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Achromobacter spp. prevalence and adaptation in cystic fibrosis lung infection

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ABSTRACT

Bacteria belonging to the genus *Achromobacter* are widely distributed in natural environments and have been recognized as emerging pathogens for their contribution to a wide range of human infections. In particular, patients with cystic fibrosis (CF) are the subjects most frequently colonized by *Achromobacter* spp., which can cause persistent infections in their respiratory tract. Although many clinical aspects and pathogenic mechanisms still remain to be elucidated, *Achromobacter* spp. have been a source of expanding interest in recent years. This review examines the current literature regarding *Achromobacter* spp. role in CF, focusing on taxonomy, prevalence in CF lung infections, genomic characteristics, and adaptation strategies including modifications of metabolism and virulence, acquisition of antibiotic resistance, exchange of mobile genetic elements and development of hypermutation.

1. Introduction

Achromobacter spp. are non-lactose fermenting, catalase and oxidase positive Gram-negative bacilli widely distributed in the environment, mainly in moist soil and water sources but also in plants (Edwards et al., 2017). These motile opportunistic pathogens are mainly found in wet environments and are increasingly isolated also in nosocomial settings. Nosocomial outbreaks are often caused by contamined disinfectant solutions, dialysis fluids, saline solutions and deionized water (Gomila et al., 2014). *Achromobacter* spp. strains are usually resistant to a variety of antibiotics and disinfectants, due to both innate and adaptive antibiotic resistance (Edwards et al., 2017). *Achromobacter* spp. colonization events have been associated with a variety of infections such as bacteraemia, meningitis, pneumonia, peritonitis and urinary tract infections (Amoureux et al., 2016a; Neidhöfer et al., 2022). In addition, other conditions such as renal disease, cancer, diabetes, and endocarditis increase the risk of *Achromobacter* infection. These infections usually occur in subjects with underlying immunodeficiency, in subjects with impaired airway clearance due to chronic lung disease and in subjects that underwent surgical procedures. In particular, *Achromobacter* strains are primarily isolated from the respiratory tract of cystic fibrosis (CF) patients (Edwards et al., 2017) where these microorganisms can persist for a long time in both lower and upper airways (Hansen et al., 2010).

CF is a monogenic autosomal recessive disorder that is strictly linked with chronic bacterial respiratory infections: persistent airways infections and the ensuing prolonged lung inflammation lead to lung insufficiency, which accounts for the majority of CF morbidity and mortality (Quon and Rowe, 2016). Many opportunistic pathogens can cause lung infections in people with CF, among them *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, *Achromobacter* spp., non-typeable *Haemophilus influenzae*, *Aspergillus* and nontuberculous mycobacteria (Gibson et al., 2003). Although some retrospective studies have found that *Achromobacter* spp. infection in people with CF has no statistically significant impact on lung function (Edwards et al., 2017; Raso et al., 2008; Lambiase et al., 2011; De Baets et al., 2007; Tan et al., 2002), this is controversial since it has been reported that infection with the type

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species *Achromobacter xylosoxidans* results in a heightened host inflammatory response (Hansen et al., 2010). Moreover, chronic infections with *A. xylosoxidans* lead to a greater number of pulmonary exacerbation events (Edwards et al., 2017) and annual hospitalizations (Firmida et al., 2016; Rønne Hansen et al., 2006), thus highlighting the growing role of this opportunistic pathogen in CF.

In the last ten years the number of publications regarding *Achromobacter* spp. has more than tripled in comparison to the preceding decade (Fig. 1), underlining both the increasing research interest for these microorganisms as well as their improved recognition and emergence in the clinical setting, especially in CF. This review examines the current literature regarding *Achromobacter* spp. role in CF, focusing on taxonomy, prevalence in CF lung infections, genomic characteristics and adaptation strategies including modifications of metabolism and virulence, acquisition of antibiotic resistance, exchange of mobile genetic elements and development of hypermutation.

2. Research interest

Although primarily isolated from the airways of people with CF, *Achromobacter* spp. can cause a broad range of infections in hosts with other underlying conditions. Not only are these bacteria able to establish chronic infections associated with lung inflammation in people with CF (Hansen et al., 2010; Lambiase et al., 2011), they also produce biofilm, resist common disinfectants (Gomila et al., 2014; Günther et al., 2016), readily acquire antibiotic resistance (Trancassini et al., 2014) and outcompete resident microbiota (Talbot and Flight, 2016; Jeukens et al., 2017). This could be some of the reasons why there has been an increase in research interest regarding *Achromobacter* spp. in the last 20 years (Fig. 1). In particular, thanks to the advent of next-generation sequencing technologies, the rise in number of publications regarding this CF emerging pathogen has been followed by a steep increase in whole genome sequencing (WGS) data availability (Vincent et al., 2017; Land et al., 2015).

3. Taxonomy

Achromobacter spp. are classified as members of the β-proteobacteria and belong to the order of *Burkholderiales*, which also includes the *Burkholderia* genus. The family name of *Achromobacter* is *Alcaligenaceae,* which is the same family *Bordetella* and *Alcaligenes* belong to (Gomila et al., 2014). Phylogenetically, *Achromobacter* has been found to be closely related to the genus *Bordetella*, most members of which are human pathogens involved in respiratory infections, and a common origin of these microorganisms has been suggested (Li et al., 2013; Gross et al., 2008; Melvin et al., 2014).

Membership within the *Achromobacter* genus is continuously evolving, with recently named novel species being described since 2016 (Vandamme et al., 2016a; Kuncharoen et al., 2017; Singh et al., 2017; Green and Jones, 2018). Indeed, recent studies have resulted in the reclassification of previously described species: *A. spiritinus* has been reclassified as *A. marplatensis,* and *A. sediminum* has been reassigned to the novel genus *Verticia* (Vandamme et al., 2015, 2016b).

Current approaches for identifying members of the *Achromobacter* genus include biochemical testing (e.g. VITEK2), *nrdA* or 16 S rRNA gene sequencing, multi-locus sequence typing (MLST), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). In routine clinical microbiology laboratories, where time-sensitive results are necessary, bacterial identification is often carried out by biochemical testing even though it allows a less accurate species determination and at times results in misidentification due to *Achromobacter* spp. biochemical similarities with other Gram-negative bacilli (Fernández-Olmos et al., 2012; Alby et al., 2013; Isler et al., 2020).

Another identification method is MALDI-TOF MS which allows an accurate identification at genus level (Fernández-Olmos et al., 2012; Alby et al., 2013; Degand et al., 2008). Nonetheless, accurate identification at species level is still hindered by the limited number of species included in MALDI-TOF databases (Isler et al., 2020). Attempts to improve MALDI-TOF accuracy by increasing the number of species

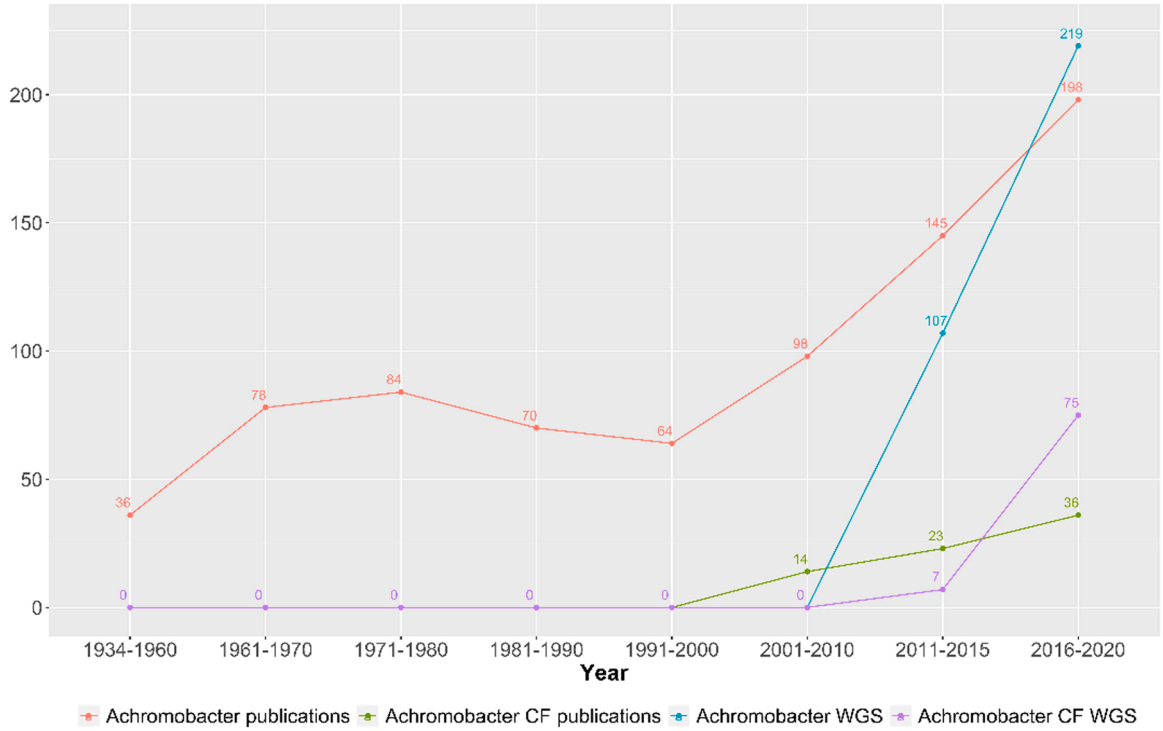

Fig. 1. *Achromobacter* research interest as of February 03, 2021. The number of publications is based on a literature search using PubMed (https://pubmed.ncbi.nlm.nih.gov) with Title=*Achromobacter* and Title=*Achromobacter* AND cystic fibrosis, while the number of whole genome sequencing (WGS) data is based on the content of NCBI (https://www.ncbi.nlm.nih.gov/Traces/wgs/) with Term=*Achromobacter* and Project type=WGS.

included in the databases presented promising results (Garrigos et al., 2021; Papalia et al., 2020a) and their incorporation into commercial databases will allow a more accurate identification at species level.

As regards sequence-based identification, *nrdA* gene sequencing or MLST (MLST scheme comprises 7 genes, namely *nusA, rpoB, eno, gltB, lepA, nuoL,* and *nrdA* - available at PubMLST (Jolley et al., 2018)) offer a more accurate species recognition (Spilker et al., 2012, 2013), whereas 16 S rRNA gene sequencing has been shown to not being able to ensure a definitive species identification due to the conserved nature of the gene (Gomila et al., 2014). With the advancement of sequencing techniques, WGS has become an affordable method for accurately identifying bacteria while obtaining a great amount of data enclosing a richness of information. The increasing use of WGS will enable correct identification of *Achromobacter* to the species level, including the reassignment of taxa that have historically been incorrectly speciated.

To date, the genus *Achromobacter* comprises 22 named species and multiple genogroups (Parte et al., 2020); WGS data is available for all 22 species but complete reference genomes are available only for 6 species, namely *Achromobacter deleyi, Achromobacter denitrificans, Achromobacter insolitus, Achromobacter pestifer, Achromobacter spanius,* and *Achromobacter xylosoxidans*. Detailed information regarding *Achromobacter* child taxa with a validly published name is reported in Table 1.

4. Genome and pan-genome

The first *Achromobacter* spp. complete genome sequence published was that of an environmental strain isolated from soil (*A. xylosoxidans* A8, RefSeq accession: GCF_000165835.1) (Strnad et al., 2022b) whereas the first complete genome assembly of a clinical isolate from a CF patient (*A. xylosoxidans* NH44784–1996, accession: GCF_000967095.2) was published two years later, in 2013 (Jakobsen et al., 2013). Complete reference genomes are available for 6 *Achromobacter* species (out of 22; Table 1).

