Volume 62 Number 5
May 2001

CURRENT THERAPEUTIC RESEARCH®

CLINICAL AND EXPERIMENTAL

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Excerpta Medica

CURRENT THERAPEUTIC RESEARCH®
VOL. 62, NO. 5, MAY 2001

Reprinted from *Current Therapeutic Research* Vol. 62, No. 5, May 2001

Modulation of the Intestinal Ecosystem by Probiotics and Lactulose in Children During Treatment with Ceftriaxone

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ABSTRACT

Background: The value of oral bacteriotherapy during antibiotic treatment is a much debated subject. Comparative studies on the effects of different probiotics on the intestinal ecosystem are lacking.

Objective: Six different commercially available preparations of probiotics and 1 prebiotic (lactulose) were compared to establish whether their action prevented or corrected imbalances in the intestinal ecosystem (dysbiosis) during parenteral therapy with ceftriaxone.

Methods: Fifty-one children (25 female, 26 male; mean age, 5.1 years) admitted to the hospital for febrile respiratory tract infections were treated. Ceftriaxone 50 mg/kg per day was administered parenterally alone (therapy 1) or with 1 of the following probiotic/prebiotic preparations: *Saccharomyces boulardii* (therapy 2); *Enterococcus* species (therapy 3); lactulose (therapy 4); *Lactobacillus casei* GG (therapy 5); *Lactobacillus rhamnosus, Lactobacillus bifidus,* and *Lactobacillus acidophilus* (therapy 6); *Bifidobacterium bifidum* and *L acidophilus* (therapy 7); or a mixture of various lactobacilli and bifidobacteria at high concentrations (therapy 8).

Intestinal microflora were evaluated by standard microbiologic methods and by biochemical assays on fecal samples collected before and after treatment.

Results: Ceftriaxone induced a decrease in *Escherichia coli* and lactobacilli counts and an increase in cocci and clostridia counts. Partial protection of the intestinal ecosystem (eubiosis) was achieved with therapies 6, 7, and 8, which contained different combinations of *Lactobacillus* and *Bifidobacterium* species. Probiotics containing lactobacilli were more active than the older *Saccharomyces* and *Enterococcus* preparations. The newer probiotics reduced β-galactosidase, β-glucosidase, and β-glucuronidase levels. Increased fecal β-lactamase activity was observed in 60% of patients treated with ceftriaxone alone and 75% of those treated with ceftriaxone and *S boulardii.* A lower incidence of beta-lactamase–positive samples (30%–40%) was observed with therapy 7 and therapy 8.

Accepted for publication March 14, 2001.

Conclusions: In this preliminary study, probiotics containing multiple species of lactobacilli and bifidobacteria administered at high concentration (20 billion to 360 billion per day) were more effective in preventing dysbiosis induced by ceftriaxone treatment than were other preparations studied. Probiotic therapy may need to be maintained for several days after discontinuation of antibiotic treatment to adequately restore balance to the intestinal ecosystem.

Key words: colonic microflora, beta-lactamases, colonic enzymes, probiotics, intestinal imbalance, children. (*Curr Ther Res Clin Exp.* 2001;62:418–435)

INTRODUCTION

The human gastrointestinal tract is host to various species of microorganisms, with populations up to 10^{11} bacteria per gram of intestinal contents. This bacterial population plays a significant role in colonic metabolism and health. The nature and the extent of this metabolism depend on the characteristics of the bacterial flora and the availability of exogenous and endogenous substrates. Products of carbohydrate fermentation are thought to benefit the host, in contrast to the potentially toxic products of protein fermentation.

Antibiotic therapy can affect the composition of the intestinal ecosystem and affect intestinal enzyme activity and metabolism. The effects of antibiotics on carbohydrate metabolism may be due, in part, to the antibiotic's ability to induce changes in saccharolytic activity (ie, β-galactosidase activity).

Reduction of fermentative activity, as might occur with the use of β-lactam antibiotics, is the primary cause of imbalance in the intestinal ecosystem. In severe infections of the gastrointestinal tract, there is a prevalence of putrefactive activity, which is performed by gram-negative microflora. Excessive activity of β-glucuronidase, an enzyme involved in the formation of toxic and carcinogenic compounds, may have a negative effect on human health. The use of β-lactam antibiotics can also induce an increase in β-lactamase production in the fecal flora, thereby increasing bacterial resistance to other β-lactam drugs and plasmidic resistance transfer among intestinal enterobacteria. This is a great problem in the use of β-lactam antibiotics, and the control of bacterial resistance has become a key objective for clinical microbiologists.

Species of *Bifidobacterium* and *Lactobacillus* have low activities of the enzymes involved in carcinogen formation and metabolism compared with the other major anaerobes in the gut, such as *Bacteroides, Eubacteria,* and *Clostridia* species. Therefore, increasing the proportion of lactic acid bacteria (LAB) in the gut could beneficially modify the levels of xenobiotic metabolizing enzymes.

The use of probiotics and their place in therapy have been subjects of debate since our first study in 1982.[1] A number of reports have been published on bacteriotherapy with probiotics during antibiotic treatment,[1–5] and related topics such as recovery from *Clostridium difficile* diarrhea,[6–9] maintenance of the

equilibrium of the intestinal ecosystem during antibiotic therapy,[10–13] recovery from acute diarrhea in children,[14,15] and probiotic activity in travelers' diarrhea.[16]

A substantial number of probiotic products have been marketed, but the results of feeding experiments are variable, possibly because of the use of nonhuman bacterial isolates, problems of strain stability, and differences in the bacterial concentrations of the various preparations. The probiotic preparations currently available on the market are so numerous and differ so substantially from one another that it is difficult to establish their efficacy.[17,18]

In this study, we compare the efficacy of commonly used probiotic preparations in preventing intestinal adverse events due to imbalances in the intestinal ecosystem in children receiving a course of ceftriaxone therapy.

PATIENTS AND METHODS

Patients

Children admitted to our hospital during the winter of 1997–1998 for severe febrile infections of the respiratory tract were eligible for this study. Respiratory tract infections were diagnosed based on chest radiographs and routine blood tests (leukocyte count, increase in neutrophil count, erythrocyte sedimentation rate, protein C reaction).

The exclusion criteria were diarrhea and vomiting following gastrointestinal viral or bacterial infection, previous administration of antibiotics, and consumption of yoghurt or LAB within 1 month before the analysis.

