0031-3998/06/5903-0451
PEDIATRIC RESEARCH
Vol. 59, No. 3, 2006
Printed in U.S.A.

Effect of a Milk Formula With Prebiotics on the Intestinal Microbiota of Infants After an Antibiotic Treatment

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ABSTRACT: Antibiotics exert deleterious effects on the intestinal microbiota, favoring the emergence of opportunistic bacteria and diarrhea. Prebiotics are nondigestible food components that stimulate the growth of bifidobacteria. Our aim was to evaluate the effects on the intestinal microbiota of a prebiotic-supplemented milk formula after an antibiotic treatment. A randomized, double-blind, controlled clinical trial was carried out in 140 infants 1–2 y of age distributed into two groups after a 1-wk amoxicillin treatment (50 mg/kg/d) for acute bronchitis. The children received for 3 wk >500 mL/d of a formula with prebiotics (4.5 g/L) or a control without prebiotics. Fecal samples were obtained on d –7 (at the beginning of the antibiotic treatment), on d 0 (end of the treatment and before formula administration), and on d 7 and 21 (during formula administration). Counts of *Bifidobacterium*, *Lactobacillus-Enterococcus*, *Clostridium lituseburiense* cluster, *Clostridium histolyticum* cluster, *Escherichia coli*, and *Bacteroides-Prevotella* were evaluated by fluorescent *in situ* hybridization (FISH) and flow cytometry. Tolerance and gastrointestinal symptoms were recorded daily. Amoxicillin decreased total fecal bacteria and increased *E. coli*. The prebiotic significantly increased bifidobacteria from 8.17 ± 1.46 on d 0 to 8.54 ± 1.20 on d 7 compared with the control 8.22 ± 1.24 on d 0 *versus* 7.95 ± 1.54 on d 7. The *Lactobacillus* population showed a similar tendency while the other bacteria were unaffected. No gastrointestinal symptoms were detected during the prebiotic administration. Prebiotics in a milk formula increase fecal bifidobacteria early after amoxicillin treatment without inducing gastrointestinal symptoms. **(*Pediatr Res* 59: 451–456, 2006)**

The newborn gastrointestinal tract is sterile at birth and is rapidly colonized by microorganisms from the maternal fecal flora. A number of factors influence the colonization pattern of the newborn gut such as the mode of delivery (vaginal or cesarean), the hygienic conditions of the delivery, and the type of feeding (maternal milk or cow's milk formula) (1,2). It is estimated that at about 2 y of age, a "mature" resident microbiota including some 200 different species has become established and that it will remain unchanged for many years, demonstrating remarkable stability.

The complex bacterial ecosystem present in the gut has been traditionally studied through bacteriological culture methods based on selective media; molecular methods based on the study of the 16S rRNA gene or the use of 16S rRNA hybridization probes have been developed in the past decade that allow the culture-independent analysis of the colonic microbiota (3,4). Such molecular methods have already been used to evaluate the infantile intestinal microbiota (5,6).

The intestinal microbiota may be qualitatively and quantitatively altered by the use of anti-inflammatory nonsteroidal anti-inflammatory drugs, laxatives, and antibiotics (1,7). When administered in adequate doses and for adequate lengths of time, antibiotics will eradicate susceptible microorganisms and stimulate the proliferation of opportunists such as *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Candida*, and some species of *Clostridium* that may occupy ecological niches previously unavailable to them (8). These bacteria may be potentially pathogenic and produce toxins, as observed with *Clostridium difficile*, resulting in episodes of diarrhea that range from mild to severe and life threatening, such as pseudomembranous enterocolitis (9). Some time after the antibiotic treatment has ended, the microbiota returns spontaneously to its previous composition.