The *Achromobacter* spp. genomes (Strnad et al., 2022b; Jakobsen et al., 2013; Badalamenti and Hunter, 2015; Li et al., 2018, 2017, 2020; Méndez et al., 2018; Reis et al., 2017; Wass et al., 2019) consists of a single chromosome comprising an average of 6.5 Mbp (range=5,876,039–7,013,095 bp), presents a relatively high GC content (mean=65.5%, range=63.8–67.7%), and a mean of 5978 (range=5328–6459) open reading frames (ORFs) have been predicted with a coding density of ~90% (information obtained from the annotation files of *Achromobacter* spp. complete genome sequences available on NCBI - n = 41 - accessed December 2021). These genomic features resemble those of major CF pathogens such as *P. aeruginosa* (median total length: 6.6 Mbp; median ORFs count: 6097; median GC: 66.2% - source: NCBI) and *Burkholderia* spp. (median total length: 7.7 Mbp; median ORFs count: 6916; median GC: 66.7% - source: NCBI). Of note, a mean of 19% (range=10–29%) of ORFs still remain classified as having hypothetical function in *Achromobacter* spp., suggesting that some aspects of metabolism, pathogenic potential or adaptation mechanisms might still need to be elucidated and characterized with further studies.

Pan-genome analysis (Jeukens et al., 2017; Li et al., 2013) revealed that *Achromobacter* spp. has an open pan-genome and its conserved core genome consists of ~30% of the genes carried in an average genome of this genus. This means that a great part of the pan-genome is categorized as accessory genome, which comprises genes that are not conserved among isolates. Typically, these features coupled with such a large genome size characterize species living in a community with frequent lateral gene transfer and high adaptability to diverse environmental conditions (Tettelin et al., 2008; Rouli et al., 2015).

5. Contribution to CF infections: prevalence and species variation

Prevalence data from the latest European CF patients annual registry (2020) (ECFS Patient Registry, 2020) showed a high number of

Table 1
Achromobacter child taxa with a validly published name (information retrieved on LPSN (Parte et al., 2020) on 23–06–2022). The RefSeq accession numbers of taxa with reference genomes having "complete genome" as assembly level on NCBI are reported. NA = not available.

Child taxa	Reference (ref)	Current taxonomic status	Ref Seq assembly accession (number of available strains)
Achromobacter aegrifaciens	Vandamme, 2013 (Vandamme et al., 2013a)	Correct name	NA
Achromobacter agilis	Vandamme, 2016 (Vandamme et al., 2016a)	Correct name	NA
Achromobacter aloeverae	Kuncharoen, 2017 (Kuncharoen et al., 2017)	Correct name	NA
Achromobacter animicus	Vandamme, 2013 (Vandamme et al., 2013b)	Correct name	NA
Achromobacter anxifer	Vandamme, 2013 (Vandamme et al., 2013a)	Correct name	NA
Achromobacter deleyi	Vandamme, 2016 (Vandamme et al., 2016a)	Correct name	GCF_016127315.1 GCF_013116765.2 GCF_021432025.1 Amoureux et al. (2016a)
Achromobacter denitrificans	Coenye, 2003 (Coenye et al., 2003a)	Correct name	GCF_013267375.1 GCF_013267395.1 GCF_003812265.1 GCF_002205315.1 GCF_013343095.1 GCF_001514355.1 Quon and Rowe (2016)
Achromobacter dolens	Vandamme, 2013 (Vandamme et al., 2013a)	Correct name	NA
Achromobacter insolitus	Coenye, 2003 (Coenye et al., 2003b)	Correct name	GCF_008245125.1 GCF_001971645.1 GCF_900637265.1 GCF_000783435.2 Neidhöfer et al. (2022)
Achromobacter insuavis	Vandamme, 2013 (Vandamme et al., 2013a)	Correct name	NA
Achromobacter kerstersii	Vandamme, 2016 (Vandamme et al., 2016a)	Correct name	NA
Achromobacter marplatensis	Gomila, 2011 (Gomila et al., 2011)	Correct name	NA
Achromobacter mucicolens	Vandamme, 2013 (Vandamme et al., 2013b)	Correct name	NA
Achromobacter pestifer	Vandamme, 2016 (Vandamme et al., 2016a)	Correct name	GCF_013267355.1 Edwards et al. (2017)
Achromobacter piechaudii	Yabuuchi, 1998 (Yabuuchi et al., 1998)	Correct name	NA
Achromobacter pulmonis	Vandamme, 2013 (Vandamme et al., 2013b)	Correct name	NA
Achromobacter ruhlandii		Correct name	NA

(*continued on next page*)

Table 1 (*continued*)

Child taxa	Reference (ref)	Current taxonomic status	Ref Seq assembly accession (number of available strains)
	Yabuuchi, 1998 (Yabuuchi et al., 1998)		
Achromobacter sediminum	Zhang, 2014 (Zhang et al., 2014)	Synonym (*Verticia* spp.)	NA
Achromobacter spanius	Coenye, 2003 (Coenye et al., 2003b)	Correct name	GCF_900636675.1 GCF_002812705.1 GCF_003994415.1 GCF_002966795.1 Neidhöfer et al. (2022)
Achromobacter spiritinus	Vandamme, 2013 (Vandamme et al., 2013b)	Synonym (*A. marplatensis*)	NA
Achromobacter veterisilvae	Dumolin, 2020 (Dumolin et al., 2020)	Correct name	NA
Achromobacter xylosoxidans	Yabuuchi, 1981 (Yabuuchi and Yan, 1971, 2022a)	Correct name	GCF_008432465.1 GCF_022870085.1 GCF_001457475.1 GCF_013343135.1 GCF_016728825.1 GCF_001558755.2 GCF_016027035.1 GCF_014490035.1 GCF_900475575.1 GCF_900010105.1 GCF_900009125.1 GCF_013282255.1 GCF_001558915.1 GCF_900009115.1 GCF_013282235.1 GCF_001559195.1 GCF_009363015.1 GCF_001051055.1 GCF_000165835.1 Land et al. (2015)

Achromobacter infections in Denmark, Belgium and Portugal (prevalence: 13.58%, 10.31% and 9.80% respectively), while other countries presented a prevalence around or lower than 10%. Moreover, a higher percentage of adults with *Achromobacter* infection has been reported when compared with data regarding pediatric patients.

Even though the European CF patients annual registry has only reported *Achromobacter* spp. infection data starting from 2018, there are national CF registries that have been reporting *Achromobacter* spp. data for a long time. One of them is the French CF registry (Menetrey et al., 2021), which reports an increase in patients colonized over 20 years (6, 7% of the patients in 2018 versus 3.1% in 1999).

Similarly to European data, the latest US annual registry (2020) showed that the prevalence of *Achromobacter* spp. varies by age group, with an increase of infections in adult patients (Patient Registry Annual Data Report, 2020). While a general trend analysis of comprehensive European data is not possible (*Achromobacter* spp. infection data are available starting from 2018), the reported prevalence for *Achromobacter* spp. appears to be stable at around 7%. Concordantly, prevalence data from the US annual registry reported that *Achromobacter* infections rose from 1.9% in 2005 to around 7% in 2011 and have since remained stable (Green and Jones, 2018). Overall, the prevalence of *Achromobacter* species in people with CF is less than 10% at the majority of centers worldwide. Although data about *Achromobacter* spp. prevalence remain rare and are not available for all countries, the distribution of *Achromobacter* species in people with CF appears to be different among countries with available data (Table 2 and Fig. 2): the type species *A. xylosoxidans* is the most often isolated *Achromobacter* species among people with CF (Spilker et al., 2013; Papalia et al., 2020b; Gade et al., 2017; Gabrielaite et al., 2021; Amoureux et al., 2016b; Coward et al., 2016; Veschetti et al., 2021a) in all countries, while *A. marplatensis* and *A. pulmonis* show the lowest prevalence. *A. insuavis* infections are reported with a similar frequency in Denmark and France (20–24% and 19%, respectively), and at a lower rate in UK (12%), Italy (8%) and Argentina (5%). *A. dolens* is most prevalent in US (17%) followed by Argentina (10%), while it has a prevalence $< 10\%$ in UK, Italy and Denmark (8%, 3% and 0–2%). Furthermore, *A. ruhlandii* prevalence is 17–25% in Argentina, US and in Denmark, where an outbreak was reported at two CF centers (Rønne Hansen et al., 2006; Ridderberg et al., 2011); *A. insolitus* seems to have a higher prevalence in Italy (12%) than in France and US (both 4%), and *A. aegrifaciens* has a prevalence of 12–15% in Italy and France while in Denmark is $< 5\%$. Although prevalence data is available in the literature, the limited number of people with CF included in some of the studies and the still suboptimal species-level identification techniques (Fernández-Olmos et al., 2012; Alby et al., 2013; Isler et al., 2020; Saiman et al., 2001; Kidd et al., 2009) coupled with the changing nomenclature hinder an accurate prevalence estimation.

The high prevalence of *A. xylosoxidans*, *A. ruhlandii*, *A. dolens* and *A. insuavis* among clinical isolates, coupled with the phylogenetic clustering of these species and their ability to develop chronic infections (Amoureux et al., 2016b; Barrado et al., 2013; Dupont et al., 2015),

Table 2
Achromobacter CF prevalence in different countries. The number of *Achromobacter* infected people with CF included in each study is reported in the last row.

Achromobacter species	Country						
	Argentina[a] (%)	Denmark - Aarhus (%)	Denmark – Copenhagen (%)	France – Dijon (%)	Italy - Verona (%)	United Kingdom[b] (%)	United States of America[c] (%)
A. aegrifaciens	–	5	2	15	12	–	–
A. dolens	10	2	–	–	3	8	17
A. insolitus	–	–	–	4	12	–	4
A. insuavis	5	24	20	19	8	12	–
A. marplatensis	2	2	–	–	–	2	–
A. pulmonis	2	–	–	–	–	–	–
A. ruhlandii	17	19	25	–	–	3	24
A. xylosoxidans	63	36	52	57	65	61	43
N. patients included in the study	41	43	51	47	26	96	341
Identification method	*nrdA* and bla_{OXA} sequencing, MLST	*nrdA* seq, MLST	WGS	*nrdA* seq, MLST	WGS	*nrdA* and bla_{OXA} seq, MLST, WGS	*nrdA* seq, MLST
Study reference	(Papalia et al., 2020b)	(Gade et al., 2017)	(Gabrielaite et al., 2021)	(Amoureux et al., 2016b)	(Veschetti et al., 2021a)	(Coward et al., 2016)	(Spilker et al., 2013)

[a] Six healthcare centers in Argentina were involved in the study.
[b] Study by the UK national reference laboratory.
[c] Eighty-six CF treatment centers in the US were involved in the study.