The children were recruited into the study after their parents had given their oral informed consent and after obtaining authorization from the hospital ethics committee.

Treatment

The children were randomly assigned to (1) ceftriaxone (Rocefin®, Roche, Milan, Italy) alone or with 1 of the following probiotic or prebiotic agents (Table I): (2) *Saccharomyces boulardii* (Codex®, SmithKline Beecham, Milan, Italy); (3) *Enterococcus* species SF68 type LAB (Bioflorin®, Bracco, Milan, Italy); (4) lactulose (Laevolac®, Boehringer Mannheim, Milan, Italy); (5) *Lactobacillus casei* subspecies *rhamnosus* GG (Dicoflor 30®, Dicofarm, Rome, Italy); (6) *Lactobacillus rhamnosus* + *Lactobacillus bifidus* + *Lactobacillus acidophilus* (Ramnoflor®, Medifood-Trufood, Genova, Italy); (7) *Bifidobacterium bifidum* and *L acidophilus* (Infloran®, Istituto Sieroterapico Berna, Como, Italy); or (8) a mixture of various LAB (Yovis®, Sigma Tau, Pomezia-Rome, Italy).

The individual probiotic dosages used were those recommended by the manufacturers, except in the case of the *B bifidum* plus *L acidophilus* preparation (therapy 7), which we had previously shown[3] should be administered at double the recommended dosage. All probiotic and prebiotic products were obtained from commercial sources and not directly from the manufacturers.

Table I. Characteristics and dosage of different commercial probiotic products administered to children undergoing intravenous ceftriaxone (Cx) therapy.

Therapy No./Agents	No. of Live Cells Per Capsule or Sachet	Dosage	No. of Patients	Mean (SD) Age of Patients, y
1. Cx alone	—	50 mg/kg per day	5	4.5 (4.3)
2. Cx + *Saccharomyces boulardii*	1×10^9	1 capsule TID	6	6.8 (2.6)
3. Cx + *Enterococcus* species SF68	75×10^6	1 capsule TID	7	3.4 (1.7)
4. Cx + lactulose	—	2.0–3.3 g BID*	7	5.7 (2.6)
5. Cx + *Lactobacillus casei* subspecies *rhamnosus* GG	3×10^9	2 sachets × 3	7	4.0 (2.3)
6. Cx + *Lactobacillus rhamnosus*	1.1×10^9	2 capsules TID	7	6.3 (3.9)
+ *Lactobacillus bifidus*	6×10^9			
+ *Lactobacillus acidophilus*	6×10^8			
7. Cx + *Bifidobacterium bifidum*	1×10^9	2 capsules TID	7	5.3 (2.9)
+ *L acidophilus*	1×10^9			
8. Cx + *Streptococcus salivarius* subspecies *thermophilus*	2.04×10^{11}	1 sachet/d	5	7.4 (3.2)
+ *Bifidobacterium breve*	93×10^9			
+ *Bifidobacterium infantis*	93×10^9			
+ *Bifidobacterium longum*	93×10^9			
+ *L acidophilus*	2×10^9			
+ *Lactobacillus plantarum*	2.2×10^8			
+ *L casei*	2.2×10^8			
+ *Lactobacillus delbrueckei* subspecies *bulgaricus*	2×10^8			
+ *Streptococcus faecium*	3×10^7			

*Dose administered according to body weight: 2.0 g for patients weighing ≤20 kg and 3.3 g for those weighing >20 kg.

Fecal samples were collected at admission to the hospital before antibiotic administration and after ~5 days (mean ± SD, 4.5 ± 1.5 days), before discharge from the pediatric ward.

All children were examined again 7 days after discharge to determine whether the different therapies produced clinical variations, in particular, variations in the number of stools per day.

Microbiology

Microflora Determination

Microbiologic and enzymatic studies were carried out on fecal samples using standard methods.[19,20] Briefly, stool samples were collected after emission and

immediately frozen and stored at −70°C until analysis and subsequent testing for microbial composition. The following media were used: blood agar for total aerobic and anaerobic count; Schaedler agar (for anaerobic total count); kanamycin-vancomycin Schaedler agar (for *Bacteroides*); reinforced clostridial agar (for *Clostridia*); Mitis Salivarius agar (for aerobic and anaerobic cocci); tomato juice agar, Rogosa SL agar, and MRS agar (for bifidobacteria and lactobacilli); bile esculine azide agar (for enterococci); mannitol salt agar (for staphylococci); MacConkey agar and SS agar (for Enterobacteriaceae); Sabouraud dextrose agar (for yeasts); and CCFA (for *Clostridium difficile*). After incubation under aerobic conditions (room air, 37°C, 24 hours) and under anaerobic conditions (85% nitrogen, 10% carbon dioxide, 5% hydrogen gas mixture, 37°C, 48–72 hours in an anaerobic chamber), different colony types were counted, isolated, and identified by morphologic and biochemical analysis (API system: 20E, 50 CHL, rapid ID 32 A, Strep, Staph, ID32 C; bioMérieux, Marcy l'Etoile, France). The lower limit of detection was 10^2 microorganisms per gram of feces. Results were expressed as $\log_{10}$ of the number of colony-forming units per gram of feces.

We used a semiquantitative micromethod to detect a range of microbial enzyme activity in fecal samples. Enzyme activity was determined at the same time as the microbiologic analysis—on the third dilution of each sample. Nineteen different common bacterial reactions were determined using the API-ZYM system (API Products, bioMérieux).[19,20]

Antibiotic and β-Lactamase Determination

The presence of ceftriaxone and β-lactamases in the feces was determined during the microbiologic and enzymatic analyses on the same sample. Antibiotic concentrations in feces were determined by the agar-well-diffusion microbiologic method using *Providencia rettgeri Sanelli* (0.02% final concentration from overnight culture) as the test microorganism in Isosensitest agar.[21] Fecal antibiotic concentrations were also measured to establish that patients had not taken antibiotics before recruitment into the study.

Beta-lactamase activity in the supernatant of fecal samples was determined spectrophotometrically at 490 nm using the nitrocefin test.[22]

Statistical Analysis

The Kruskal-Wallis test was used to determine potential differences between treatment groups at baseline. The Student *t* test for paired observations was used for pretreatment versus posttreatment comparisons. Statistical significance was set at $P < 0.05$.