The autochthonous microbiota may also be modified by the intake of dietary components such as probiotics or prebiotics. Prebiotics are nondigestible dietary ingredients such as fructooligosaccharides (FOS) that exert positive influences on the host because they selectively stimulate in the colon the growth and/or metabolic activities of bacterial species with health-promoting properties, mainly *Bifidobacterium* and *Lactobacillus* (10). Prebiotics exert regulatory effects on colonic functions, increasing fecal bulk and water retention through the increased bacterial mass because they are a suitable substrate for fermentation. Prebiotics also increase calcium absorption independently of intake, probably because the acidification of the luminal milieu makes calcium more soluble and more easily absorbable (11). Fructans are generally considered safe for infants and children; however, some concerns have been raised due to the increased frequency of loose stools observed in infants fed FOS-supplemented formula and the possibility

Received July 20, 2005; accepted October 3, 2005.
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Supported by NESTEC, Vers-chez-les-Blancs, Switzerland.

DOI: 10.1203/01.pdr.0000198773.40937.61

Abbreviations: FISH, fluorescent *in situ* hybridization; **FOS,** fructooligosaccharides

of increased risk of dehydration (12). Probably more studies are required to further clarify this aspect.

In view of the negative repercussions of antibiotic treatments on the intestinal microbiota, the aim of this study was to evaluate the effect of a milk formula supplemented with a prebiotic mixture including inulin and oligofructose on the recovery of fecal bifidobacteria after treatment of an episode of acute bronchitis with amoxicillin in infants.

SUBJECTS AND METHODS

Population. The study was a prospective, randomized, double-blind, placebo-controlled trial that was carried out in a single health center (La Faena) located in the Southeastern part of Santiago, Chile. This center provides medical care to a population of about 42,000 inhabitants, the majority of whom belong to the low and medium-low socioeconomic strata. The study population was formed by infants of either sex 12–24 mo of age on admission to the protocol. Their parents or legal guardians received careful explanations about the aim and scope of the study, and if they agreed, they signed a standard written consent form. The project had been approved by the Committee on Ethics in Research in Humans, INTA, University of Chile.

It was calculated that 56 subjects must complete the protocol in each of the study groups; because a 25% dropout rate in each group was estimated from previous studies, the total recruitment was originally estimated to require 75 infants per group for a total of 150 infants. However, because the dropout rate was low, we completed the numbers required for a meaningful statistical analysis after we enrolled 140 infants.

All infants admitted to the study had to fulfill the following inclusion criteria: age between 12 and 24 mo on admission, born at term with weight between 2.5 and 4.2 kg, with normal bowel function, and on full oral feeding. Furthermore, they had to have a diagnosis of acute bronchitis that warranted treatment with an antibiotic. Acute bronchitis was defined by the presence of productive cough and occasional wheezing and the absence of overt bronchial obstruction, lung consolidation, or pleural involvement; patients may have had fever during the first few days but usually this disappeared spontaneously or with the beginning of the treatment. Children were excluded if they fulfilled one or more of the following criteria: antibiotic use in the preceding 4 wk, allergy to antibiotics, suspected to require antibiotic treatment for >7 d, total or partial breastfeeding, unwillingness on the part of the parents to interrupt any regular intake of fermented foodstuffs during the study period, symptoms or signs of respiratory insufficiency or of immune deficiency, congenital malformations, inborn errors of metabolism or chronic diseases, cow's milk allergy or lactose intolerance, and treatment with steroids or prokinetic drugs. Criteria for exclusion after admission to the protocol included noncompliance with the 7-d antibiotic treatment or the need for extending it beyond 7 d.

