/39) were female. The average working time required for virus isolation and identification was 4.9 d, and that for the anti-adenovirus ELISA IgM test was 4 d. Using kappa statistics, we assessed the consistency of the 2 methods in analyzing the incidence of lower respiratory tract diseases. The results showed a satisfactory consistency between the 2 methods ($\kappa = 0.73$).Regarding the epidemic trend, the virus isolation rates for the months from January 2014 to June 2014 was 3.7%, 6.7%, 9.3%, 17.1%, 22.9%, and 24.4%, respectively. Compared with the statistics for the same period in 2013, the HAdVs positive rates exhibited monthly increases. The results of a SPSS analysis showed that the differences were statistically significant ($P < 0.0001$). Regarding the age groups most vulnerable to infections, the number of children between 1 to 5 y of age infected by HAdVs was significantly greater ($P = 0.0001$) than that of people in other age groups, accounting for 53.8% (21/39) of the total number of people that were HAdVs positive.

Discussions: HAdVs infection is a type of viral infection that occurs frequently. Young children and immunocompromised people infected by the virus can develop lethal adenovirus pneumonia. Based on the study results, we identified an abnormal increase in the number of people infected by adenoviruses. In addition to issuing warnings to the public regarding the epidemic trend of the virus, we monitored the infections continuously and conducted analyses using various testing techniques. The methods used in this study can serve as novel options that physicians and laboratory personnel employ to efficiently and accurately detect adenoviruses in clinical practice.

Keywords: Human adenoviruses (HAdVs); Community outbreak; Virus isolation techniques; anti-adenovirus ELISA IgM tests

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Synthetic biology for the design of antimicrobial peptides

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The number of multi drug resistant pathogens is constantly growing and novel antibiotic substances are desperately needed in order to at least maintain the status quo. Ribosomally synthesized antimicrobial peptides are not yet exploited for human applications despite an indisputable potential. Among those, lantibiotics, a class of posttranslationally modified and thioether-ring bearing antimicrobial peptides, show desirable properties as high but specific antimicrobial activity and excellent stability.

We employ a synthetic biology approach for the generation of novel lantibiotics employing the blueprint of natural lantibiotics. Based on their structural and functional features, natural lantibiotics are dissected into modular subunits. These subunits are then shuffled to generate thousands of novel, putative active, chimeric lantibiotics. The lantibiotic genes are subsequently produced by combinatorial DNA *de novo* synthesis.

These libraries will then be screened for molecules with high antimicrobial activity. To enable for high screening rates we developed a platform based on nL-sized reaction vessels (nL-reactors) that are used for peptide production and activity-screening in a single step and at rates of 10^5 variants per day. During screening, library cells are grown to microcolonies within nL-reactors along with a sensor strain serving as a model for a pathogen. Library cells secreting an active antimicrobial peptide will deactivate the sensor cells within the encircling nL-reactor. Clearance of an nL-reactor from the sensor thus indicates the presence of a strain secreting a highly active peptide. We use large particle flow cytometry and fluorescently labeled cells in order to isolate promising candidates.

We will present results from the screening of a peptide library containing 10^4 different chimeric variants derived from the module-shuffling of ten natural lantibiotics. Several interesting antimicrobials have been isolated, demonstrating for the first time the generation of new-to-nature lantibiotics by module-shuffling.

Keywords: lantibiotics; peptide-modules; module-shuffling; nL-reactor; screening; antimicrobial peptides; AMP

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The value of morphological characterisation of bacterial colonies in microbial diagnosis and clinical decision-making

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During the course of infection, microorganisms go through genetic and physiological changes to survive the selective pressures imposed by the human immune system and the antibiotic treatments. Colony morphological manifestations of such antimicrobial responses are fairly immediate and inexpensive to obtain experimentally, and can be a very useful tool in clinical decision making. Several morphotypes have already been associated to chronic infections and device-associated infections. For example, *P. aeruginosa* mucoid variants are typically isolated from cystic fibrosis lungs at chronic stages. These colony variants are markedly resistant to common antibiotics, such as gentamicin, aminoglycosides, ciprofloxacin and imipenem. Likewise, *S. aureus* small colony variants, often isolated from several chronic device-associated infections, display augmented resistance to several classes of antibiotics and, able to live intracellularly, and therefore surviving the action of both antibiotics and host immune defences.