RESULTS

Clinical Course

A total of 51 patients (25 female, 26 male, mean age 5.1 years) were enrolled in the study. All patients showed a distinct improvement in their febrile respira-

tory tract infections, with disappearance of fever, reduction of cough, and substantial normalization of indicators of inflammation on blood tests. None of the patients recruited dropped out during the study as a result of gastrointestinal complications. No differences in intestinal complaints were observed among the various groups of patients during probiotic treatment.

At the end of treatment, children receiving ceftriaxone alone had a significantly higher number of stools per day (mean 3 ± 1 bowel movements; bulky, brown, and with normal odor) than those who received probiotic therapies 7 and 8 (mean 1 ± 1 bowel movements; normal; $P \leq 0.01$).

Microbiologic Findings

We observed substantial intersubject variability of fecal flora before therapy. The Kruskal-Wallis test revealed a high, though not statistically significant ($P > 0.05$) degree of variability among the various patient groups before treatment. This high variability may be a function of the pathologic condition, namely respiratory tract infections.

The different treatments induced a number of fairly homogeneous changes for each treatment group (Table II).

Treatment with ceftriaxone alone induced a significant reduction in the concentration of *E coli* ($P = 0.03$) and a decrease in lactobacilli and bifidobacteria counts (Figure 1A). The changes in the concentration of *E coli* and LAB were paralleled by a nonsignificant increase in the number of *Clostridia* species (from log 4.6 to log 6.0) (1 log). The *Enterococcus faecium* 1 and *E faecium* 2 counts also increased significantly ($P = 0.05$) compared with pretreatment levels.

The effects of ceftriaxone on the composition of microflora were associated with a reduction in the activities of fermentative enzymes such as β-galactosidase and β-glucosidase. In turn, there was a trend, though not statistically significant, toward increased β-glucuronidase activity (2 of 5 patients became positive). Overall, these findings were indicative of a state of dysbiosis.[1,20]

In the groups of patients who received probiotic/prebiotic therapy (therapies 2–8), the most relevant microbiologic effect was a reduction in the high number of specific *Clostridia* species induced by ceftriaxone (*C perfringens, C paraputrificum, C ramosum, C innocuum, C clostridioforme, C bifermentans),* with a return to baseline levels; after treatment, *C clostridioforme, C fallax,* and *C perfringens* were the most frequently isolated species. However, none of the probiotic/prebiotic preparations tested in this study modified the inhibition of *E coli* caused by ceftriaxone (Table II and Figure 1).

The mixture of probiotic lactobacilli and bifidobacteria at high concentrations (therapy 8) had the most significant impact on the changes in microflora composition caused by ceftriaxone treatment. Therapy 8 induced a marked increase in enterococci mean count; lactobacilli and bifidobacteria count showed a 2 log increase, as did the anaerobic cocci count. Aerobic and anaerobic total counts also increased, mainly reflecting a general increase in gram-positive components (Figure 1B). Specifically, the recorded increases were rep-

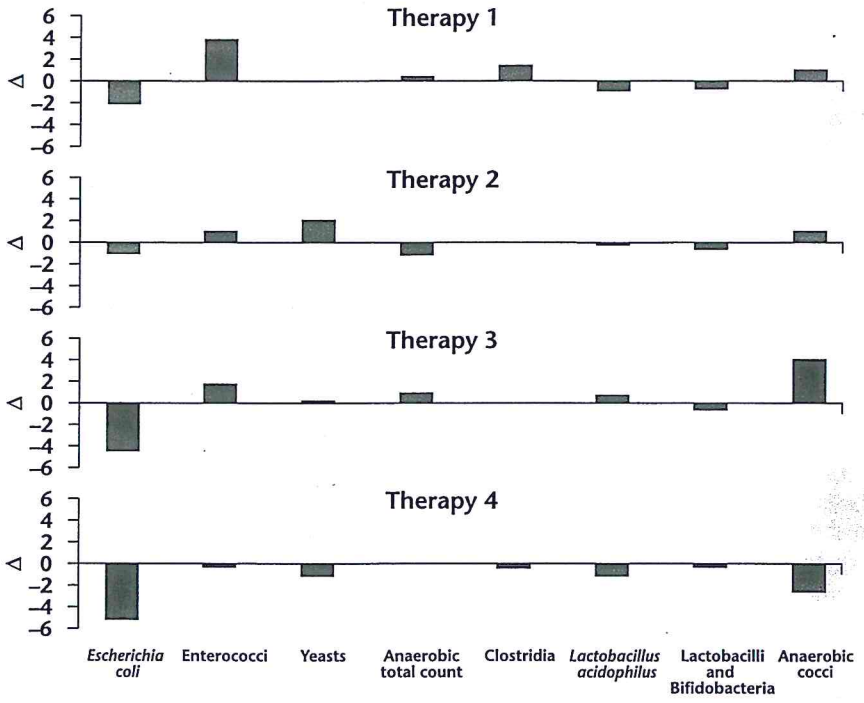

Figure 1A. Changes in fecal microflora composition in children treated with different probiotics. Results are expressed as the difference in mean $\log_{10}$ count values after treatment versus mean count before treatment (Δ). Therapy 1: ceftriaxone alone; therapy 2: ceftriaxone + *Saccharomyces boulardii;* therapy 3: ceftriaxone + *Enterococcus* species; therapy 4: ceftriaxone + lactulose.

resented mainly by *E faecium* 1, *Lactobacillus plantarum, Lactobacillus pentosus, Lactococcus lactis* subpecies *lactis* 1, and *Bifidobacterium* species. We encountered discrepancies and had difficulty identifying *Lactobacillus* and *Bifidobacterium* at the species level using the API system.

The effects of other probiotic preparations containing lactobacilli and bifidobacteria (therapies 5 and 6) were less marked than those obtained with therapy 8. Microflora imbalance caused by ceftriaxone was partially corrected in most of the patients who received therapy 5 or 6 (Figure 1B). There was a trend toward an increase in the number of lactobacilli and bifidobacteria, without reversal of the changes caused by ceftriaxone.