Management of patients and study design. The antibiotic treatment was started at the health center on the day of admission to the protocol and consisted of an amoxicillin suspension (Amoval, Laboratorios Saval, Chile) at a dosage of 50 mg/kg/d divided into three daily doses and administered for 7 d. On enrollment, patients were assigned at random to either an experimental or a control group. The experimental group received a growing-up formula with Prebio 1 (Nestlé de Chile, Santiago, Chile); the latter was a mixture of oligofructose and inulin (Raftilose P95 and Raftiline, respectively; Orafti, Tienen, Belgium) in 70/30 proportion by weight, which provided 4.5 g/L after formula reconstitution. The control formula was similar except that it lacked the Prebio mixture. The formula with Prebio 1 consisted of whole milk, sugar, honey, corn oil, soy lecithin, Prebio 1, and a complete vitamin and mineral mixture; its composition was as follows: fat mix, 4.97 g; lecithin, 4.27 g; linoleic acid, 0.04 g; α-linolenic acid, 0.44 g; protein, 4.42 g; available carbohydrates, 9.42 g of which 6.50 g was lactose, 1.89 was sucrose and other carbohydrates 1.03 g; fiber as oligosaccharides was 0.61 g; total solids: 20.4 g and 0.83 g moisture.

All formula products were letter coded by the manufacturer so that the investigators, field personnel, and the parents receiving them were unaware of its composition. The code was broken only after the data analysis was concluded. Formulas were kept in cold storage and delivered to the field station as required, where they were also kept refrigerated.

The children received the product allocated for 3 wk after completion of the antibiotic treatment with a minimum volume of 500 mL/d; it was calculated that 500 mL of the growing-up formula with Prebio 1 provided 2.25 g of the prebiotic mixture per day. Formula intake was recorded daily by the mother or legal guardian in an *ad hoc* form and was then compared with the advice provided by the pediatrician and the field nurses. While they were taking the respective formulas, children were allowed their usual diet and mothers were advised not to provide other formulas, yogurt or acidified milk, or iron-containing preparations.

Fecal samples were obtained on d −7 (just before the antibiotic therapy), d 0 (end of the antibiotic therapy and beginning of the formula feeding), d 7 (after 1 wk of formula feeding), and d 21 (end of the administration of the allocated formula). If an episode of diarrhea occurred during the period in which the formula was being administered, a stool culture was performed to detect potential enteropathogens. Possible adverse events were registered in *ad hoc* forms. In addition to *Bifidobacterium*, fecal *Lactobacillus-Enterococcus*, *Bacteroides-Prevotella*, *Escherichia coli*, and *Clostridium* spp were also counted by using FISH and flow cytometry, as previously described (3). Furthermore, the presence of enteropathogenic *E. coli* (EPEC), *Campylobacter*, *Salmonella* spp, and *Shigella* was also evaluated by culture methods.

Gastrointestinal symptoms (flatulence, abdominal pain, nausea, vomiting) as well as diaper rash, and the number and characteristics of the stools were recorded daily by the pediatrician at the health center or at home by the mother.

Laboratory procedures. Fecal samples were collected under sterile conditions immediately after emission and transported to the laboratory within at the most 3 h after their passage. An additional sample for pathogen detection was obtained by rectal swabbing and placed in transport medium.

About 1 g of feces was diluted to 10% (wt/vol) in sterile phosphate-buffered saline (PBS), homogenized with glass beads, and centrifuged at 300 $\times$ *g* for 1 min to eliminate large particles. One milliliter of the supernatant was added to 3 mL of 4% paraformaldehyde and fixed overnight at 4°C; 1.5 mL of the fixed sample was centrifuged twice at 13,000 $\times$ *g* for 5 min and the pellet was resuspended in 1 mL of sterile PBS. Finally, the pellet was suspended in 0.3 mL of a 50% solution of 95% ethanol in PBS and stored at −30°C until analyzed. For permeabilization of Gram-positive bacteria, 100 μL of the fixed bacteria was diluted to 10% in sterile PBS and centrifuged at 8000 rpm for 3 min. The pellet was then resuspended in Tris-ethylenediamine tetraacetic acid (EDTA) buffer, pH 8.8, and centrifuged again for 3 min at 8000 rpm; the pellet was then suspended in 1 mL of Tris-EDTA buffer with 1 mg/mL of lysozyme and incubated for 10 min at room temperature. After centrifuging, the pellet was washed once with PBS.