Therefore, the aim of this work is to introduce a novel computer-assisted microbial morphotyping platform in support of microbial diagnosis and further clinical decision-making. A dataset of morphotypes, extracted from the publicly available at MorphoCol database (http://morphocol.org), exemplifies how the platform assists in the manual morphological characterisation, collects data from automatic image processing tools, clusters colonies that show observable similar morphologies and describes the antibiotic susceptibility of the individual groups. Results show that key colony features, such as size, consistency and texture, can be in fact predictors of pathogenic potential of bacteria. Therefore, new colonies may be matched against the described groups, enabling the formulation of a preliminary diagnosis and therapeutics based on the previous reports..

Keywords: clinical decision making; data mining; colony morphology; antibiotic susceptibility

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TLR expression in dendritic cells under the influence virus vaccines in combination with chitosan as adjuvant

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The aim of the investigation was to study the effect of live and inactivated virus vaccines combined with chitosan on intracellular TLR expression in human DCs. Experiments involved the use of 1% chitosan glutamate solution that was added in equal volume to a vaccine (final concentration of preparation 0,5%). There were applied: 1) trivalent inactivated split subunit vaccine "Agrippal" (Novartis vaccines and diagnostics, Italy) and 2) live cold-adapted virus (CA) A/Krasnodar/101/35/59 (I.I. Mechnikov Research Institute of Vaccines and Sera, RAMS, Moscow); 3) inactivated "Immovac" and 4) live polio vaccine for oral administration of 1, 2, and 3-type. To obtain DCs the suspension of human mononuclear cells were cultivated in the presence of recombinant GM-CSF and IL-4 ("BioSource International Inc.", Belgium). On the 3rd day the same cytokines were added. On the 6th day of incubation the medium was changed and supplemented with vaccines under study (50 µl/ml each), chitosan derivatives (1% of chitosan glutamate solution, 50 µl/ml) or vaccines with chitosan solution (50 µl/ml + 50 µl/ml) for induction of TLR expression. Two days later DCs were washed from the culture medium and used for experiment. TLR expression in DCs was realized via flow cytometry using monoclonal antibodies against antigens to TLR3, 7, 8, 9. DC culture obtained from peripheral blood monocytes demonstrated the pool of both plasmacytoid and myeloid DCs that were activated in the presence of viral preparations, as well as combination of above vaccines with chitosan. Yielded DCs can influence the direction of immune response as the direction of CD4+ cell differentiation depended on TLR type being involved in signal initiation, as well as the nature of the pathogen or antigen invaded the body. Currently it is determined that specific TLRs differently induce Th1 and Th2 immune responses and definite DC subpopulations differentially respond to various PAMPs as these express different TLRs. Thus, the direction of immune response is determined by DC-expressed TLR type [Colonna M]. The study of mechanisms of TLR ligand recognition, TLR expression and effector molecules, signal transduction, as well as TLR gene polymorphism is essential in revealing the immunodeficiency states related to impairment of TLR functional activity including the progression of severe infectious diseases (sepsis, meningitis), autoimmune diseases, atherosclerosis and allergic pathology. Besides, methods of evaluation of TLR components appear to be an important issue in the development of various immunomodulating and vaccine preparations and adjuvants.

Keywords: virus vaccines; chitosan; dendritic cells

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Tools of testing efficacy of photodynamic inactivation to pathogenic microorganisms *in vitro* and *ex vivo*