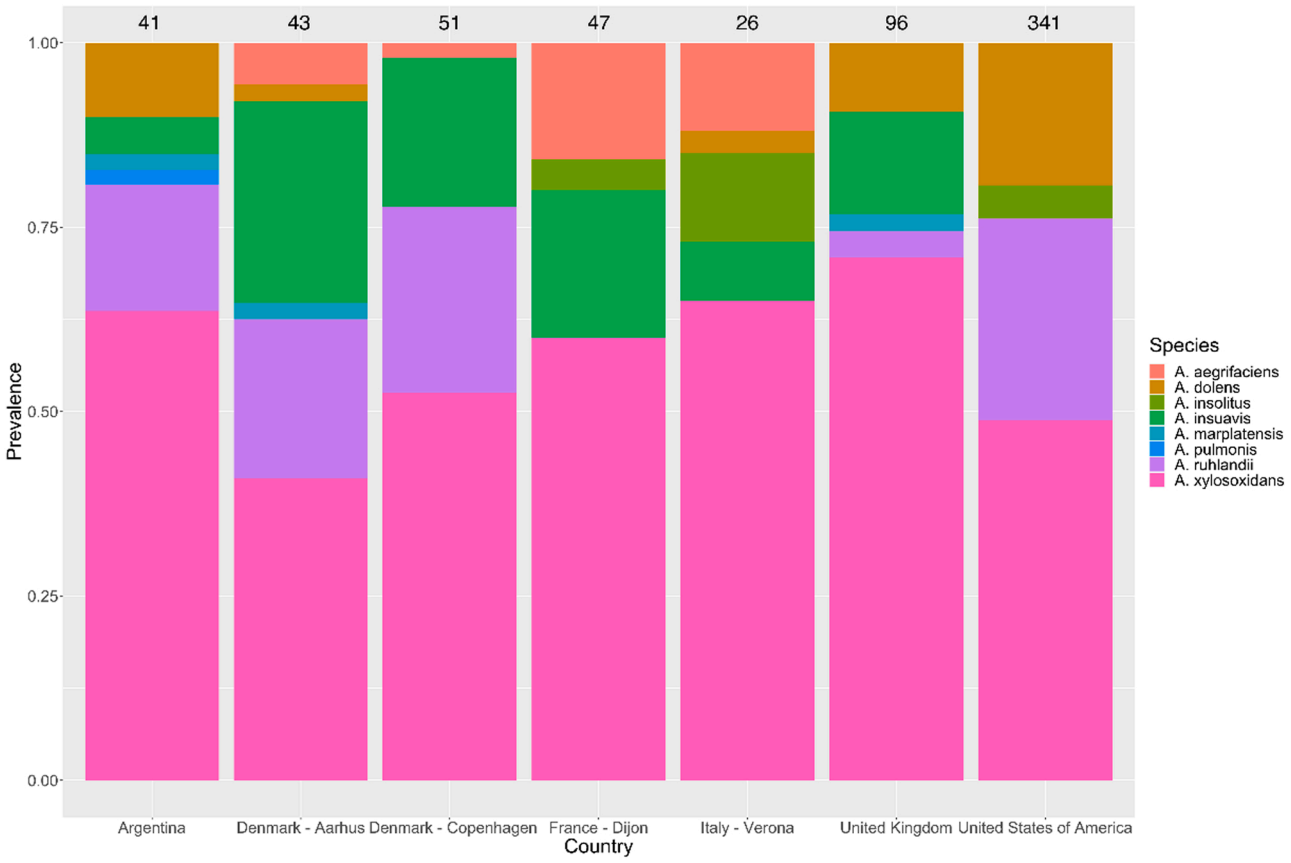

Fig. 2. *Achromobacter* CF prevalence in different countries. The number of *Achromobacter* infected people with CF included in each study is reported over each bar.

might indicate that they could be better adapted to cause opportunistic chronic infections (Jeukens et al., 2017). Even though more than half of people with CF with airway colonization by *A. xylosoxidans* develop chronic infections, usually associated with decline in respiratory function and lung inflammation (Hansen et al., 2010; Lambiase et al., 2011; Firmida et al., 2016; Gade et al., 2017; Amoureux et al., 2016b; Pereira et al., 2011), the clinical impact of different *Achromobacter* species is still not well characterized.

According to the available longitudinal studies (Gabrielaite et al., 2021; Ridderberg et al., 2011; Amoureux et al., 2013), the majority of people with CF with *Achromobacter* spp. chronic lung infection seem to harbor a unique strain or clone type (identified by WGS or PFGE). Interestingly, two of these studies (Gabrielaite et al., 2021; Amoureux et al., 2013) observed that 20–24% of patients were infected with more than one *Achromobacter* species and/or clone types over the sampling period, suggesting that not all *Achromobacter* spp. strains lead to chronic lung infections.

Cases of cross-infection among patients have been reported, even by indirect person-to-person transmission (Rønne Hansen et al., 2006; Hansen et al., 2013). For example, the *A. ruhlandii* Danish epidemic strain has been identified in multiple patients attending the same CF center and, more recently, patient-to-patient transmission was verified also for *A. xylosoxidans* and *A. insuavis* strains. In some cases, clear epidemiological connections (e.g. sibling pairs, visit-based) were found, but in other instances no epidemiological connection to support cross-infection could be identified (Green and Jones, 2018; Gabrielaite et al., 2021). In all the reported cases, WGS proved to be essential for *Achromobacter* species typing and identification of patient-to-patient transmission.

6. Adaptation strategies

The increase in the number of sequenced *Achromobacter* spp. genomes enabled researchers to focus at first on the genomic differences among clinical and environmental isolates and afterwards on the genomic determinants of pathogenicity and adaptation during persistent infections, allowing to identify a variety of adaptation mechanisms in the CF lung environment. A phylogenetic study aimed at evaluating differences among environmental and clinical strains showed that in the latter 35 genes involved in metabolism (COG functional categories: Amino acid transport and metabolism, Carbohydrate transport and metabolism, Cell wall/membrane/envelope biogenesis, Coenzyme transport and metabolism, Energy production and conversion, Inorganic ion transport and metabolism, Secondary metabolites biosynthesis, transport and catabolism, Lipid transport and metabolism), regulation, and efflux pumps were positively selected, and that this group of isolates carried a greater number of antibiotic resistance genes, namely for resistance against aminoglycosides, β-lactams, chloramphenicol and sulfonamides (Jeukens et al., 2017). Interestingly, it was shown that the most frequently mutated genes were involved in general metabolism (COG functional categories: Energy production and conversion, Amino Acid metabolism and transport, Carbohydrate metabolism and transport, Lipid metabolism, Inorganic ion transport and metabolism), which is the key to adapt to host conditions and outcompete the resident microbiota as well as other opportunistic pathogens (Sandri et al., 2021; Menetrey et al., 2020; Olive and Sassetti, 2016). Indeed, during CF lung colonization, bacteria survive under the selective pressure imposed by the host immune system and antibiotic therapies by increasing the efficiency in nutrient acquisition, developing the ability to avoid toxic compounds and to evade immune response, and promoting the colonization of new areas (Houry et al., 2012). An overview of *Achromobacter* spp. main adaptation strategies reported to date is represented in Fig. 3.

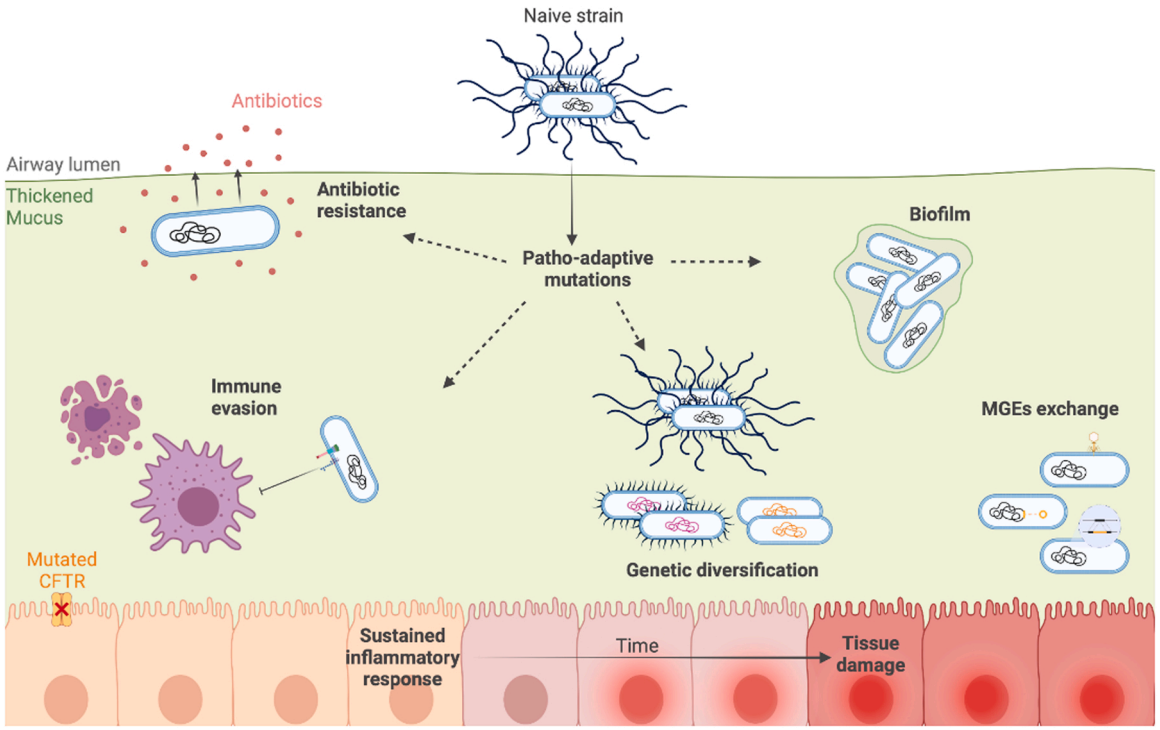

Fig. 3. *Achromobacter* spp. adaptation strategies reported in the literature to date: antibiotic resistance, immune evasion, genetic diversification, biofilm production and exchange of mobile genetic elements. MGEs = mobile genetic elements, CFTR = cystic fibrosis conductance transmembrane regulator. This figure was created on BioRender.com.

6.1. Metabolism

Studies on within-host evolution of *Achromobacter* spp. in people with CF have found that the most mutated genes during adaptation are mainly involved in general metabolism, leading to attenuation of functions not essential for survival in the CF lung environment, such as amino acid synthesis (since amino acid concentrations are elevated in CF sputum and bacteria have sufficient supply) (Ridderberg et al., 2015). In particular, the ability to survive with limited oxygen has been identified as one of the possible adaptive mechanisms favouring persistence of *Achromobacter* in the CF airways environment (Jeukens et al., 2017). Indeed, hypoaerobic/anaerobic growth ability confers to microorganisms the possibility to locate deeper within the mucous layer and within biofilm structures or in more hypoxic regions of the lung, where some antibiotics and penetration default can be dramatically less effective due to anaerobic conditions (Borriello et al., 2004). The use of denitrification for energy production in oxygen depleted environments, such as CF mucus, has been demonstrated for *P. aeruginosa*, another CF pathogen (Schobert, 2010), and there is evidence that molybdenum uptake, upon which denitrification depends, is essential for anaerobic proliferation and influences virulence in this pathogen (Pederick et al., 2014; Périnet, 2016). Results suggesting that *Achromobacter* and *P. aeruginosa* may share this adaptive mechanism have been reported in a study regarding CF clinical isolates (Jeukens et al., 2017), and anaerobic growth ability has also been suggested as a key difference between occasional and chronic infection isolates (Veschetti et al., 2021a).

6.2. Virulence

The *Achromobacter* genus has been found to be phylogenetically related to *Bordetella* and this strong link highlights the potentially pathogenic nature of this CF emerging pathogen from the phylogenetic perspective (Li et al., 2013; Gross et al., 2008; Melvin et al., 2014). The mechanisms underlying *Achromobacter* spp. ability to colonize the respiratory tract as well as other sites of the human body are still not fully clear, but a number of virulence factors have been described that likely support their invasiveness and survival in hostile environments. Similar to better-studied pathogens, biofilm production, motility, lower oxygen tolerance, and virulence factor secretion have been shown to play important roles in *Achromobacter* (mainly *A. xylosoxidans*) infection, adaptation, and persistence.