For FISH, the pellet was resuspended in 450 μL of hybridization buffer (0.9 mol/L NaCl, 20 mmol/L Tris HCl, 0.01% SDS, 30% formamide), and 50 μL of the suspension were distributed into nine tubes. Four microliters of the universal probe Eub338 (S-D-Bact-0338-a-A-18: 5′-GCTGCCTCCCGTAGGAGT-3′) labeled with fluorescein isothiocyanate (FITC) and 4 μL of one of the following species-specific oligonucleotide probes labeled with indodicarbocyanin (Cy5): Bac303 (S-*-Bac-0303-a-A-16; 5′-CCAATGTGGGGACTT-3′) for *Bacteroides*/*Prevotella*; Bif164 (S-G-Bif-0164-b-A-18; 5′-CATCCGGCATTACCACCC-3′) for *Bifidobacterium*; Lac158 (S-*-Lac-0158-a-A-20; 5′-GGTATTAGCAYCTGTTTCCA-3′) for *Lactobacillus*/*Enterococcus*; Eco1531 (L-S-Eco-1531-a-A-21; 5′-CACCGTAGTGCCTCGTCATCA-3′) for *E. coli*; Clit135 (S-*-Clit-0135-a-A-19; 5′-GTTATCCGTGTGTACAGGG-3′) for *C. lituseburense* cluster including *Clostridium difficile* and Chis150 (S-*-Chis-0150-a-A-23; 5′-TTATGCGGTATTAATCTYCCTTT-3′) for the cluster of *Clostridium histolyticum,* including *Clostridium perfringens*. The concentration of each of the probes was 50 ng/μL. Control tubes were prepared adding the Eub338 and nonEub (5′-ACATCCTACGGGAGGC-3′) probes labeled with either FITC or Cy5 to the remaining three tubes. After overnight hybridization at 35°C, 150 μL of the hybridization buffer was added to each tube. After centrifugation, the pellet was resuspended in 200 μL of wash solution (0.065 mol/L NaCl, 20 mmol/L Tris-HCl, 5 mmol/L EDTA, 0.01% sodium dodecylsulfate), incubated for 20 min at 37°C, centrifuged at 8000 rpm for 3 min, and resuspended in 200 μL of PBS.

Counting of the labeled bacteria was carried out by flow cytometry. Of every hybridized sample, 25 μL was added to a polystyrene tube containing 1 mL of FacsFlow, and 2 μL of a solution of fluorescent polystyrene beads 6 μm in diameter (Microsphere Standard, Invitrogen, Carlsbad, CA) to allow the absolute quantification of the hybridized bacteria. The samples were analyzed in a FacsScalibur flow cytometer (Becton-Dickinson, Franklin Lakes, NJ). Forward scatter (FSC), side scatter (SSC), and two fluorescent signals were measured. A 530-nm band pass filter (500–560 nm) was used to detect the green fluorescence (FL1, FITC), and a 661 band pass filter (645–677 nm) was used to detect the red fluorescence (FL4, Cy5). After calibration, every sample was read until 100,000 fluorescent events had been counted. Individual files were analyzed using WinMDI 2.8. Results obtained for every probe were expressed as percentage of the total count obtained with the universal Eub338 probe, and as $\log_{10}$ of cell counts, calculated by applying the relative quantification and the absolute counts obtained with the glass beads. These results are shown for every child who completed its four samples (S1 to S4) only, giving a total of 115 analyzable samples.

Statistical analysis. Demographic and anthropometric data were analyzed using t and χ^2 tests. Counts and percentages of the different bacterial populations were expressed as means ± SD; results obtained before and after the antibiotic treatment were analyzed by a paired t test and those obtained during the follow-up were evaluated by analysis of variance (ANOVA) for repeated measurements followed by a *post hoc* test if the ANOVA was positive.