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Photodynamic inactivation (PDI) is one of promising methods useful for an effective eradication of pathogenic microorganisms. PDI is an efficient tool for inactivation of microorganisms via an excitation of photosensitive compounds with light of appropriate wavelength resulting in the production of cytotoxic reactive oxygen species causing damage of microorganisms. Methylene blue (MB) is a cationic phenothiazine dye binding to the chemical groups with a negative charge. It is widely used because of its low price, high photoactivity, and ability to enter into oxidation-reduction processes. Several steps are important for *in vitro* PDI testing. Selection of appropriate conditions, like medium, source of light, duration of excitation, and evaluation of results is critical for the optimization of experiment. The choice of media can be important because of possible interference between media components and MB. For example, in the *Candida* research (the most frequently isolated genus of clinically relevant yeasts), RPMI-MOPS with phenol red is the generally used medium for testing an efficiency of different drugs to *Candida* yeasts growing like planktonic culture or forming biofilm. Experiments are usually evaluated using spectrophotometer at OD_{560} (planktonic culture) or OD_{490} when biofilm is determined using tetrazolium salts (Ramage et al., 2001). In both cases, phenol red interfered with MB; moreover, tetrazolium salts also interacted with MB at higher concentrations. Therefore, in our experiments, only RPMI-MOPS without phenol red was used and spectrophotometry was accompanied by the determination of live cells through the calculation of the colonies formed on solid media - colony forming units (CFU) (Bujdak et al., 2009). Concerning light source, it was necessary to take into account absorption and emission spectrum of MB. This was a reason, why we changed light source of wide-spectrum (400-700 nm, 7.404 $W.m^{-2}$) to red monochromatic LED light (580-670 nm, 16.7 $W.m^{-2}$) despite of the fact that both light sources covered excitation maximum of MB. Optimal distance of the light was also important, because PDI decreased with higher distance. Duration of light irradiation should be kept the shortest as possible, but still efficient for PDI effect to be observed. It was in the range of several seconds to some hours. We optimized a distance to 15 cm, but the duration of light application depended on tested microorganisms; from several min (*Streptococcus mutans* and *Staphylococcus auresus*) to 2 h (*Escherichia coli* and *Candida albicans*). An effect of PDI was also tested using *ex vivo* model that was originally developed for this purpose. This model employed mouse tongues from 7-8 week old BALB/c female mice. The control tongue was cultivated only with the yeast suspension and compared with those incubated with yeasts and 1 $mmol.L^{-1}$ MB and then irradiated for 2.5 h. Then cryo cuts of mouse tongues stained with Periodic Acid Schiff were evaluated by light microscopy. In this experiment, amount of inoculum and optimal duration of pre-incubation with microorganisms were important parameters in order to form sufficient biofilm in a control sample. In summary, this study developed methods which are useful in a correct evaluation of PDI of microorganisms using MB like photoactive compound.

Keywords: photodynamic inactivation, methylene blue, microorganisms, biofilm

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Use of the collections of pathogenic bacteria from the Microbial Resource Centre CIRM-BP to evaluate the antibacterial potential of candidate molecules

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The International Centre for Microbial Resource dedicated to pathogenic bacteria (CIRM-BP) is located within the French National Institute for Agricultural Research (INRA) centre of Val de Loire. The activity of the CIRM-BP is dedicated to the conservation and the distribution of strains of bacterial pathogens isolated from animals or from human as well as the distribution of their genomic DNAs. The CIRM-BP is ISO 9001 certified since 2008.

Its collection comprises around 2500 strains belonging to 57 genera and 167 species of bacteria of risk groups 2 and 3. The CIRM-BP offers services related to strain identification, characterization and preservation. The CIRM-BP also develops scientific partnerships for studying biodiversity.

Beside these activities, the CIRM-BP takes advantage of its collections of pathogenic strains to study the antibacterial potential of different molecules proposed by its collaborative partners.

In that aim, a panel of pathogenic bacterial strains is first constituted considering the intended target for the candidate molecules (for example pathogenic bacteria specific of the production systems of farm animal). This leads to the choice of the bacterial species to be tested and of the strains, in function of their origin and context of isolation.

Different techniques are then used to evaluate the Minimum Inhibitory Concentration (MIC) depending of the available quantity of candidate molecules. In each case, the MIC is determined as the lowest concentration of molecules or extract, inhibiting visible growth of bacteria.

When the quantity is not a limiting factor, the evaluation begins by a screening on a panel of tens of strains. Each tested molecule is thus embedded in an agar growth media, on which, the standardized bacterial inocula (0.5 McFarland, 10 fold diluted) are then spotted using a Denley multipoint inoculator before incubation (10^5 CFU/spot).

To control the obtained results or when the available quantity of candidate molecules is low, for directly investigating the MIC, a turbidimetric micromethod employing the Bioscreen C apparatus (Thermo Fisher Scientific, Saint-Herblain, France) is used. This automated growth curve analysis system follows multiple kinetic bacterial growths in 100-well honeycomb microplates (100-300 µl final volume). The candidate molecules are serially two-fold diluted in broth and then bacterial suspension of mid-exponential growth phase are added to each well leading to a final bacterial concentration of $5x10^5$ CFU/mL. The plates are then incubated in the Bioscreen.

In case of very limited quantities of molecules to be tested, the radial *diffusion agar* overlay/underlay assay developed by Lehrer *et al.* (1991) is used to get a first insight of the bacterial inhibition spectrum [1] before to precisely determine the MICs with the Bioscreen on suitable species only.