The combination of lactulose and ceftriaxone (therapy 4) induced a general decrease in mean count of all the bacterial species studied and a significant

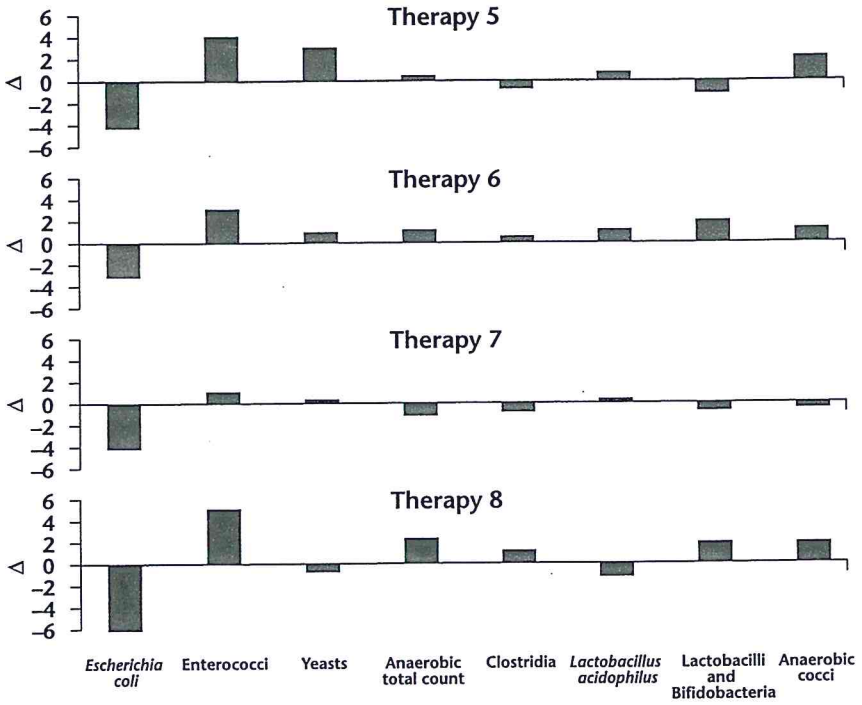

Figure 1B. Changes in fecal microflora composition in children treated with different probiotics. Results are expressed as the difference in mean $\log_{10}$ count values after treatment versus mean count before treatment (Δ). Therapy 5: ceftriaxone + *Lactobacillus casei* subspecies *rhamnosus* GG; therapy 6: ceftriaxone + *Lactobacillus rhamnosus* + *Lactobacillus bifidus* + *Lactobacillus acidophilus;* therapy 7: ceftriaxone + *Bifidobacterium bifidum* + *L acidophilus;* therapy 8: ceftriaxone + *Streptococcus salivarius* subspecies *thermophilus* + *Bifidobacterium breve* + *Bifidobacterium infantis* + *Bifidobacterium longum* + *L acidophilus* + *Lactobacillus plantarum* + *L casei* + *Lactobacillus delbrueckei* subspecies *bulgaricus* + *Streptococcus faecium.*

($P = 0.03$) selective decrease in pH, thus enhancing the strong inhibitory effect of the antibiotic. A significant decrease ($P = 0.02$) in stool pH was observed with the *B bifidum* plus *L acidophilus* preparation (therapy 7), as well as with the preparations containing a high concentration of LAB (therapy 8) ($P = 0.04$) and lactulose (therapy 4) ($P = 0.02$).

In the group of patients treated with *S boulardii,* the intestinal ecosystem was essentially unchanged, except for an increase in fungi (Figure 1A); this finding was not surprising since *S boulardii* is itself a yeast. The probiotic preparation containing *Enterococcus* species SF68 type LAB (therapy 3) did not correct the

Table II. Changes in microflora composition and intestinal pH in children treated with ceftriaxone (Cx) alone or with probiotic agents.*

Therapy No./Agents	*Escherichia coli*	Enterococci	Lactobacilli	Clostridia	β-Glucuronidase	pH
	log	log	log	log	nmol/g feces	
1. Cx alone	−4	+4	0	+1	+1	−0.1
2. Cx + *Saccharomyces boulardii*	−1	+1	0	0	+4	−0.6
3. Cx + *Enterococcus* species SF68	−4	+2, 3	−1	0	−2, 3	−0.5
4. Cx + lactulose	−4	0	+0.5, 1	0	−1	−0.6
5. Cx + *Lactobacillus casei* subspecies *rhamnosus* GG	−4	+4	+0.5, 1	+0.5, 1	−1	−0.6
6. Cx + *Lactobacillus rhamnosus* + *Lactobacillus bifidus* + *Lactobacillus acidophilus*	−4	+2, 3	+2, 3	+0.5, 1	−2, 3	−0.3
7. Cx + *L acidophilus* + *Bifidobacterium bifidum*	−4	+1	+1	−1	−1	−0.7
8. Cx + mixture of LAB	−4	+4	+2, 3	+1	−1	−0.6

LAB = lactic acid bacteria.
*0 = no changes before and after treatment; +1 = 1 log increase; +2, 3 = 2–3 log increase; +4 = 4 log increase; −1 = 1 log decrease; −2, 3 = 2–3 log decrease; −4 = 4 log decrease; +0.5, 1 = 0.5–1 log increase.