RESULTS

Compliance and symptoms. The 140 subjects enrolled in the study protocol had a normal clinical examination except for the diagnosis of acute bronchitis, which was treated with amoxicillin; 130 of them completed successfully the 7-d antibiotic treatment and were randomly assigned to the Prebio group ($n = 64$) or the control group ($n = 66$). Seventeen subjects (13%), seven in the Prebio group (10.9%) and 10 in the control group (15.1%), did not complete the protocol. The main reasons for dropout were loss to follow-up in seven cases (41.2%), decision of the parents to stop participating (four cases, 23.5%), and additional antibiotic treatment (three cases, 17.6%). As a result, 57 children completed the protocol in the Prebio group and 56 children in the control group. One child was hospitalized, one was lost to follow-up because the parents moved to another area, and one was withdrawn by the physician. Demographic and anthropometric data of both groups at baseline are presented in Table 1.

Both formulas were well tolerated by the children. The average weekly volume ingested by the children was higher than the minimum of 500 mL required by the protocol, and no differences in volume intake were observed between weeks or groups. None of the participating children experienced deterioration of their nutritional status during the study and no differences in the frequency diaper rashes were observed. Gastrointestinal symptoms including flatulence, restlessness, cramping pain, crying, and vomiting were similar in both groups. No differences between the groups were observed in their daily stool frequency and consistency during the administration of the milk formulas (data not shown).

Twelve children developed episodes of diarrhea during the study after the antibiotic treatment, four in the Prebio group and seven in the Control group. All but two episodes were of short duration (<72 h) and were treated with oral fluids and advice to the mothers to maintain feeding as usual. Of the 137 stool samples obtained in this study, a total of 24 enteropathogens were isolated (17.5%); by far the predominant microorganism was *Campylobacter jejuni*, detected in 14 children (10.3%); none of these detections was associated with episodes of acute diarrhea. *E. coli* O44 was detected in two cases and *Salmonella enterica*, *Aeromonas* spp, and other EPEC serotypes (O26, O55, O86, O111, O119, and O126) in one subject each. No differences between the groups were observed in relation to asymptomatic enteropathogen shedding.

Evaluation of the fecal microbiota by FISH and flow cytometry. As shown in Table 2, the total number of bacteria detected in the children with the universal Eub338 probe after the antibiotic treatment and before inclusion in the protocol decreased 30.8% compared with d −7 ($p < 0.0000$). The bacterial populations not evaluated in this study represented 55.9 ± 24.9% of the total of bacteria detected with Eub338 at d −7; this percentage significantly decreased to 41.7 ± 25.5% after the 1-wk antibiotic treatment ($p < 0.000$), suggesting that these other bacterial populations were more sensitive to amoxicillin than those we studied. The antibiotic administration also modified the population of *E. coli*, which increased significantly ($p = 0.015$), this change being reflected by a significant increase of its proportion in the total population ($p = 0.004$). Other populations, such as *Bacteroides* and *Bifidobacterium,* were only altered in their percentages, which were significantly higher after amoxicillin ($p < 0.000$ and $p = 0.013$, respectively).

Table 3 shows the evolution of the bacterial populations from d 0, just after the amoxicillin treatment and before the administration of the formulas tested, and after 7 and 21 d of formula intake. The total counts of bacteria increased rapidly

Table 1. *Demographic and anthropometric data of children from the Prebio and control groups at baseline*

	Intention-to-treat analysis		Per-protocol analysis	
	Prebio	Control	Prebio	Control
No.	64	66	57	56
Girls	25 (39.0%)	26 (39.4%)	24 (42.1%)	20 (35.7%)
Age (mo)	17.5 ± 3.3	17.0 ± 3.4	17.2 ± 3.4	17.0 ± 3.4
Weight (kg)	11.0 ± 1.3	10.9 ± 1.3	10.9 ± 1.3	10.9 ± 1.3
Length (cm)	82 ± 5	80 ± 4	82 ± 5	81 ± 4
BMI (kg/m^2)	16.4 ± 1.5	16.9 ± 1.4	16.3 ± 1.4	16.8 ± 1.4