Biofilms consist of bacterial microcolonies encased in a matrix of polysaccharides, DNA and proteins and they can have variable morphology, growing attached to a surface or as unattached aggregates in mucus or in sputum (Mantovani et al., 2012; Høiby et al., 2017). Biofilm formation is a growth phenotype that many bacteria causing CF infections use for survival and proliferation in hostile environment; this bacterial ability allows to shield pathogens against environmental stress and increase tolerance towards antibiotics and host defences (Høiby et al., 2017; Nielsen et al., 2016). The presence of genes linked to biofilm formation in other bacteria has also been reported in *Achromobacter* spp. genomes. Among them, the *flgB* gene, which encodes a flagellar basal body rod protein implicated in *Bordetella bronchiseptica* and *P. aeruginosa* biofilm formation (Nicholson et al., 2012; de la Fuente-Núñez et al., 2012), and the pgaABCD operon, which encodes the polysaccharide β-1, 6-GlcNAc involved in both cell-cell adherence and cell-surface adherence in other CF pathogens (Jakobsen et al., 2013). Members of *Achromobacter* genus also present peritrichous flagella that enable swimming motility, contributing to biofilm formation and host cell invasion (Swenson and Sadikot, 2015). A study of 52 *A. xylosoxidans* strains isolated during an outbreak at a CF Centre in Rome (Italy) found that the great majority of strains were motile and biofilm producers; moreover, a significant prevalence of strong biofilm-producing strains was found in patients with severely impaired lung function (Trancassini et al., 2014). These results were explained with an enhanced adaptation of *A. xylosoxidans* to the CF nosocomial environment. In particular, biofilm production seems to play an important role in bacterial persistence as it has been reported that gene expression profiles and antimicrobial susceptibility at biofilm stage differ from planktonic cells. Indeed, in biofilm stage, *Achromobacter* spp. genes associated with

anaerobic respiration were found to be upregulated, suggesting the adaptation to the microaerobic and anaerobic conditions prevalent in the late stage of CF chronic infections (Nielsen et al., 2017). Interestingly, a reduced biofilm formation has been observed in chronic strains over time of infection, which may result from within-host adaptation to the CF lung during chronic colonization (Nielsen et al., 2016). *Achromobacter* spp. has also been reported to form mixed biofilms with *P. aeruginosa* in vitro, where it can affect *P. aeruginosa* biofilm formation (Sandri et al., 2021; Menetrey et al., 2020).

Similarly to other Gram-negative pathogens, *Achromobacter* spp. express membrane-bound virulence factors. Among them, the Vi capsular polysaccharide, which enables surface adhesion and protection from environmental toxins; the O-antigen, involved in eliciting the host immune response (Li et al., 2013); and lipopolysaccharide (LPS), which induces the production of key inflammatory cytokines such as IL-6, IL-8 and TNF-α (Mantovani et al., 2012). The accumulation of mutations in genes involved in LPS production has been suggested to be involved in *Achromobacter* spp. persistence in CF lungs, probably leading to a reduced recognition by the host defense system (Veschetti et al., 2021a). Indeed, a reduction in the number of LPS lipid A acyl chains by other bacteria was shown to modulate the recognition of LPS by toll-like receptors (Qureshi et al., 1991).

Among cell membrane components, secretion systems also have an important function in bacterial pathogenicity since they are involved in the release of toxins, proteases and other virulence factors. Numerous genes encoding different types of secretion systems have been identified in *Achromobacter* genome, including: type II secretion system (T2SS), which is widely conserved among γ-proteobacteria and is involved in the release of extracellular toxins and proteases; type III secretion system (T3SS) that delivers virulence factors directly into the host cell; type VI secretion system (T6SS) that mediates the transport by direct contact with the target cells; and type VII secretion system (T7SS) which includes sigma-fimbriae encoding genes (Green and Mecsas, 2016). In particular, T3SS is known to enhance the bacterial ability to infect host cells with effector proteins and to contribute in immune evasion (Li et al., 2013; Jakobsen et al., 2013; Swenson and Sadikot, 2015). Recently, a phospholipase A2 (PLA2) encoded by the majority of *A. xylosoxidans* genomes, termed AxoU, was identified as a T3SS substrate that induces cytotoxicity in macrophages suggestive of a pathogenic or inflammatory role in the CF lung (Pickrum et al., 2020). The presence of T3SS has also been considered as a key discriminant among clinical and environmental *Achromobacter* strains. Comparative genomics analyses showed that virulence genes related to T3SS are more common in *Achromobacter* CF isolates rather than in environmental strains (Jeukens et al., 2017; Li et al., 2013), linking this feature with the infection ability of these microorganisms. Furthermore, the presence of functional T3SS genes seems to be associated with the establishment of chronic infections in the CF lung, while occasional infection isolates show a lack of functional T3SS genes (Veschetti et al., 2021a).

As concerns secreted virulence factors, genomic studies reported the presence of genes encoding colicin V, a cytotoxic protein that likely gives *Achromobacter* spp. environmental advantages by eliminating competing flora and enabling tissue invasion, and AepA, which facilitates the production of cellulases and proteases enabling mucosal invasion (Jakobsen et al., 2013). The presence of secreted proteases was also recently assessed (Veschetti et al., 2020). In addition, production of phospholipase C was observed, which allows hydrolysis of phospholipids of the alveolar surfactants and tissue disruption (Pederick et al., 2014), and a heat-stable cytotoxic factor has been identified and associated with increase of pro-inflammatory cytokines in vitro (Mantovani et al., 2012).

Interestingly, it was also found that some *Achromobacter* spp. strains are able to inactivate *P. aeruginosa* quinolone signal (PQS), participating in the Quorum Sensing (QS) mechanisms used for coordination of gene expression when bacterial cells reach a critical cell density (Papenfort and Bassler, 2016). The QS creates a global regulatory network and is believed to regulate the expression of up to 12% of the *P. aeruginosa* genome (Lin et al., 2018). Therefore, the ability to disrupt the QS could give *Achromobacter* spp. a competitive advantage over *P. aeruginosa* and maybe over other CF pathogens like *S. maltophilia* during co-habitation in the same lung environment, e.g., by affecting their growth, motility and /or biofilm formation (Sandri et al., 2021; Menetrey et al., 2020; Soh et al., 2015).

6.3. Antibiotic resistance

An important factor for the survival of *Achromobacter* spp. within the host is its resistance to antibiotics, mediated by naturally occurring and acquired systems of defense, rendering infections particularly hard to eradicate. A variety of mechanisms contribute to the resistance patterns of bacteria such as production of degrading enzymes, efflux pump system or changes in the antibiotic target. Isler et al (Isler et al., 2020). recently wrote a comprehensive overview of *Achromobacter* antibiotic resistance mechanisms, so we shall report here a brief summary regarding this matter.

Achromobacter spp. show an innate resistance to many classes of antibiotics, especially to aminoglycosides, some monobactams (aztreonam), tetracyclines, some penicillins (penicillin G, ticarcillin) and cephalosporins, which include antibiotics relevant to CF lung infection treatment (Trancassini et al., 2014; Swenson and Sadikot, 2015; Almuzara et al., 2010). In particular, trimethoprim-sulfamethoxazole, ceftazidime, piperacillin, and carbapenems are the most active agents against *Achromobacter* spp. isolates. Among carbapenems, several studies showed imipenem to be more active than meropenem against *Achromobacter* isolates (Amoureux et al., 2019; Caverly et al., 2019; Díez-Aguilar et al., 2019).

The most conserved genes conferring antibiotic resistance among *Achromobacter* spp. can be classified in 5 groups: class B β-lactamase, group B chloramphenicol acetyltransferase, rRNA methylases, class A β-lactamase and resistance-nodulation-cell division (RND) efflux pump (Hu et al., 2015). Particularly, many members belonging to the RND efflux pump group are associated with intrinsic resistance. Among them, AxyABM, which is able to extrude most cephalosporins, fluoroquinolones, aztreonam and chloramphenicol, and AxyXY-Opr, which extrudes aminoglycosides but can also accommodate cefepime, tetracyclines and carbapenems (Swenson and Sadikot, 2015; Bador et al., 2013, 2011).

Another important resistance mechanism is the production of β-lactamases. Interestingly, bla_{OXA} genes follow a species-specific distribution: while the specificity of $bla_{OXA-114}$, $bla_{OXA-243}$, $bla_{OXA-364}$ for *A. xylosoxidans*, *A. insuavis* and *A. dolens* respectively was already reported in literature (Bador et al., 2011), no bla_{OXA} genes were recently reported for *A. aegrifaciens* and some *A. insolitus* isolates, whereas bla_{OXA-2} was identified in *A. insolitus* strains (Menetrey et al., 2021). OXA-114-like enzymes show great activity in vitro against penicillin G, early cephalosporins, piperacillin, and ticarcillin (Isler et al., 2020). However, phenotypic piperacillin susceptibility results common among OXA-114-positive *A. xylosoxidans* isolates (Isler et al., 2020). Moreover, extended-spectrum β-lactamase, AmpC type β-lactamase, and metallo-β-lactamase have been observed in *A. xylosoxidans* CF isolates and appear to contribute to resistance to β-lactams including carbapenems (Traglia et al., 2012; Filipic et al., 2017; Vali et al., 2014; Neuwirth et al., 2006; Shibata et al., 2003; Riccio et al., 2001; Sofianou et al., 2005; Shin et al., 2005).

Many studies have focused on the role of antibiotic resistance in *Achromobacter* CF infections. Among them, Jeukens et al (Jeukens et al., 2017). performed a pan-genomic analysis of publicly available *Achromobacter* spp. and reported that clinical strains carry more resistance genes than other stains, namely for resistance against aminoglycosides (six additional genes), β-lactams (six additional genes), chloramphenicol (three additional genes) and sulfonamides (two additional genes). These additional genes presumably contribute to acquired resistance, but no

genomic information about their origin (e.g. mobile genetic elements, recombination) is reported. In addition, clinical strains showed positive selection of three genes encoding efflux pump components: *emrA*, *macA* and *mexW* (Jeukens et al., 2017). The protein products of these genes are elements of a major facilitator superfamily multidrug export complex, an ABC efflux pump that exports macrolides and an RND-type efflux pump, respectively. Positive selection of these genes suggest that efflux pumps represent another key mechanisms for adaptation to a pathogenic lifestyle as they are implicated in bacterial virulence (Alcalde-Rico et al., 2016) and show a tendency to favor loss of specificity, which translates into multi-drug resistance (Lewis, 1994; Vargiu et al., 2016).

Analysis of 54 *Achromobacter* genomes from people with CF presenting chronic and occasional infections found that there is no significant difference in resistance genes between chronic and occasional isolates. Moreover, 54% of isolates presenting deleterious variants in antibiotic resistance genes carried mutations in at least one *bla* gene (Veschetti et al., 2021a). Another recent study of 101 *Achromobacter* clinical isolates showed that development of antibiotic resistance is associated with chronic infections; in particular, late isolates were statistically significantly less susceptible than early and single isolates (Gabrielaite et al., 2021). Moreover, nearly all isolates were resistant or intermediate resistant to aztreonam, ceftriaxone, cefuroxime, ciprofloxacin, moxifloxacin, penicillin, rifampicin, tobramycin and trimethoprim. Interestingly, it was also reported that isolates belonging to the Danish epidemic strain (*A. ruhlandii*) were resistant or intermediate resistant to a median of 20 antibiotics, while the median was 14 for other *Achromobacter* isolates (*A. xylosoxidans* and *A. insuavis*), which could be one of the reasons this strain has become so widespread among people with CF in Denmark.

Taken together, acquired antimicrobial resistance in chronic *Achromobacter* CF infections, either by chromosomal mutation or horizonal gene transfer, is a growing concern, especially as it can be shared among genetically similar pathogens like *P. aeruginosa*, *Ralstonia* spp. and *Burkholderia* spp. Current knowledge deficits could be addressed by greater antimicrobial susceptibility testing of *Achromobacter* clinical isolates to enable better diagnosis, monitoring and treatment of antimicrobial resistance emergence and persistence in people with CF.