Currently, the CIRM-BP works on the evaluation of the antibacterial properties of some molecules originating from natural products such as eggs [2], wood or algae.

Keywords: pathogenic bacteria; culture collection, MIC

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VITEK® 2: An automated antimicrobial susceptibility testing for detection of yeasts

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Yeast infections have acquired great importance due to increasing frequency of immunocompromised patients or patients undergoing aggressive diagnostic and therapeutic techniques, and because of the high morbidity and mortality rates associated with these infections. Additionally, an increase in the emergence of new pathogenic species that are difficult to diagnose and treat has been observed. The aim of this study is to determine and compare the *in vitro* susceptibility of 89 strains of yeasts to the antifungals: amphotericin B, voriconazole, fluconazole and flucytosine, with the VITEK 2® system. The antifungigram was performed automatically by the Vitek 2 Systems ®. The origin of the yeasts was: Group 1 - microbiota of wild animals (W) (26/89), 2 - milk from subclinical mastitis (M) (27/89) and 3 – hospital setting (H) (36/89). Of the 89 yeasts submitted to test Vitek 2®, 25 (20.9%) were resistant to fluconazole, eleven (12.36%) to amphotericin B, three (3.37%) to voriconazole, and no sample was resistant to flucytosine. There was a broader spectrum of action, in decreasing order, of antifungal flucytosine, voriconazole, amphotericin B and fluconazole, where the sensitivity rates were respectively 95.5%, 80%, 80% and 58.8%. Regarding the minimum inhibitory concentration (MIC) of the yeasts studied, fluconazole showed an MIC between one and 64 mg / mL for the three groups, voriconazole had an MIC between 0.12 and 8 mg / mL, amphotericin B had an MIC between 0.25 and 4 mg / mL for group H and the group W, and between 0.25 and 16 mg / mL for group M and flucytosine had an MIC equal to 1µg/mL for all groups. The yeasts isolated from the human hospital (H) showed the greatest resistance to fluconazole 12/89 (13.49%), followed by group W (7.87%) and group M (5.62%). The more resistant group to voriconazole was followed by the M and H groups, the W group showed no resistance to this antifungal. Group H was the least resistant (2.25%) to amphotericin. Results suggest that increased exposure of yeasts isolated from a human hospital environment to the drugs studied, in relation to those isolated from animals, causes them a greater resistance to fluconazole especially where significant difference ($p < 0.05$), was observed. This study proves resistance against the available antifungal agents has been increasing over time, while confirming the importance of determining sensitivity of the yeast to specific antifungals to help prevent resistance or potential drug overdose.

Keywords: antifungical; resistence; yeast

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Ellagic acid derivatives from *Terminalia chebula* Retz. increase the susceptibility of *Pseudomonas aeruginosa* to stress by inhibiting polyphosphate kinase

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Polyphosphate kinase 1(PPK1) plays an important role in the virulence, survival under stress conditions and maintenance of cellular structure and ,therefore, is an attractive therapeutic target to control the infections caused by MDR *P. aeruginosa* strains.

Ellagic acid derivatives (EADs), fractionated from the fruit of *T. chebula* Retz., caused 93% reduction in *P. aeruginosa* PAO1 *ppk1*gene expression ($p<0.05$) and complete inhibition of its activity ($p<0.01$), at 0.5mg/ml. Transmission electron microscopy of EADs-treated *P. aeruginosa* also showed marked reduction in polyphosphate granules in the cytosol and thinning of cell envelope and cytoplasm. Expression of *rpoS*, the downstream master stress response regulator, was also reduced by 94% ($p<0.05$) and the sensitivity of *P. aeruginosa* PAO1increased to different *in vitro* stresses.

0.14% of bacterial cells population was recovered after one day of desiccation which reduced to 0.06% on treatment with EADs. PAO1 showed 0.45% resistant cells when exposed to oxidative stress generated by hydrogen peroxide and this population reduced to 0.13% in the presence of EADs which could be because of decreased catalase and superoxide dismutase (91 and 81% reduction, $p<0.001$) activities.

Also, persister cells formed in response to piperacillin (5XMIC) stress, decreased in the presence of EADs from 1.3 to 0.03 %. PPK-regulated swimming, swarming and twitching motilities and biofilm formation were also reduced significantly ($p\leq0.05$) in PAO1 and the clinical strains of *P. aeruginosa* by EADs. The results show that natural compounds like EADs can be very effective against antibiotic resistant pathogen.