Table 2. *Effect of 7-d administration of amoxicillin on the fecal microbiota of children*

	D−7	D 0	p
Total bacteria (Eub 338)			
Log	9.57 ± 0.50	9.40 ± 0.57	<0.000
%	100	100	
Bifidobacterium (Bif164)			
Log	8.18 ± 1.36	8.20 ± 1.39	NS
%	16.9 ± 17.9	21.2 ± 16.7	0.013
Bacteroides (Bac303)			
Log	7.46 ± 1.64	7.65 ± 1.53	NS
%	8.05 ± 9.16	12.9 ± 12.8	<0.000
Lactob./Enteroc. (Lac158)			
Log	6.75 ± 1.78	6.72 ± 1.87	NS
%	3.91 ± 5.57	3.74 ± 4.40	NS
E. coli (Eco1531)			
Log	4.77 ± 0.96	5.10 ± 1.39	0.015
%	0.12 ± 0.49	2.78 ± 9.73	0.004
C. lituseburense (Chis150)			
Log	4.60 ± 0.60	4.53 ± 0.77	NS
%	0.02 ± 0.01	0.03 ± 0.02	NS
C. histolyticum (Clit135)			
Log	8.23 ± 1.23	8.13 ± 1.27	NS
%	16.5 ± 12.9	18.5 ± 13.9	NS
Other bacterial populations (%)	55.9 ± 24.9	41.7 ± 25.5	<0.000

Results are expressed as means ± SD of log (fluorescent events) and percentage of the population in relation to the total bacteria counted by the Eub338 probe. Differences between d 7 and d 0 were compared applying a paired t test.

Lactob./Enteroc., *Lactobacillus/Enterococcus*; NS, not significant.

Table 3. *Evolution of the different fecal bacterial populations in children receiving the milk formula supplemented with prebiotics or the control formula after a 7-d amoxicillin treatment*

Bacterial populations	D 0	D 7	D 21	*p*
Total bacteria (Eub 338)				
Prebio	9.38 ± 0.56	9.54 ± 0.52	9.47 ± 0.44	NS
Control	9.42 ± 0.57	9.59 ± 0.45	9.63 ± 0.48	
Bifidobacterium				
Prebio				
Log	8.17 ± 1.46	8.54 ± 1.20*	8.51 ± 0.88	
%	20.8 ± 17.9	23.7 ± 16.6*	20.0 ± 15.6	
Control				
Log	8.22 ± 1.24	7.95 ± 1.54	7.95 ± 1.68	0.029
%	21.5 ± 15.8	16.3 ± 18.9	17.8 ± 18.3	<0.000
Bacteroides				
Prebio				
Log	7.70 ± 1.46	7.34 ± 1.70	7.40 ± 1.63	
%	12.0 ± 11.0	8.26 ± 9.18	8.11 ± 8.94	
Control				
Log	7.61 ± 1.60	7.46 ± 1.70	7.37 ± 1.67	NS
%	13.3 ± 14.5	10.01 ± 9.01	7.03 ± 9.01	NS
Lactob./Enteroc.				
Prebio				
Log	6.55 ± 1.95	7.22 ± 1.62	7.02 ± 1.59	
%	3.68 ± 4.55	4.74 ± 6.93	4.33 ± 6.94	
Control				
Log	6.84 ± 1.82	6.74 ± 1.01	.67 ± 1.84	0.057
%	3.69 ± 4.34	3.16 ± 4.81	2.00 ± 3.92	0.068
E. coli				
Prebio				
Log	5.23 ± 1.58	4.72 ± 0.85	4.66 ± 0.82	
%	1.92 ± 5.60	0.06 ± 0.30	0.07 ± 0.34	
Control				
Log	4.98 ± 1.40	4.83 ± 1.05	4.70 ± 0.86	NS
%	3.67 ± 12.11	0.19 ± 0.70	0.16 ± 0.88	NS
C. lituseburense				
Prebio				
Log	4.52 ± 0.84	4.66 ± 0.76	4.66 ± 0.77	
%	0.05 ± 025	0.03 ± 017	0.16 ± 1.06	
Control				
Log	4.46 ± 0.50	4.60 ± 0.68	4.67 ± 0.78	NS
%	0.03 ± 0.10	0.04 ± 0.27	0.07 ± 0.35	NS
C. histolyticum				
Prebio				
Log	8.14 ± 1.23	8.20 ± 1.19	8.48 ± 0.47	
%	19.2 ± 13.4	14.8 ± 12.3	15.3 ± 12.3	
Control				
Log	8.12 ± 1.32	8.23 ± 1.33	8.40 ± 1.06	NS
%	17.8 ± 14.1	15.9 ± 11.5	17.0 ± 12.9	NS