6.4. Mobilome

Horizontal gene transfer is the transfer of genetic elements among microorganisms by means other than vertical transmission and is a well-described mechanism that has been increasingly studied due to its role in the rapid dissemination of genetic elements among bacteria (Soucy et al., 2015). In particular, the acquisition of MGEs harboring genes related to virulence and antibiotic resistance can enable their microbial host to synthesize products that affect the fitness of resident microbiota and co-infecting pathogens or confer antibiotic resistance (Botelho et al., 2020). MGEs have been detected in the great majority of prokaryotic organisms and a rich variety of MGEs carrying resistance genes have been identified in *A. xylosoxidans* clinical isolates, such as plasmids, IS26, IS440, and class I and class II integrons (Hu et al., 2015; Traglia et al., 2012). Nevertheless, literature about the scale and importance of mobilome is still scarce for *Achromobacter* species.

A bioinformatic study found through pan-genome analysis that the most likely candidates to be involved in horizontal transfer with *Achromobacter* spp. were *Sinorhizobium* sp. as well as *Ralstonia*, *Pseudomonas* and *Burkholderia* sp (Jeukens et al., 2017)., which share a similar GC content with *Achromobacter* and are soil microorganisms that are also responsible for CF opportunistic infections (Mahmood et al., 2016; LiPuma, 2015). These results have been confirmed in a recent study that identified MGEs - phages, insertion sequence (IS) elements, integrative and conjugable elements (ICEs), and integrative and mobilizable elements – through genome analysis of 54 *Achromobacter* spp. clinical isolates from occasional and chronic CF lung infection (Veschetti et al., 2021b).

Among MGEs, phages (viruses which infect bacteria) are drivers of bacterial evolution (Clokie et al., 2011). Interestingly, most of the conserved phages in all *Achromobacter* species were previously described in other pathogens and carried genes related to MGE stability, biofilm formation and stress responses, highlighting the importance of MGE in *Achromobacter* pathogenicity. Moreover, type II toxin-antitoxin systems, which have been reported to occur more often in pathogenic bacteria and have been evaluated as antimicrobial targets (Williams and Hergenrother, 2012), were identified in *Achromobacter* isolates (Veschetti et al., 2021b). Additionally, an ancestral uptake of the phage Bcep176 from *Burkholderia* has been proposed for *A. xylosoxidans* (Veschetti et al., 2021b).

As another type of MGEs, different classes of ISs, which are frequently associated to antibiotic resistance genes and to class I and II integrons (Hu et al., 2015; Traglia et al., 2012), were also detected in *Achromobacter* spp., either inside of or in proximity to pathogenicity islands. In particular, ISs from a wide variety of microorganisms have been identified, especially from species of clinical interest including *B. cepacia* complex, *P. aeruginosa*, and *S. maltophilia* (Veschetti et al., 2021b).

Also, a great number of ICEs carrying genes related to a variety of functions such as secretion, motility, quorum sensing, metabolism, mismatch repair, and resistance to different classes of antimicrobial molecules have also been found in *Achromobacter* genomes (Veschetti et al., 2021b). In particular, the most represented antibiotic resistance genes were the sulfonamide resistance gene *sul1* and the *aac(6')* family aminoglycoside acetyltransferase, which are frequently found within MGEs such as integrons, plasmids and transposons carried by other Gram-negative opportunistic pathogens (Domingues et al., 2012). Additionally, while phages and ISs have shown high consistency in longitudinal isolates, variations in the presence and pathogenic content of ICEs over time were observed, indicating a frequent exchange of MGEs within the CF lungs (Veschetti et al., 2021b).

Little is still known about *Achromobacter* spp. plasmid content, especially regarding strains isolated from people with CF. Indeed, the majority of the available literature concerns environmental strains from aquacultures and soil. In particular, plasmids that have been reported in *Achromobacter* spp. are the wide host range IncP plasmids (Traglia et al., 2012), a 27 kbp plasmid coding for nitrite reductase and nitrous oxide reductase (Kathiravan and Krishnani, 2014), the 70 kbp 2,4-dichlorophenoxyacetic acid-degradative pEST4011 plasmid (Vedler et al., 2000), and the 98 kbp plasmid pA81 harboring genes encoding heavy metal resistance determinants (Jencova et al., 2008).

Overall, these findings underline MGEs contribution to the genomic plasticity of *Achromobacter* isolates and support that MGEs might play an important role in pathogenesis and adaptation during chronic infections, highlighting the need for further studies.

6.5. Hypermutation and clonal diversification

Among the variety of adaptation mechanisms identified in the CF lung environment, another important aspect is the high-rate accumulation of pathoadaptive mutations leading to hypermutation (Marvig et al., 2013). Typically, short-term adjustments are believed to be the result of regulatory alterations in gene expression whereas long-term adaptation is the result of the accumulation of pathoadaptive mutations (Ridderberg et al., 2015). Interestingly, the generation rate of mutations can be accelerated due to defects in DNA repair or error avoidance systems in hypermutable strains, thus giving rise to clonal diversification within the host (Oliver et al., 2000). Some of the genes involved in this phenomenon, also referred to as mutator genes, are *mutL*, *mutS*, *pfpI*, *superoxide dismutase*, *radA*, *radC*, *rad50*, *uvrA*, *uvrB*, *uvrC*, and *uvrD* (Oliver, 2010). The occurrence of hypermutation has been demonstrated for various CF pathogens, such as *Pseudomonas aeruginosa* (Oliver et al., 2000; Ciofu et al., 2010; Hogardt et al., 2007; Mena et al., 2008) and

Burkholderia cepacia complex (Martina et al., 2014), and more recently for *Achromobacter* (Veschetti et al., 2020; Oliver, 2010). In particular, *Achromobacter* spp. hypermutation events appear to be observed frequently (60–78%, (Veschetti et al., 2021a)) in strains isolated from the lungs of chronically infected patients while no occasional infection isolate showed hypermutator characteristics to date (Gabrielaite et al., 2021; Veschetti et al., 2021a, 2020; Oliver, 2010), thus suggesting that hypermutation might constitute an advantageous adaptive mechanism in the lung environment. Interestingly, a recent study reported the presence of two copies of *mutS* gene in *A. dolens* genome and a variable copy number of this gene in *A. insuavis* (Veschetti et al., 2021a). Both species were isolated from chronically infected CF patients, but these findings were validated by analyzing publicly available reference genomes. Moreover, in the same study, *A. dolens* hypermutator isolates carrying mutations in a single *mutS* gene have been identified, thus suggesting that both genes are needed for effective mismatch repair in this species.

Hypermutation is a key feature for within-host evolution of clonal lineages leading to clonal diversification, which results from co-evolution of several subpopulations from an original infecting isolate (Winstanley et al., 2016). During this process, mutation of genes involved mainly in the general metabolism, but also in virulence and antimicrobial resistance, was observed in CF chronic infections (Ridderberg et al., 2015). Interestingly, genes required for initiation of acute infection were found to be selected against, e.g. genes of the type I and type III secretion systems and genes related to pilus and flagellum formation or function, while mutations of antimicrobial resistance genes or their regulatory genes were found, that likely caused increased resistance to meropenem. In particular, *A. insuavis* has been reported to show higher diversification compared to other species (Dupont et al., 2015). In addition to hypermutation, other mechanisms might also contribute to clonal diversification, such as IS-related genomic rearrangements (Dupont et al., 2015).

7. Concluding remarks: challenges and areas of further research

Achromobacter spp. are a subject of increasing interest for their pathogenic characteristics and their growing prevalence in people with CF; nonetheless, many clinical aspects and pathogenic mechanisms remain to be elucidated. This genus still suffers from difficulties in diagnosis due to misidentification caused by its biochemical similarity to other Gram-negative bacilli and continuously evolving taxonomy. Some advances have already been made regarding the identification of *Achromobacter* species by the introduction of *nrdA* gene analysis, MLST (Spilker et al., 2012, 2013) and the creation of databases for MALDI-TOF-MS (Fernández-Olmos et al., 2012; Alby et al., 2013), which is the most employed technique in routine clinical microbiology laboratories for this aim. The growing clinical concern and research interest for *Achromobacter* spp. is determining and will continue to determine an increase in the number of collected isolates, both in CF centers and across other diseases. This could lead to the creation of new or updated MALDI-TOF databases that will allow a more accurate identification at species level if incorporated into commercial databases or made publicly available. Moreover, with the advancement of sequencing techniques, WGS could become feasible even in routine clinical microbiology laboratories and the growth of sequences in public repositories (e.g. NCBI, RefSeq) will allow a comprehensive analysis in terms of phylogeny, virulome, resistome and mobilome. In such circumstances, *Achromobacter spp.* genomic analysis could offer a plethora of information which might assist clinicians in choosing the best course of action. Additionally, we still have a limited understanding of *Achromobacter* adaptation to the CF lungs environment attributable to the restricted number of comparative studies, especially involving isolates from occasionally infected patients. In fact, the comparison between chronic and occasional infection isolates may allow the identification of genetic markers of persistence, while studies of less clinically characterized *Achromobacter* species could help in understanding whether some species are more likely to establish a chronic colonization of the CF airways. Studies focusing on *Achromobacter* interactions with other opportunistic pathogens or with lung microbiota also lack, limiting our understanding of the interplays occurring in the airway environment and of their participation in adaptation and persistance.

Another problem contributing to the difficult understanding of *Achromobacter* spp. adaptation is the large number of ORFs classified as having hypothetical function. Bioinformatic and functional studies regarding the product of these genes could give further insights on their role and contribution to *Achromobacter* metabolism, pathogenic potential and adaptation mechanisms.

Noteworthily, the variety of MGEs identified in *Achromobacter* genomes and their diverse virulence and antibiotic resistance profiles have confirmed *Achromobacter* spp. as a reservoir of MGEs. Not only they do contribute to genomic plasticity, but some of these elements can also even become a constitutive part of the bacterial genome (Veschetti et al., 2021a), thus highlighting the need for further studies to better elucidate MGEs clinical impact and their potential to become antimicrobial targets in treatment regimens. For example, the exploitation of type II toxin-antitoxin systems as an antibacterial strategy via artificial activation of the toxin has been proposed (Williams and Hergenrother, 2012).

Overall, the increasing number of studies focusing on this emerging pathogen and the continuous refinement of research techniques will likely allow a deeper knowledge of *Achromobacter* species that could successfully be translated into the clinical setting to the benefit of the patients.

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Author Contributions

L.V. performed literature search and wrote the original draft. M.B., G.M.S. and A.S. contributed to the text of the article. R.P.M., G.M., A.S. and M.M.L. supervised and reviewed the content of the article. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

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References

2020 Patient Registry Annual Data Report, 2020.

Alby, K., Gilligan, P.H., Miller, M.B., 2013. Comparison of matrix-assisted laser desorption ionization-time of flight (maldi-tof) mass spectrometry platforms for the identification of gram-negative rods from patients with cystic fibrosis. J. Clin. Microbiol. 51 (11), 3852–3854.

Alcalde-Rico, M., Hernando-Amado, S., Blanco, P., Martínez, J.L., 2016. Multidrug efflux pumps at the crossroad between antibiotic resistance and bacterial virulence. Front. Microbiol. 7, 1483.

Almuzara, M., Limansky, A., Ballerini, V., Galanternik, L., Famiglietti, A., Vay, C., 2010. In vitro susceptibility of *Achromobacter* spp. isolates: comparison of disk diffusion, Etest and agar dilution methods. Int. J. Antimicrob. Agents 35 (1), 68–71.

Amoureux, L., Bador, J., Siebor, E., Taillefumier, N., Fanton, A., Neuwirth, C., 2013. Epidemiology and resistance of *Achromobacter xylosoxidans* from cystic fibrosis patients in Dijon, Burgundy: first French data. J. Cyst. Fibros. 12 (2), 170–176.

Amoureux, L., Bador, J., Verrier, T., Mjahed, H., De Curraize, C., Neuwirth, C., 2016a. *Achromobacter xylosoxidans* is the predominant *Achromobacter* species isolated from diverse non-respiratory samples. Epidemiol. Infect. 144 (16), 3527–3530 (Dec).