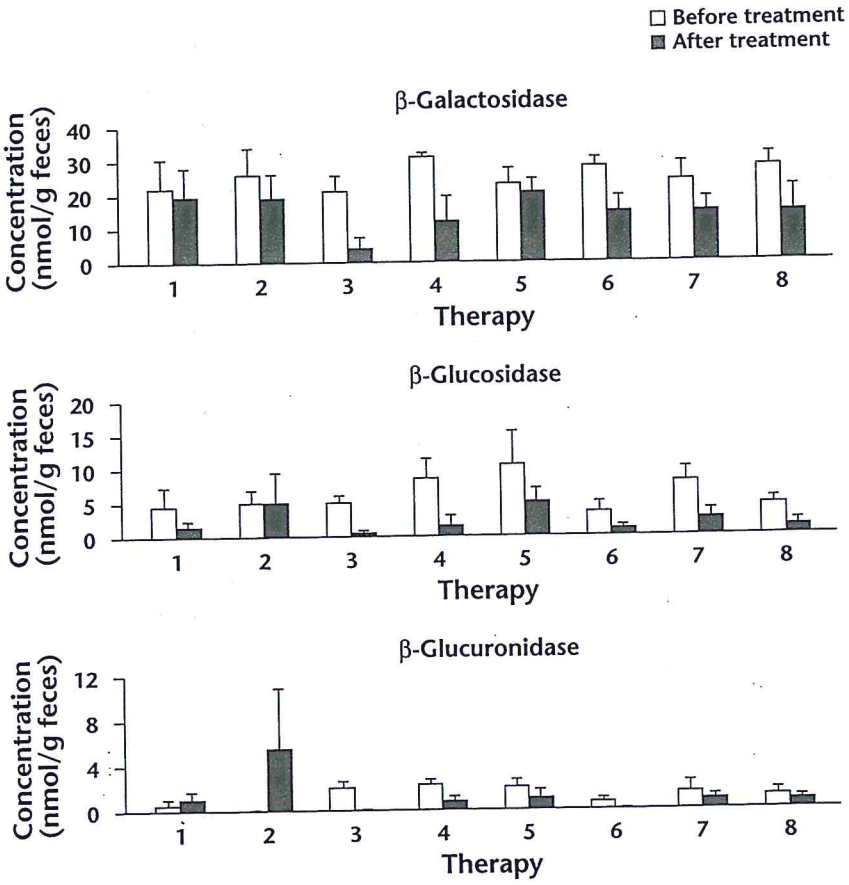

Figure 2. Enzymatic activities of β-galactosidase, β-glucosidase, and β-glucuronidase before and after administration of therapies 1 through 8. Therapy 1 = ceftriaxone alone; therapy 2 = ceftriaxone + *Saccharomyces boulardii;* therapy 3 = ceftriaxone + *Enterococcus* species; therapy 4 = ceftriaxone + lactulose; therapy 5 = ceftriaxone + *Lactobacillus casei* subspecies *rhamnosus* GG; therapy 6 = ceftriaxone + *Lactobacillus rhamnosus* + *Lactobacillus bifidus* + *Lactobacillus acidophilus;* therapy 7 = ceftriaxone + *Bifidobacterium bifidum* + *Lactobacillus acidophilus;* therapy 8 = ceftriaxone + *Streptococcus salivarius* subspecies *thermophilus* + *Bifidobacterium breve* + *Bifidobacterium infantis* + *Bifidobacterium longum* + *L acidophilus* + *Lactobacillus plantarum* + *L casei* + *Lactobacillus delbrueckei* subspecies *bulgaricus* + *Streptococcus faecium.*

dysbiosis induced by administration of ceftriaxone, even though the *Enterococcus* administered was able to compete with and replace the other *Enterococcus* species and colonize the gastrointestinal tract. The same biotype (API codes 5157511 and 5357511) was isolated and identified in fecal samples and in the commercial preparation.

Appreciable changes were also observed in fecal enzymatic activity after treatment. All of the probiotic/prebiotic preparations in our experimental conditions induced a decrease in β-galactosidase and β-glucosidase activity and an increase in esterase activities (ie, esterase-lipase activity and leucine and valine arylamidase activity; data not shown). All the probiotic preparations, with the exception of *S boulardii,* also induced a decrease in β-glucuronidase activity and in the number of patients with β-glucuronidase activity (Figure 2). No significant difference was observed between different probiotic/prebiotic regimens in the effects on fecal enzymatic activity, with 1 exception: the increase in β-glucuronidase activity induced by *S boulardii* plus ceftriaxone was substantially greater than that induced by ceftriaxone alone or other probiotic treatments.

Beta-Lactamase and Ceftriaxone Levels in Feces

Fecal β-lactamases increased after antimicrobial therapy in all groups studied (Table III). In the group of children treated with ceftriaxone alone (therapy 1), β-lactamase activity was detectable in 3 of 5 (60%) patients after a short period of therapy (compared with no activity before treatment). In the groups treated with therapies 3, 4, 6, 7, and 8, β-lactamase activity was found in 1 or 2 more children after treatment compared with before treatment. In groups that received therapy 2 (*S boulardii*) and therapy 5 (*L casei* subspecies *rhamnosus* GG), β-lactamase activity was detected in 83% (5/6) and 85% (6/7) of children, respectively. The administration of the newer probiotics (therapies 7 and 8) in combination with ceftriaxone appears to maintain a low percentage of positive samples (30%–40%).

Ceftriaxone concentrations in feces were detectable in 36% of samples. This frequency is probably due to the pharmacokinetic characteristics of ceftriaxone, such as its enterohepatic circulation, and to microbial inactivation. Samples with detectable fecal concentrations of the antibiotic tested negative for β-lactamases in the majority of children.

DISCUSSION

The aim of this study was to compare simultaneously the effects of different probiotics on the intestinal ecosystem during antimicrobial therapy. We present these data as preliminary results obtained in a relatively small number of children for each treatment. However, the relatively small and homogenous population is the consequence of the restrictive exclusion criteria, including the short period of investigation (winter of 1997–1998), the common pathologic condition (respiratory tract infections), and the defined antimicrobial treatment (ceftriaxone).

Ceftriaxone is known to cause an appreciable degree of dysbiosis in the intestinal ecosystem.[23] In our study, treatment with ceftriaxone alone had a significant impact on the number of stools per day in infants compared with ceftriaxone treatment in combination with probiotics containing mixtures of *Bifidobacterium* and *Lactobacillus* species.

Table III. Composition of the intestinal ecosystem before and after therapy in patients treated with ceftriaxone alone (therapy 1) or with *Saccharomyces boulardii* (therapy 2); *Enterococcus* species (therapy 3); lactulose (therapy 4); *Lactobacillus casei* GG (therapy 5); *Lactobacillus rhamnosus, Lactobacillus bifidus,* and *Lactobacillus acidophilus* (therapy 6); *Bifidobacterium bifidum* and *L acidophilus* (therapy 7); or a mixture of various lactobacilli and bifidobacteria at high concentrations (therapy 8).*