Key words: Ellagic acid derivatives, *Terminalia chebula*, *P.aeruginosa*, *ppk*, *rpoS*, stress, persister cells, motilities

The growth and toxigenic potential of *Bacillus cereus* during storage temperature abuse in cooked irradiated rice

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Rice is probably the food most associated with food poisoning by *Bacillus cereus*. Given that temperature abuse can occur at different stages of production and distribution of rice. The objective of this study was to evaluate the effect of storage temperature abuse on the growth and potential toxicity of *B. cereus* for combined treatments involving low doses of gamma irradiation in combination with antimicrobial agents (nisin and / or carvacrol). Treated and none treated rice spiked with endospores was incubated at 10 °C for 2 weeks. Microbial population was examined using plate counting on MYP agar. Concurrently, toxigenic potential was measured through recording enterotoxins and phosphatidylcholine-specific phospholipase C (PC-PLC) activity. The results showed that compared to rice samples processed only with higher concentrations of antibacterial agents, the application of reduced concentration of antimicrobials to half in combination with low gamma irradiation doses resulted in a significant decrease ($p \leq 0.05$) of *B. cereus* accounts and this decrease was proportional to the irradiation dose. Toxin production was delayed by irradiation and a total absence of enterotoxin was observed in irradiated rice at 1.8 kGy in the presence of nisin alone or in combination with carvacrol until the 12^{th} day of storage at 10 °C. PC-PLC expression in rice samples inoculated with *B. cereus* was found closely related to the cell density. Reduced proliferation of *B. cereus* obtained by combined treatment was associated with a limitation of toxin production and led systematically to significant decrease ($p \leq 0.05$) of PC-PLC activity during storage.

Keywords: *Bacillus cereus*; sub-lethal stresses; radio-sensitization; carvacrol; nisin; combined treatments.

Effect of storage duration on microbial load of Orange pomace

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Introduction

Residue from the processing of fruits and vegetables, traditionally considered as an environmental problem are being increasingly recognized as sources for obtaining high-phenolic products. The polyphenolics from waste materials, being derived from agro-industrial production, may be used as functional food ingredients and as natural antioxidants (Zhou *et al*., 2009). It is well known that by-products represent an important source of sugars, minerals, organic acid, dietary fibre and phenolics which have a wide range of action which includes antitumoral, antiviral, antibacterial, cardioprotective and antimutagenic activities (Djilas et al., 2009). Thus the objective of this work is to determine the extent of microbial load of orange pomace with time in order to establish its safety for human consumption.

Materials and methods

Orange pomace was collected after juice extraction from the Product Development Programme of the National Horticultural Research Institute, Ibadan, Nigeria. Pomace samples were kept at ambient temperature of $32^{O}C$, representative samples were taken at every two hours over a period of eight hours for microbial analysis. The culture media used were Potato Dextrose Agar (PDA) to enumerate fungi and Nutrient Agar (NA) to enumerate bacteria. Petri dishes containing the media were inoculated with 1Ml of serially diluted samples. Dishes containing NA were incubated at $27^{0}C$ for 24hrs while those with PDA were incubated at $27^{0}C$ for 48hrs before enumeration.

Result and Discussion

Progressive increase in total bacterial count was observed from samples collected from 0 to 8hr, the highest count was 8hrs with total count of 2.6×10^{3}cfu/g while the least was at 0hr with total count of 1.1×10^{2}cfu. The difference in the count was statistically significant at $p < 0.05$. After 48hr of incubation, there was decline in total fungal count from 0hr (5.4×10^{2} cfu/g) to 8hr (0 cfu/g), this could be attributed to release of phytotoxic substances from the pomace which has inhibitory effect on the growth of fungi. Progressive decrease was observed in *Aspergillus flavus* count from 0hr to 8hr of sampling which suggest that phytotoxic substances produced by the pomace inhibit growth of *A. flavus* which eventually led to the death of *A flavus* that initially grew on the pomace. A significant difference was observed in microbial load generally at $p < 0.05$.

Conclusion

A very rapid increase in bacteria count was observed from 6hr to 8hr, this suggests that the pomace can be utilized up to six hours after juice extraction to avoid the high bacterial load, though the fungal growth inhibition continues afterwards.

Keywords: Orange pomace; microbial load

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