Amoureux, L., Bador, J., Bounoua Zouak, F., Chapuis, A., de Curraize, C., Neuwirth, C., 2016b. Distribution of the species of *Achromobacter* in a French Cystic Fibrosis Centre and multilocus sequence typing analysis reveal the predominance of *A. xylosoxidans* and clonal relationships between some clinical and environmental isolates. J. Cyst. Fibros. 15 (4), 486–494.

Amoureux, L., Sauge, J., Sarret, B., Lhoumeau, M., Bajard, A., Tetu, J., 2019. Study of 109 *Achromobacter* spp. isolates from 9 French CF centres reveals the circulation of a multiresistant clone of *A. xylosoxidans* belonging to ST 137. J. Cyst. Fibros. J. Eur. Cyst. Fibros. Soc. 18 (6), 804–807.

Badalamenti, J.P., Hunter, R.C., 2015. Complete genome sequence of *Achromobacter xylosoxidans* MN001, a cystic fibrosis airway isolate. Genome Announc. 3 (4), e00947–15. Aug 27.

Bador, J., Amoureux, L., Blanc, E., Neuwirth, C., 2013. Innate aminoglycoside resistance of *Achromobacter xylosoxidans* is due to AxyXY-OprZ, an RND-type multidrug efflux pump. Antimicrob. Agents Chemother. 57 (1), 603–605.

Bador, J., Amoureux, L., Duez, J.M., Drabowicz, A., Siebor, E., Llanes, C., 2011. First description of an RND-type multidrug efflux pump in *Achromobacter xylosoxidans*, AxyABM. Antimicrob. Agents Chemother. 55 (10), 4912–4914.

Barrado, L., Branas, P., Orellana, M.A., Martinez, M.T., Garcia, G., Otero, J.R., 2013. Molecular characterization of *Achromobacter* isolates from cystic fibrosis and non-cystic fibrosis patients in Madrid, Spain. J. Clin. Microbiol. 51 (6), 1927–1930. Jun 1.

Borriello, G., Werner, E., Roe, F., Kim, A.M., Ehrlich, G.D., Stewart, P.S., 2004. Oxygen limitation contributes to antibiotic tolerance of *Pseudomonas aeruginosa* in biofilms. Antimicrob. Agents Chemother. 48 (7), 2659–2664.

Botelho, J., Mourão, J., Roberts, A.P., Peixe, L., 2020. Comprehensive genome data analysis establishes a triple whammy of carbapenemases, ICEs and multiple clinically relevant bacteria. Microb. Genomics 6 (10).

Caverly, L.J., Spilker, T., Kalikin, L.M., Stillwell, T., Young, C., Huang, D.B., 2019. In vitro activities of β-lactam-β-lactamase inhibitor antimicrobial agents against cystic fibrosis respiratory pathogens. Antimicrob. Agents Chemother. 64 (1). Dec 20.

Ciofu, O., Mandsberg, L.F., Bjarnsholt, T., Wassermann, T., Høiby, N., 2010. Genetic adaptation of *Pseudomonas aeruginosa* during chronic lung infection of patients with cystic fibrosis: strong and weak mutators with heterogeneous genetic backgrounds emerge in *mucA* and/or *lasR* mutants. Microbiol. Read. 156 (Pt 4), 1108–1119.

Clokie, M.R., Millard, A.D., Letarov, A.V., Heaphy, S., 2011. Phages in nature. Bacteriophage 1 (1), 31–45.

Coenye, T., Vancanneyt, M., Falsen, E., Swings, J., Vandamme, P., 2003b. *Achromobacter insolitus* sp. nov. and *Achromobacter spanius* sp. nov., from human clinical samples. Int. J. Syst. Evol. Microbiol. 53 (6), 1819–1824.

Coenye, T., Vancanneyt, M., Cnockaert, M.C., Falsen, E., Swings, J., Vandamme, P., 2003a. *Kerstersia gyiorum* gen. nov., sp. nov., a novel *Alcaligenes faecalis*-like organism isolated from human clinical samples, and reclassification of *Alcaligenes denitrificans* Rüger and Tan 1983 as *Achromobacter denitrificans* comb. nov. Int. J. Syst. Evol. Microbiol. 53 (6), 1825–1831.

Coward, A., Kenna, D.T.D., Perry, C., Martin, K., Doumith, M., Turton, J.F., 2016. Use of *nrdA* gene sequence clustering to estimate the prevalence of different *Achromobacter* species among Cystic Fibrosis patients in the UK. J. Cyst. Fibros. 7.

De Baets, F., Schelstraete, P., Van Daele, S., Haerynck, F., Vaneechoutte, M., 2007. *Achromobacter xylosoxidans* in cystic fibrosis: prevalence and clinical relevance. J. Cyst. Fibros. 6 (1), 75–78.

Degand, N., Carbonnelle, E., Dauphin, B., Beretti, J.L., Le Bourgeois, M., Sermet-Gaudelus, I., 2008. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of nonfermenting gram-negative bacilli isolated from cystic fibrosis patients. J. Clin. Microbiol. 46 (10), 3361–3367.

Díez-Aguilar, M., Ekkelenkamp, M., Morosini, M.I., Merino, I., de Dios Caballero, J., Jones, M., 2019. Antimicrobial susceptibility of non-fermenting Gram-negative pathogens isolated from cystic fibrosis patients. Int. J. Antimicrob. Agents 53 (1), 84–88.

Domingues, S., da Silva, G.J., Nielsen, K.M., 2012. Integrons: vehicles and pathways for horizontal dissemination in bacteria. Mob. Genet. Elem. 2 (5), 211–223.

Dumolin, C., Peeters, C., Ehsani, E., Tahon, G., De Canck, E., Cnockaert, M., 2020. *Achromobacter veterisilvae* sp. nov., from a mixed hydrogen-oxidizing bacteria enrichment reactor for microbial protein production. Int. J. Syst. Evol. Microbiol. 70 (1), 530–536.

Dupont, C., Michon, A.L., Jumas-Bilak, E., Nørskov-Lauritsen, N., Chiron, R., Marchandin, H., 2015. Intrapatient diversity of *Achromobacter* spp. involved in chronic colonization of Cystic Fibrosis airways. Infect. Genet. Evol. 32, 214–223.

ECFS Patient Registry, 2020;163.

Edwards, B.D., Greysson-Wong, J., Somayaji, R., Waddell, B., Whelan, F.J., Storey, D.G., et al., 2017. Prevalence and Outcomes of *Achromobacter* species infections in adults with cystic fibrosis: a North American cohort study. J. Clin. Microbiol. 55(7) 2074–2085.

Fernández-Olmos, A., García-Castillo, M., Morosini, M.I., Lamas, A., Máiz, L., Cantón, R., 2012. MALDI-TOF MS improves routine identification of non-fermenting Gram negative isolates from cystic fibrosis patients. J. Cyst. Fibros. J. Eur. Cyst. Fibros. Soc. 11 (1), 59–62.

Filipic, B., Malesevic, M., Vasiljevic, Z., Lukic, J., Novovic, K., Kojic, M., 2017. Uncovering differences in virulence markers associated with *Achromobacter* species of CF and non-CF origin. Front. Cell Infect. Microbiol. 7, 224.

Firmida, M.C., Pereira, R.H.V., Silva, E.A.S.R., Marques, E.A., Lopes, A.J., 2016. Clinical impact of *Achromobacter xylosoxidans* colonization/infection in patients with cystic fibrosis. Braz. J. Med. Biol. Res. 49 (4).

de la Fuente-Núñez, C., Korolik, V., Bains, M., Nguyen, U., Breidenstein, E.B.M., Horsman, S., 2012. Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. Antimicrob. Agents Chemother. 56 (5), 2696–2704.

Gabrielaite, M., Bartell, J.A., Nørskov-Lauritsen, N., Pressler, T., Nielsen, F.C., Johansen, H.K., 2021. Transmission and antibiotic resistance of *Achromobacter* in cystic fibrosis. J. Clin. Microbiol. JCM.02911-20, jcm;JCM.02911-20v1.

Gade, S.S., Nørskov-Lauritsen, N., Ridderberg, W., 2017. Prevalence and species distribution of *Achromobacter* sp. cultured from cystic fibrosis patients attending the Aarhus centre in Denmark. J. Med. Microbiol. 66 (5), 686–689.

Garrigos, T., Neuwirth, C., Chapuis, A., Bador, J., Amoureux, L., 2021. Development of a database for the rapid and accurate routine identification of *Achromobacter* species by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS). Clin. Microbiol. Infect. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. 27 (1), 126.e1–126.e5.

Gibson, R.L., Burns, J.L., Ramsey, B.W., 2003. Pathophysiology and management of pulmonary infections in cystic fibrosis. Am. J. Respir. Crit. Care Med. 168 (8), 918–951. Oct 15.

Gomila, M., Tvrzová, L., Teshim, A., Sedláček, I., González-Escalona, N., Zdráhal, Z., et al., 2011. *Achromobacter marplatensis* sp. nov., isolated from a pentachlorophenol-contaminated soil. Int. J. Syst. Evol. Microbiol. 61 (9), 2231–2237. Sep 1.

Gomila, M., Prince-Manzano, C., Svensson-Stadler, L., Busquets, A., Erhard, M., Martínez, D.L., et al., 2014. Genotypic and phenotypic applications for the differentiation and species-level identification of Achromobacter for clinical diagnoses. PLoS One 9(12) e114356.

Green, E.R., Mecsas, J., 2016. Bacterial secretion systems – an overview. Microbiol. Spectr. 4 (1).

Green, H., Jones, A.M., 2018. Emerging Gram-negative bacteria: pathogenic or innocent bystanders. Curr. Opin. Pulm. Med. 24 (6), 592–598.

Gross, R., Guzman, C.A., Sebaihia, M., Martins dos Santos, V.A., Pieper, D.H., Koebnik, R., 2008. The missing link: *Bordetella petrii* is endowed with both the metabolic versatility of environmental bacteria and virulence traits of pathogenic *Bordetellae*. BMC Genom. 9 (1), 449.

Günther, F., Merle, U., Frank, U., Gaida, M.M., Mutters, N.T., 2016. Pseudobacteremia outbreak of biofilm-forming *Achromobacter xylosoxidans* – environmental transmission. BMC Infect. Dis. 16 (1), 584.

Hansen, C.R., Pressler, T., Ridderberg, W., Johansen, H.K., Skov, M., 2013. *Achromobacter* species in cystic fibrosis: cross-infection caused by indirect patient-to-patient contact. J. Cyst. Fibros. 12 (6), 609–615.

Hansen, C.R., Pressler, T., Nielsen, K.G., Jensen, P.Ø., Bjarnsholt, T., Høiby, N., 2010. Inflammation in *Achromobacter xylosoxidans* infected cystic fibrosis patients. J. Cyst. Fibros. 9 (1), 51–58.

Hogardt, M., Hoboth, C., Schmoldt, S., Henke, C., Bader, L., Heesemann, J., 2007. Stage-specific adaptation of hypermutable *Pseudomonas aeruginosa* isolates during chronic pulmonary infection in patients with cystic fibrosis. J. Infect. Dis. 195 (1), 70–80.

Høiby, N., Bjarnsholt, T., Moser, C., Jensen, P.Ø., Kolpen, M., Qvist, T., 2017. Diagnosis of biofilm infections in cystic fibrosis patients. In: APMIS Acta Pathol. Microbiol. Immunol. Scand., 125, pp. 339–343.

Houry, A., Gohar, M., Deschamps, J., Tischenko, E., Aymerich, S., Gruss, A., 2012. Bacterial swimmers that infiltrate and take over the biofilm matrix. Proc. Natl. Acad. Sci. USA 109 (32), 13088–13093. Aug 7.

Hu, Y., Zhu, Y., Ma, Y., Liu, F., Lu, N., Yang, X., 2015. Genomic insights into intrinsic and acquired drug resistance mechanisms in *Achromobacter xylosoxidans*. Antimicrob. Agents Chemother. 59 (2), 1152–1161.