	Therapy 1		Therapy 2		Therapy 3		Therapy 4	
	Before	After	Before	After	Before	After	Before	After
Total aerobic count	6.2 (3.3)	7.2 (3.1)	11.5 (2.5)	11.5 (0.5)	11.5 (0.8)	10.5 (2.8)	9.4 (3.6)	7.4 (4.3)
Escherichia coli	4.4 (1.1)	2.2 (0.4)†	8.2 (4.8)	7.2 (5.5)	8.4 (3.7)	4.0 (3.7)†	7.4 (3.7)	2.3 (0.5)‡
Enterobacteria (other)	2.0 (0.0)	2.4 (0.9)	2.0 (0.0)	2.0 (0.0)	2.7 (1.2)	2.3 (0.8)	2.7 (0.9)	2.1 (0.4)
Enterococci	4.6 (2.2)	8.2 (2.2)†	7.5 (5.3)	8.5 (4.4)	6.0 (1.7)	7.6 (3.6)	8.4 (3.7)	7.9 (4.2)
Microaerophilic lactobacilli	4.2 (1.6)	4.2 (1.3)	3.0 (0.8)	3.0 (0.8)	4.3 (3.1)	3.6 (2.1)	4.3 (2.4)	3.1 (1.8)
Fungi	4.4 (1.5)	4.4 (1.5)	6.2 (2.9)	8.2 (3.3)	8.4 (4.2)	8.7 (4.1)	7.6 (4.1)	6.3 (4.0)
Total anaerobic count	7.8 (2.6)	8.2 (1.3)	10.5 (1.6)	10.7 (2.5)	9.3 (2.6)	10.1 (2.9)	9.6 (3.1)	9.6 (3.7)
Bacteroides	5.2 (4.0)	4.2 (1.9)	4.8 (2.9)	3.3 (1.3)	4.7 (2.1)	3.6 (1.6)	3.9 (2.4)	3.1 (1.1)
Clostridia	4.6 (0.9)	6.0 (1.9)	6.5 (4.0)	6.5 (3.3)	6.6 (2.8)	6.6 (4.3)	7.4 (2.4)	6.9 (4.4)
Lactobacillus acidophilus	4.8 (1.9)	3.8 (1.3)	3.0 (0.8)	2.7 (0.5)	3.4 (1.5)	4.0 (3.2)	4.1 (1.8)	2.9 (0.7)
Lactobacilli-Bifidobacteria	7.2 (2.8)	6.4 (2.3)	6.2 (2.6)	5.7 (4.3)	7.6 (3.1)	7.0 (3.8)	7.0 (2.3)	6.6 (3.4)
Anaerobic cocci	5.2 (1.3)	6.2 (0.8)	6.2 (3.8)	7.0 (5.8)	5.4 (2.4)	9.4 (3.6)†	8.6 (3.3)	6.0 (3.5)
pH	6.9 (0.4)	6.8 (0.7)	6.8 (0.5)	6.2 (0.5)	7.2 (0.5)	6.7 (0.8)	6.9 (0.4)	6.3 (0.5)†
Ceftriaxone concentration mg/g feces	—	0.46	—	0.25	—	1.28; 0.012; 1.42; 2.1; 2.3	—	3.4; 1.3; 2.1; 2.3
No. of positive samples	—	1/5	—	3/6	—	5/7	—	4/7
No. of β-lactamase–positive samples	0/5	3/5	3/6	5/6	2/7	3/7	3/7	4/7

(continued)

Table III. Continued.

	Therapy 5		Therapy 6		Therapy 7		Therapy 8	
	Before	After	Before	After	Before	After	Before	After
Total aerobic count	10.0 (2.3)	10.2 (3.5)	6.8 (1.2)	8.6 (3.4)	9.6 (1.7)	8.1 (4.8)	9.8 (3.5)	12.0 (0.0)
Escherichia coli	6.8 (2.8)	2.6 (0.9)†	5.6 (1.4)	2.6 (0.8)†	7.7 (3.6)	3.7 (3.7)	8.2 (3.9)	2.4 (0.5)†
Enterobacteria (other)	2.6 (1.3)	2.4 (0.9)	2.0 (0.0)	2.1 (0.4)	2.0 (0.0)	2.0 (0.0)	4.8 (3.6)	2.4 (0.9)
Enterococci	7.6 (3.6)	11.4 (0.9)†	6.7 (2.5)	9.6 (4.0)	7.1 (5.0)	8.1 (3.6)	5.0 (2.5)	9.8 (4.4)
Microaerophilic lactobacilli	3.2 (1.1)	3.6 (1.5)	2.9 (1.1)	3.0 (1.4)	2.7 (1.0)	3.9 (1.9)	4.4 (1.5)	4.4 (2.1)
Fungi	7.6 (3.6)	10.4 (3.6)	5.4 (3.1)	6.3 (3.2)	7.6 (3.5)	7.9 (4.4)	7.8 (4.0)	7.2 (4.4)
Total anaerobic count	11.4 (1.3)	11.8 (0.4)	9.3 (2.6)	10.6 (2.5)	9.0 (4.0)	8.0 (4.4)	9.4 (3.7)	12.0 (0.0)
Bacteroides	4.8 (1.3)	5.0 (2.0)	3.6 (1.5)	4.4 (1.3)	3.0 (0.6)	3.1 (1.5)	4.0 (1.9)	3.4 (1.7)
Clostridia	8.4 (2.3)	7.8 (4.0)	6.1 (2.1)	6.6 (2.9)	7.3 (4.0)	6.6 (4.0)	9.2 (3.7)	10.4 (3.6)
Lactobacillus acidophilus	4.2 (1.1)	4.8 (1.3)	4.1 (1.8)	5.4 (3.5)	3.1 (0.6)	3.4 (1.6)	5.2 (1.8)	3.8 (1.1)
Lactobacilli-Bifidobacteria	8.2 (2.5)	7.0 (2.6)	7.7 (3.2)	9.6 (3.9)	6.7 (3.6)	6.1 (4.3)	8.6 (3.4)	10.6 (3.1)
Anaerobic cocci	6.9 (3.2)	9.4 (3.6)	8.7 (3.2)	10.1 (3.2)	5.9 (3.1)	5.4 (3.4)	7.0 (3.0)	9.0 (4.5)
pH	6.9 (0.8)	6.3 (0.7)	6.8 (0.8)	6.5 (0.5)	6.8 (0.3)	6.1 (0.5)†	6.9 (0.3)	6.3 (0.8)
Ceftriaxone concentration mg/g feces	—	0.7	—	0.005; 1.5	—	1.41; 1.38	—	1.26; 0.75
No. of positive samples	—	2/7	—	2/7	—	2/7	—	2/5
No. of β-lactamase–positive samples	2/7	6/7	1/7	3/7	0/7	2/7	1/5	2/5

*Values are expressed as log n bacteria per gram of fresh feces, mean (SD).
†$P \leq 0.05$ versus values before therapy (Student t test).
‡$P \leq 0.01$ versus values before therapy (Student t test).

Our results suggest that the probiotic/prebiotic agents studied are quite different in the degree of protection they afford from the intestinal microbial imbalance caused by ceftriaxone, even though all of them reversed the ceftriaxone-induced increase in the number of *Clostridia* species.