Isler, B., Kidd, T.J., Stewart, A.G., Harris, P., Paterson, D.L., 2020. *Achromobacter* infections and treatment options. Antimicrob. Agents Chemother. 64 (11), e01025–20. /aac/64/11/AAC.01025-20.atom.

Jakobsen, T.H., Hansen, M.A., Jensen, P.Ø., Hansen, L., Riber, L., Cockburn, A., et al., 2013. Complete genome sequence of the cystic fibrosis pathogen Achromobacter xylosoxidans NH44784–1996 complies with important pathogenic phenotypes. PLoS One 8(7) e68484.

Jencova, V., Strnad, H., Chodora, Z., Ulbrich, P., Vlcek, C., Hickey, W.J., 2008. Nucleotide sequence, organization and characterization of the (halo)aromatic acid catabolic plasmid pA81 from *Achromobacter xylosoxidans* A8. Res. Microbiol. 159 (2), 118–127.

Jeukens, J., Freschi, L., Vincent, A.T., Emond-Rheault, J.G., Kukavica-Ibrulj, I., Charette, S.J., 2017. A pan-genomic approach to understand the basis of host adaptation in *Achromobacter*. Genome Biol. Evol. 9 (4), 1030–1046.

Jolley, K.A., Bray, J.E., Maiden, M.C.J., 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 3, 124.

Kathiravan, V., Krishnani, K.K., 2014. *Pseudomonas aeruginosa* and *Achromobacter* sp.: nitrifying aerobic denitrifiers have a plasmid encoding for denitrifying functional genes. World J. Microbiol. Biotechnol. 30 (4), 1187–1198.

Kidd, T.J., Ramsay, K.A., Hu, H., Bye, P.T.P., Elkins, M.R., Grimwood, K., 2009. Low rates of *Pseudomonas aeruginosa* misidentification in isolates from cystic fibrosis patients. J. Clin. Microbiol. 47 (5), 1503–1509.

Kuncharoen, N., Muramatsu, Y., Shibata, C., Kamakura, Y., Nakagawa, Y., Tanasupawat, S., 2017. *Achromobacter aloeverae* sp. nov., isolated from the root of Aloe vera (L.) Burm.f. Int. J. Syst. Evol. Microbiol. 67 (1), 37–41.

Lambiase, A., Catania, M.R., del Pezzo, M., Rossano, F., Terlizzi, V., Sepe, A., 2011. *Achromobacter xylosoxidans* respiratory tract infection in cystic fibrosis patients. Eur. J. Clin. Microbiol. Infect. Dis. 30 (8), 973–980.

Land, M., Hauser, L., Jun, S.R., Nookaew, I., Leuze, M.R., Ahn, T.H., 2015. Insights from 20 years of bacterial genome sequencing. Funct. Integr. Genom. 15 (2), 141–161.

Lewis, K., 1994. Multidrug resistance pumps in bacteria: variations on a theme. Trends Biochem. Sci. 19 (3), 119–123.

Li, G., Zhang, T., Yang, L., Cao, Y., Guo, X., Qin, J., 2017. Complete genome sequence of *Achromobacter insolitus* type strain LMG 6003T, a pathogen isolated from leg wound. Pathog. Dis. 75 (4).
Li, G., Yang, L., Zhang, T., Guo, X., Qin, J., Cao, Y., 2018. Complete genome sequence of *Achromobacter spanius* type strain DSM 23806T, a pathogen isolated from human blood. J. Glob. Antimicrob. Resist. 14, 1–3.
Li, X., Hu, Y., Gong, J., Zhang, L., Wang, G., 2013. Comparative genome characterization of *Achromobacter* members reveals potential genetic determinants facilitating the adaptation to a pathogenic lifestyle. Appl. Microbiol. Biotechnol. 97 (14), 6413–6425.
Li, Y., Tian, Y., Hao, Z., Ma, Y., 2020. Complete genome sequence of the aromatic-hydrocarbon-degrading bacterium *Achromobacter xylosoxidans* DN002. Arch. Microbiol. 202 (10), 2849–2853.
Lin, J., Cheng, J., Wang, Y., Shen, X., 2018. The *Pseudomonas* Quinolone Signal (PQS): not just for quorum sensing anymore. Front. Cell Infect. Microbiol. 8.
LiPuma, J.J., 2015. Assessing airway microbiota in cystic fibrosis: what more should be done? J. Clin. Microbiol. 53 (7), 2006–2007.
Mahmood, A., Turgay, O.C., Farooq, M., Hayat, R., 2016. Seed biopriming with plant growth promoting rhizobacteria: a review. FEMS Microbiol. Ecol. 92 (8).
Mantovani, R.P., Levy, C.E., Yano, T., 2012. A heat-stable cytotoxic factor produced by *Achromobacter xylosoxidans* isolated from Brazilian patients with CF is associated with in vitro increased proinflammatory cytokines. J. Cyst. Fibros. J. Eur. Cyst. Fibros. Soc. 11 (4), 305–311.
Martina, P., Feliziani, S., Juan, C., Bettiol, M., Gatti, B., Yantorno, O., 2014. Hypermutation in *Burkholderia cepacia* complex is mediated by DNA mismatch repair inactivation and is highly prevalent in cystic fibrosis chronic respiratory infection. Int. J. Med. Microbiol. Ijmm 304 (8), 1182–1191.
Marvig, R.L., Johansen, H.K., Molin, S., Jelsbak, L., 2013. Genome analysis of a transmissible lineage of Pseudomonas aeruginosa reveals pathoadaptive mutations and distinct evolutionary paths of hypermutators. PLoS Genet. 9(9) e1003741.
Melvin, J.A., Scheller, E.V., Miller, J.F., Cotter, P.A., 2014. *Bordetella pertussis* pathogenesis: current and future challenges. Nat. Rev. Microbiol. 12 (4), 274–288.
Mena, A., Smith, E.E., Burns, J.L., Speert, D.P., Moskowitz, S.M., Perez, J.L., 2008. Genetic adaptation of *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients is catalyzed by hypermutation. J. Bacteriol. 190 (24), 7910–7917.
Méndez, V., Hernández, L., Salvà-Serra, F., Jaén-Luchoro, D., Durán, R.E., Barra, B., et al., 2018. Complete genome sequence of the hydrocarbon-degrading strain Achromobacter sp. B7, isolated during petroleum hydrocarbon bioremediation in the Valparaiso Region, Chile. Microbiol. Resour. Announc. 7(19) e01326–18, e01326–18.
Menetrey, Q., Dupont, C., Chiron, R., Jumas-Bilak, E., Marchandin, H., 2020. High occurrence of bacterial competition among clinically documented opportunistic pathogens including *Achromobacter xylosoxidans* in cystic fibrosis. Front. Microbiol. 11, 558160.
Menetrey, Q., Sorlin, P., Jumas-Bilak, E., Chiron, R., Dupont, C., Marchandin, H., 2021. *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia*: emerging Pathogens Well-Armed for Life in the Cystic Fibrosis Patients' Lung. Genes 12 (5), 610.
Neidhöfer, C., Berens, C., Parčina, M., 2022. An 18-year dataset on the clinical incidence and MICs to antibiotics of *Achromobacter* spp. (labeled biochemically or by MAL-DI-TOF MS as A. xylosoxidans), largely in patient groups other than those with CF. Antibiotics 11 (3), 311.
Neuwirth, C., Freby, C., Ogier-Desserrey, A., Perez-Martin, S., Houzel, A., Péchinot, A., 2006. VEB-1 in *Achromobacter xylosoxidans* from cystic fibrosis patient, France. Emerg. Infect. Dis. 12 (11), 1737–1739.
Nicholson, T.L., Conover, M.S., Deora, R., 2012. Transcriptome profiling reveals stage-specific production and requirement of flagella during biofilm development in *Bordetella bronchiseptica*. PLoS One 7 (11), e49166.
Nielsen, S.M., Meyer, R.L., Nørskov-Lauritsen, N., 2017. Differences in gene expression profiles between early and late isolates in monospecies *Achromobacter* Biofilm. Pathogen 6 (2).
Nielsen, S.M., Nørskov-Lauritsen, N., Bjarnsholt, T., Meyer, R.L., 2016. *Achromobacter* species isolated from cystic fibrosis patients reveal distinctly different biofilm morphotypes. Microorganisms 4 (3).
Olive, A.J., Sassetti, C.M., 2016. Metabolic crosstalk between host and pathogen: sensing, adapting and competing. Nat. Rev. Microbiol. 14 (4), 221–234.
Oliver, A., 2010. Mutators in cystic fibrosis chronic lung infection: prevalence, mechanisms, and consequences for antimicrobial therapy. Int. J. Med. Microbiol. IJMM 300 (8), 563–572.
Oliver, A., Cantón, R., Campo, P., Baquero, F., Blázquez, J., 2000. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science 288 (5469), 1251–1253. May 19.
Papalia, M., Figueroa-Espinosa, R., Steffanowski, C., Barberis, C., Almuzara, M., Barrios, R., 2020a. Expansion and improvement of MALDI-TOF MS databases for accurate identification of *Achromobacter* species. J. Microbiol. Methods 172, 105889.
Papalia, M., Steffanowski, C., Traglia, G., Almuzara, M., Martina, P., Galanternik, L., 2020b. Diversity of *Achromobacter* species recovered from patients with cystic fibrosis, in Argentina. Rev. Argent. Microbiol. 52 (1), 13–18.
Papenfort, K., Bassler, B.L., 2016. Quorum sensing signal–response systems in Gram-negative bacteria. Nat. Rev. Microbiol. 14 (9), 576–588.
Parte, A.C., Sardà Carbasse, J., Meier-Kolthoff, J.P., Reimer, L.C., Göker, M., 2020. List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. Int. J. Syst. Evol. Microbiol. 70 (11), 5607–5612.
Pederick, V.G., Eijkelkamp, B.A., Ween, M.P., Begg, S.L., Paton, J.C., McDevitt, C.A., 2014. Acquisition and role of molybdate in *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. 80(21) 6843–6852.
Pereira, R.H.V., Carvalho-Assef, A.P., Albano, R.M., Folescu, T.W., Jones, M.C.M.F., Leao, R.S., et al., 2011. *Achromobacter xylosoxidans*: characterization of strains in Brazilian cystic fibrosis patients. J. Clin. Microbiol. 49 (10), 3649–3651.
Périnet, S., 2016. Molybdate transporter ModABC is important for Pseudomonas aeruginosa chronic lung infection;9.
Pickrum, A.M., DeLeon, O., Dirck, A., Tessmer, M.H., Riegert, M.O., Biller, J.A., 2020. *Achromobacter xylosoxidans* cellular pathology is correlated with activation of a type III secretion system. Infect. Immun. 88 (7).
Quon, B.S., Rowe, S.M., 2016. New and emerging targeted therapies for cystic fibrosis. BMJ 352, i859. Mar 30.
Qureshi, N., Takayama, K., Kurtz, R., 1991. Diphosphoryl lipid A obtained from the nontoxic lipopolysaccharide of *Rhodopseudomonas sphaeroides* is an endotoxin antagonist in mice. Infect. Immun. 59 (1), 441–444.
Raso, T., Bianco, O., Grosso, B., Zucca, M., Savoia, D., 2008. *Achromobacter xylosoxidans* respiratory tract infections in cystic fibrosis patients. APMIS 116 (9), 837–841.
Reis, A.C., Kroll, K., Gomila, M., Kolvenbach, B.A., Corvini, P.F.X., Nunes, O.C., 2017. Complete genome sequence of Achromobacter denitrificans PR1. Genome Announc. 5 (31), e00762–17.
Riccio, M.L., Pallecchi, L., Fontana, R., Rossolini, G.M., 2001. In70 of plasmid pAX22, a *bla*(VIM-1)-containing integron carrying a new aminoglycoside phosphotransferase gene cassette. Antimicrob. Agents Chemother. 45 (4), 1249–1253.
Ridderberg, W., Nielsen, S.M., Nørskov-Lauritsen, N., 2015. Genetic adaptation of *Achromobacter* sp. during persistence in the lungs of cystic fibrosis patients. PLoS One 10 (8).
Ridderberg, W., Bendstrup, K.E.M., Olesen, H.V., Jensen-Fangel, S., Nørskov-Lauritsen, N., 2011. Marked increase in incidence of *Achromobacter xylosoxidans* infections caused by sporadic acquisition from the environment. J. Cyst. Fibros. 10 (6), 466–469.
Rønne Hansen, C., Pressler, T., Høiby, N., Gormsen, M., 2006. Chronic infection with *Achromobacter xylosoxidans* in cystic fibrosis patients; a retrospective case control study. J. Cyst. Fibros. 5 (4), 245–251.
Rouli, L., Merhej, V., Fournier, P.E., Raoult, D., 2015. The bacterial pangenome as a new tool for analysing pathogenic bacteria. New Microbes New Infect. 7, 72–85.
Saiman, L., Chen, Y., Tabibi, S., San Gabriel, P., Zhou, J., Liu, Z., et al., 2001. Identification and antimicrobial susceptibility of *Alcaligenes xylosoxidans* isolated from patients with cystic fibrosis. J. Clin. Microbiol. 39 (11), 3942–3945. Nov 1.
Sandri, A., Haagensen, J.A.J., Veschetti, L., Johansen, H.K., Molin, S., Malerba, G., et al., 2021. Adaptive interactions of *Achromobacter* spp. with *Pseudomonas aeruginosa* in cystic fibrosis chronic lung co-infection. Pathogens 10 (8), 978. Aug 3.
Schobert, M., 2010. Anaerobic physiology of *Pseudomonas aeruginosa* in the cystic fibrosis lung. Int. J. Med. Microbiol. 8.
Shibata, N., Doi, Y., Yamane, K., Yagi, T., Kurokawa, H., Shibayama, K., et al., 2003. PCR typing of genetic determinants for metallo-beta-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. J. Clin. Microbiol. 41 (12), 5407–5413.
Shin, K.S., Han, K., Lee, J., Hong, S.B., Son, B.R., Youn, S.J., et al., 2005. Imipenem-resistant *Achromobacter xylosoxidans* carrying *bla*VIM-2-containing class 1 integron. Diagn. Microbiol. Infect. Dis. 53 (3), 215–220.
Singh, P., Kim, Y.J., Singh, H., Farh, M.E.A., Yang, D.C., 2017. *Achromobacter panacis* sp. nov., isolated from rhizosphere of *Panax ginseng*. J. Microbiol. 55 (6), 428–434.
Sofianou, D., Markogiannakis, A., Metzidie, E., Pournaras, S., Tsakris, A., 2005. VIM-2 metallo-beta-lactamase in *Achromobacter xylosoxidans* in Europe. Eur. J. Clin. Microbiol. Infect. Dis. Publ. Eur. Soc. Clin. Microbiol. 24 (12), 854–855.
Soh, E.Y.C., Chhabra, S.R., Halliday, N., Heeb, S., Müller, C., Birmes, F.S., et al., 2015. Biotic inactivation of the *Pseudomonas aeruginosa* quinolone signal molecule. Environ. Microbiol. 17 (11), 4352–4365.
Soucy, S.M., Huang, J., Gogarten, J.P., 2015. Horizontal gene transfer: building the web of life. Nat. Rev. Genet. 16 (8), 472–482.
Spilker, T., Vandamme, P., LiPuma, J.J., 2012. A multilocus sequence typing scheme implies population structure and reveals several putative novel *Achromobacter* Species. J. Clin. Microbiol. 50 (9), 3010–3015. Sep 1.
Spilker, T., Vandamme, P., LiPuma, J.J., 2013. Identification and distribution of *Achromobacter* species in cystic fibrosis. J. Cyst. Fibros. 12 (3), 298–301.
Strnad, H., Ridl, J., Paces, J., Kolar, M., Vlcek, C., Paces, V., 2022b. Complete Genome Sequence of the Haloaromatic Acid-Degrading Bacterium Achromobacter xylosoxidans A8.:2.
Swenson, C.E., Sadikot, R.T., 2015. *Achromobacter* respiratory infections. Ann. Am. Thorac. Soc. 12 (2), 252–258.
Talbot, N.P., Flight, W.G., 2016. Severe *Achromobacter xylosoxidans* infection and loss of sputum bacterial diversity in an adult patient with cystic fibrosis. Paediatr. Respir. Rev. 20, 27–29.
Tan, K., Conway, S.P., Brownlee, K.G., Etherington, C., Peckham, D.G., 2002. *Alcaligenes* infection in cystic fibrosis. Pediatr. Pulmonol. 34 (2), 101–104.
Tettelin, H., Riley, D., Cattuto, C., Medini, D., 2008. Comparative genomics: the bacterial pan-genome. Curr. Opin. Microbiol. 6.
Traglia, G.M., Almuzara, M., Merkier, A.K., Adams, C., Galanternik, L., Vay, C., 2012. *Achromobacter xylosoxidans*: an emerging pathogen carrying different elements involved in horizontal genetic transfer. Curr. Microbiol. 65 (6), 673–678.
Trancassini, M., Iebba, V., Citerà, N., Tuccio, V., Magni, A., Varesi, P., 2014. Outbreak of *Achromobacter xylosoxidans* in an Italian Cystic fibrosis center: genome variability, biofilm production, antibiotic resistance, and motility in isolated strains. Front. Microbiol. 5.
Vali, P., Shahcheraghi, F., Seyfipour, M., Zamani, M.A., Allahyar, M.R., Feizabadi, M.M., 2014. Phenotypic and genetic characterization of carbapenemase and ESBLs producing Gram-negative Bacteria (GNB) isolated from patients with cystic fibrosis (CF) in Tehran Hospitals. J. Clin. Diagn. Res. JCDR 8 (1), 26–30.