The probiotic preparations that appear to have the most positive effects on the intestinal ecosystem (particularly via a marked, though statistically non-significant increase in lactobacilli and bifidobacteria) are the mixture containing *B bifidum* and *L acidophilus* (therapy 7) and the preparation containing 360 billion live LAB cells (therapy 8). The *L rhamnosus* + *L bifidus* + *L acidophilus* preparation (therapy 6) induced an increase in microaerophilic lactobacilli, whereas the preparation containing *L casei* subspecies *rhamnosus* GG (therapy 5) produced no significant effect in preventing dysbiosis during ceftriaxone therapy. These data seem to indicate that organisms physiologically present in the gastrointestinal tract of healthy children and very high concentrations of probiotic (20 billion to 360 billion per day) are necessary to restore the balance of the intestinal ecosystem during antibiotic therapy. The changes in the intestinal ecosystem induced by high concentrations of probiotic are, however, relatively limited compared with the results obtained with lower-dose preparations. This may be due to the greater susceptibility of these bacteria to ceftriaxone, that is, the probability of obtaining a modification of intestinal microflora may be strongly increased when preparations containing a substantial number of bacteria belonging to several different species and strains are used for probiotic therapy. This hypothesis is further supported (though indirectly) by the results obtained with probiotics that do not contain LAB. The preparations containing only *S boulardii* or *Enterococcus* had no relevant effect on the dysbiosis caused by ceftriaxone.

All the probiotics studied induced a marked general decrease in stool pH (–0.5). This finding can be interpreted as a positive effect, since an acid environment in the intestinal ecosystem inhibits the growth of proteolytic bacteria, which are generally harmful. Lactulose, which is considered a bifidogenic factor,[24] appears to stimulate production of organic acids by the intestinal microflora, resulting in a decrease in intestinal pH.[25]

The levels of some enzyme activities in children with respiratory tract infections seem to be altered compared with those recorded in healthy children of similar age.[20]

In this study, we found that probiotics induced partial normalization of a number of fermentative activities, according to their specific composition. The decrease in some enzyme activities, such as β-galactosidase activity, after probiotic administration represents a return to normal levels (mean value 0.8 nmol/g feces),[20] whereas other activities (eg, leucine arylamidase) remained altered. Beta-glucuronidase activity, for example, reverted to baseline levels during the administration of therapies 5, 6, and 7. The reduction in β-glucosidase and β-glucuronidase activities is a characteristic effect of LAB probiotics that has been described in various studies in vivo[19,26,27] and in

vitro[28]. Indeed, only a few strains of *Lactobacillus* and *Bifidobacteria* were able to induce such modifications.[29] In contrast, the administration of *S boulardii* caused an increase in β-glucuronidase activity. A number of biochemical activities, including β-glucosidase and β-glucuronidase activity, are related to cancer risk and to the types of bacteria present in the intestinal microflora.[30]

An additional relevant finding of our work was the unexpected and never previously described ability of probiotics to limit the activity of β-lactamases in children treated with β-lactam antibiotics. The β-lactamases produced by intestinal bacteria can increase after the administration of β-lactam antibiotics,[31] thereby increasing the risk of bacterial resistance and transfer of resistance to other intestinal bacteria. This effect appears to differ according to the probiotic administered and appears to be peculiar to certain bacterial species, such as *L acidophilus* and *B bifidum,* which were used in preparations 7 and 8.

Probiotics may have a beneficial effect on human health because of their ability to modify the composition and metabolic capability of the intestinal ecosystem, limit the adverse effects caused by drugs, and possibly reduce the transfer of bacterial resistance. However, the risk of causing infection is intrinsic to their use. Although a number of species of lactobacilli have been associated with endocarditis and localized infections,[32,33] mainly in special populations, this incidence is very low, particularly in relation to their widespread use. LAB are therefore considered relatively safe.[29,34–36] *Saccharomyces* species, administered as live microorganisms, can induce fungemia in some patients.[37,38]

The *E faecium* preparation significantly increases the mean anaerobic cocci count, thereby worsening the dysbiosis caused by ceftriaxone. *Enterococcus* appears to share the main characteristics of LAB, but also comprises pathogenic species. Enterococci show an extensive range of resistance to various antibiotics, including β-lactams, lincosamides, and polymyxins; the evolution of virulence has been documented.[32,39] These considerations suggest caution in the use of enterococci as probiotics.

CONCLUSIONS

Despite the small number of patients in each treatment group, the results of this study suggest that newer probiotic preparations containing lactobacilli and bifidobacteria species, which are more similar to the organisms present in the gastrointestinal tract of healthy children, are better tolerated and more effective in preventing dysbiosis than the other agents studied.

Dysbiosis in children recorded after 4 or 5 days of drug treatment is partly remedied by the administration of probiotics. It is probable, however, that therapy with effective probiotics should be maintained for several days after discontinuation of ceftriaxone therapy to restore the eubiosis of the intestinal ecosystem.

Further studies with larger patient populations are necessary to assess the net effects of different probiotic preparations on the dysbiosis associated with antimicrobial therapy.

ACKNOWLEDGMENTS

This study was supported by a grant from Consorzio per gli Studi Universitari, Verona, Italy. This paper was presented in part at the Second World Congress on Anaerobic Bacteria and Infection, October 3–6, 1998, Nice, France. The authors are grateful to Chiara Caveiari for her technical assistance and excellent microbiologic analysis. The authors also thank Anthony Steele, MA, lecturer in medical English, University of Verona, for his linguistic editing of this paper.

REFERENCES

1. Zoppi G, Deganello A, Benoni G, Saccomani F. Oral bacteriotherapy in clinical practice. I. The use of different preparations in infants treated with antibiotics. *Eur J Pediatr.* 1982;139:18–21.
2. Zoppi G. Probiotics and prebiotics in the treatment of infections due to vancomycin-dependent *Enterococcus faecalis* and of imbalance of the intestinal ecosystem (dysbiosis). *Acta Paediatr.* 1997;86:1148–1150.
3. Zoppi G, Balsamo V, Deganello A, et al. Oral bacteriotherapy in clinical practice. II. The use of different preparations in the treatment of acute diarrhoea. *Eur J Pediatr.* 1982;139:22–24.
4. Gorbach SLI, Chang TW, Goldin B. Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. *Lancet.* 1987;2:1519.
5. Fuller R. Probiotics in man and animals. *J Appl Bacteriol.* 1989;66:365–378.
6. Tvede M, Rask-Madsen J. Bacteriotherapy for chronic relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. *Lancet.* 1989;1:1156–1160.
7. Surawicz CM, McFarland LV, Elmer GW, Chinn J. Treatment of recurrent *Clostridium difficile* colitis with vancomycin and *Saccharomyces boulardii. Am J Gastroenterol.* 1989;84:1285–1287.
8. Biller JA, Katz AJ, Flores AF, et al. Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. *J Pediatr Gastroenterol Nutr.* 1995;21:224–226.
9. Bennett RG, Gorbach S, Goldin BR, Chang TW. Treatment of relapsing *Clostridium difficile* diarrhea with *Lactobacillus* GG. *Nutr Today.* 1995;31(Suppl):355–415.
10. Colombel JP, Cortot A, Neut C, Romond C. Yoghurt with *Bifidobacterium longum* reduces erythromycin-induced gastrointestinal effects. *Lancet.* 1987;2:43.
11. Siitonen S, Vapaatalo H, Salminen S, et al. Effect of *Lactobacillus* GG yoghurt in prevention of antibiotic associated diarrhoea. *Ann Med.* 1990;22:57–59.
12. Tankanow RM, Ross MB, Ertel IJ, et al. A double-blind placebo-controlled study of the efficacy of Lactinex in the prophylaxis of amoxicillin-induced diarrhea. *DICP.* 1990;24:382–384.
13. Orrhage K, Brismar B, Nord CE. Effects of supplements of *Bifidobacterium longum* and *Lactobacillus acidophilus* on the intestinal microbiota during administration of clindamycin. *Microb Ecol Health Dis.* 1994;7:17–25.
14. Isolauri E, Juntunen M, Rautanen T, et al. A human *Lactobacillus* strain (*Lactobacillus casei* sp strain GG) promotes recovery from acute diarrhea in children. *Pediatrics.* 1991;88:90–97.
15. Kaila M, Isolauri E, Soppi E, et al. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. *Pediatr Res.* 1992; 32:141–144.
16. Hilton E, Kilakowski P, Singer C, Smith M. Efficacy of *Lactobacillus* GG as a diarrheal preventive in travelers. *J Travel Med.* 1997;4:41–43.

17. Dellaglio F, Bertazzoni Minelli E, Binelli A, et al. Plate counting of probiotic products. *Gastroenterol Int.* 1998;11(Suppl 1):137.
18. Hamilton-Miller JM, Shah S, Smith CT. "Probiotic" remedies are not what they seem. *Br Med J.* 1996;312:55–56.
19. Bertazzoni Minelli E, Benini A, Vicentini L, et al. Effects of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* administration on colonic microbiota and its metabolic activity in premenstrual syndrome. *Microb Ecol Health Dis.* 1996;9:247–260.
20. Zoppi G, Cinquetti M, Luciano A, et al. The intestinal ecosystem in chronic functional constipation. *Acta Paediatr.* 1998;87:836–841.
21. Bertazzoni Minelli E, Benini A, Vicentini L, Muner A. Antibiotics and their interaction with intestinal contents. *Microecol Ther.* 1995;25:360–367.
22. Bryan LE, Gogfrey AJ. β-Lactam antibiotics: Mode of action and bacterial resistance. In: Lorian V, ed. *Antibiotics in Laboratory Medicine.* Baltimore: Williams & Wilkins; 1991:599–664.
23. de Vries-Hospers HG, Tonk RH, van der Waaij D. Effect of intramuscular ceftriaxone on aerobic oral and faecal flora of 11 healthy volunteers. *Scand J Infect Dis.* 1991;23: 625–633.
24. Modler HW. Bifidogenic factors—sources, metabolism and applications. *Int Dairy J.* 1994;4:383–407.
25. Kiyosawa I, Takase M, Yamauchi K, et al. Lactulose and intestinal microflora in infant nutrition. *Bifidobacteria Microflora.* 1986;5:27–35.
26. Bouhnik Y, Flourié B, D'Agay-Abensour L, et al. Administration of transgalacto-oligosaccharides increases fecal Bifidobacteria and modifies colonic fermentation metabolism in healthy humans. *J Nutr.* 1997;127:444–448.
27. Djouzi Z, Andrieux C, Degivry MC, et al. The association of yoghurt starters with *Lactobacillus casei* DN 114.001 in fermented milk alters the composition and metabolism of intestinal microflora in germ-free rats and in human flora-associated rats. *J Nutr.* 1997;127:2260–2266.
28. Jiang T, Savaiano DA. In vitro lactose fermentation by human colonic bacteria is modified by *Lactobacillus acidophilus* supplementation. *J Nutr.* 1997;127:1489–1495.
29. Havenaar R, Spanhaak S. Probiotics from an immunological point of view. *Curr Opin Biotechnol.* 1994;5:320–325.
30. Hughes R, Rowland IR. Metabolic activities of the gut microflora in relation to cancer. *Microb Ecol Health Dis.* 2000;Suppl 2:179–185.
31. Edlund C, Stark C, Nord CE. The relationship between an increase in beta-lactamase activity after oral administration of three new cephalosporins and protection against intestinal ecological disturbances. *J Antimicrob Chemother.* 1994;34:127–138.
32. Aguirre M, Collins MD. Lactic acid bacteria and human clinical infection. *J Appl Bacteriol.* 1993;75:95–107.
33. Mackay AD, Taylor M, Kibbler CC, Hamilton-Miller JMT. Endocarditis caused by a probiotic lactobacillus. 2nd European Congress Chemother and BICON 7. *Antiinfect Drugs Chemother.* 1998;16(Suppl 1):36. Abstract M243.
34. Gasser F. Safety of lactic acid bacteria and their occurrence in human clinical infections. *Bull Inst Pasteur.* 1994;92:45–67.
35. Driessen FM, de Boer R. Fermented milks with selected intestinal bacteria: A healthy trend in new products. *Neth Milk Dairy J.* 1989;43:367–382.
36. Salminen S, von Wright A. Current probiotics—safety assured? *Microb Ecol Health Dis.* 1998;10:68–77.

37. Pletincx M, Legein J, Vandenplas Y. Fungemia with *Saccharomyces boulardii* in a 1-year-old girl with protracted diarrhea. *J Pediatr Gastroenterol Nutr.* 1995;21:113–115.
38. Eddy JT, Stamatakis MK, Makela EH. *Saccharomyces boulardii* for the treatment of *Clostridium difficile*-associated colitis. *Ann Pharmacother.* 1997;31:919–921.
39. Farrag N, Eltringham I, Liddy H. Vancomycin-dependent *Enterococcus faecalis*. *Lancet.* 1996;348:1581–1582.

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