Vandamme, P., Moore, E.R.B., Cnockaert, M., Peeters, C., Svensson-Stadler, L., Houf, K., et al., 2013a. Classification of Achromobacter genogroups 2, 5, 7 and 14 as Achromobacter insuavis sp. nov., Achromobacter aegrifaciens sp. nov., Achromobacter anxifer sp. nov. and Achromobacter dolens sp. nov., respectively. Syst. Appl. Microbiol. 36 (7), 474–482.

Vandamme, P., Moore, E.R.B., Cnockaert, M., De Brandt, E., Svensson-Stadler, L., Houf, K., et al., 2013b. *Achromobacter animucus* sp. nov., *Achromobacter mucicolens* sp. nov., *Achromobacter pulmonis* sp. nov. and *Achromobacter spiritinus* sp. nov., from human clinical samples. Syst. Appl. Microbiol. 36 (1), 1–10.

Vandamme, P.A., Peeters, C., Cnockaert, M., Inganäs, E., Falsen, E., Moore, E.R.B., et al., 2015. *Bordetella bronchialis* sp. nov., *Bordetella flabilis* sp. nov. and *Bordetella sputigena* sp. nov., isolated from human respiratory specimens, and reclassification of *Achromobacter sediminum* Zhang et al. 2014 as *Verticia sediminum* gen. nov., comb. nov. Int. J. Syst. Evol. Microbiol. 65 (Pt_10), 3674–3682. Oct 1.

Vandamme, P.A., Peeters, C., Inganäs, E., Cnockaert, M., Houf, K., Spilker, T., et al., 2016a. Taxonomic dissection of Achromobacter denitrificans Coenye et al. 2003 and proposal of Achromobacter agilis sp. nov., nom. rev., Achromobacter pestifer sp. nov., nom. rev., Achromobacter kerstersii sp. nov. and Achromobacter deleyi sp. nov. Int. J. Syst. Evol. Microbiol. 66 (9), 3708–3717. Sep 1.

Vandamme, P.A., Peeters, C., Cnockaert, M., Gomila, M., Moore, E.R.B., Spilker, T., et al., 2016b. Reclassification of *Achromobacter spiritinus* Vandamme et al. 2013 as a later heterotypic synonym of *Achromobacter marplatensis* Gomila et al. 2011. Int. J. Syst. Evol. Microbiol. 66 (4), 1641–1644.

Vargiu, A.V., Pos, K.M., Poole, K., Nikaido, H., 2016. Editorial: Bad bugs in the XXIst century: resistance mediated by multi-drug efflux pumps in gram-negative bacteria. Front. Microbiol. 7, 833.

Vedler, E., Kõiv, V., Heinaru, A., 2000. Analysis of the 2,4-dichlorophenoxyacetic acid-degradative plasmid pEST4011 of *Achromobacter xylosoxidans* subsp. *denitrificans* strain EST4002. Gene 255 (2), 281–288. Sep 19.

Veschetti, L., Sandri, A., Krogh Johansen, H., Lleò, M.M., Malerba, G., 2020. Hypermutation as an evolutionary mechanism for *Achromobacter xylosoxidans* in cystic fibrosis lung infection. Pathogens 9 (2), 72.

Veschetti, L., Sandri, A., Patuzzo, C., Melotti, P., Malerba, G., Lleo, M.M., 2021a. Genomic characterization of *Achromobacter* species isolates from chronic and occasional lung infection in cystic fibrosis patients. Microb. Genom. 7 (7).

Veschetti, L., Sandri, A., Patuzzo, C., Melotti, P., Malerba, G., Lleò, M.M., 2021b. Mobilome analysis of *Achromobacter* spp. isolates from chronic and occasional lung infection in cystic fibrosis patients. Microorganisms 9 (1), 130. Jan 8.

Vincent, A.T., Derome, N., Boyle, B., Culley, A.I., Charette, S.J., 2017. Next-generation sequencing (NGS) in the microbiological world: how to make the most of your money. J. Microbiol. Methods 138, 60–71.

Wass, T.J., Syed-Ab-Rahman, S.F., Carvalhais, L.C., Ferguson, B.J., Schenk, P.M., 2019. Complete genome sequence of Achromobacter spanius UQ283, a soilborne isolate exhibiting plant growth-promoting properties. Microbiol. Resour. Announc. 8(16) MRA.00236–19, e00236–19.

Williams, J.J., Hergenrother, P.J., 2012. Artificial activation of toxin-antitoxin systems as an antibacterial strategy. Trends Microbiol. 20 (6), 291–298.

Winstanley, C., O'Brien, S., Brockhurst, M.A., 2016. *Pseudomonas aeruginosa* evolutionary adaptation and diversification in cystic fibrosis chronic lung infections. Trends Microbiol. 24 (5), 327–337.

Yabuuchi, E., Kawamura, Y., Kosako, Y., Ezaki, T., 1998. Emendation of Genus *Achromobacter* and *Achromobacter xylosoxidans* (Yabuuchi and Yano) and Proposal of *Achromobacter ruhlandii* (Packer and Vishniac) Comb. Nov., *Achromobacter piechaudii* (Kiredjian et al.) Comb. Nov., and *Achromobacter xylosoxidans* Subsp. *denitrificans* (Rüger and Tan) Comb. Nov. Microbiol. Immunol. 42 (6), 429–438.

Yabuuchi', E., Yan, I., 2022a. Achromobacter gen. nov. and Achromobacter xylosoxidans (e x Yabuuchi and Ohyama 1971) norn. rev.:2.

Zhang, Z., Fan, X., Gao, X., Zhang, X.H., 2014. Achromobacter sediminum sp. nov., isolated from deep subseafloor sediment of South Pacific Gyre. Int. J. Syst. Evol. Microbiol. 64 (Pt_7), 2244–2249. Jul 1.