Fecal samples were studied before (d 0) and after 1 wk (d 7) and 3 wk (d 21) of receiving the formulas. Results are expressed as means ± SD of log (fluorescent events) and percentage of the population in relation to the total bacteria counted by the Eub338 probe. Data were compared by ANOVA for repeated measurements and results corresponding to the interaction group/time are given as F and p.

* Significant difference with the corresponding control group

to return on d 7 to their basal levels observed before amoxicillin ($F = 15.44$, $p < 0.000$); no differences were observed between both groups. The evolution of *Bifidobacterium* population during the time course of the study was significantly different between both groups, with higher counts and percentages on d 7 in the group receiving the prebiotic. A similar tendency was observed for the *Lactobacillus/Enterococcus* population, but without reaching statistical significance ($p = 0.057$ and $p = 0.068$ for the log count and the percentage, respectively). The analysis of variance for repeated measurements showed a significant decrease of *E. coli* during the time course of the study, but without differences between both formula groups ($F = 6.97$, $p = 0.001$ and $F = 12.05$, $p < 0.000$, for the log counts and percentage respectively). A decrease in percentage of *Bacteroides* was also observed ($F = 12.4$, $p < 0.000$).

DISCUSSION

The present study was carried out to evaluate whether prebiotic administration stimulates the recovery of the intestinal microbiota, including the population of *Bifidobacterium*, when it has been altered by an antibiotic treatment. This is a relevant problem as disturbances of the resident microbiota

may favor the growth of opportunist and potentially pathogenic microorganisms such as *C. difficile*, *C. perfringens*, *Klebsiella*, *Candida*, and the subsequent development of antibiotic-associated diarrhea (1,7–9). In addition, the disturbance of the homeostasis of the gut microbiota may lead to significant changes in the colonic microenvironment, especially in relation with the concentration and distribution of carbohydrates, short-chain fatty acids, and bile acids in different segments of the large intestine (13). Our results show that the oral administration of amoxicillin for 7 d induced a significant decrease, close to 30%, in the count of total fecal bacteria detected by the universal probe Eub338. The bacterial populations evaluated by the FISH method in this study represented about 44% of the total bacteria, and this percentage increased to about 58% after 1 wk of amoxicillin. This indicates that other dominant populations of the microbiota such as *Fusobacterium*, *Eubacterium*, and *Atopobium* were probably more affected by the antibiotic treatment than those evaluated in this study. Our results also show that all the populations evaluated were not equally affected by the antibiotic, the greatest change being associated with *E. coli*, which increased significantly during this period, as already described by other authors (14). The significance of an increase of this magnitude in the counts of *E. coli* is unknown; however, it has been postulated that modifications in the interrelations between bacterial species in the colon may have the potential for profound effects on health (15). We also observed that the counts of *Bifidobacterium* and *Bacteroides* were not altered by amoxicillin, although these bacteria became a higher percentage of the total population, possibly due to the decrease of other dominant, albeit not evaluated, populations. The effect of antibiotics on resident and exogenous bifidobacteria has been investigated in different settings. *In vitro* studies evaluating their susceptibility to antibiotics have shown that most *Bifidobacterium* species are sensitive to penicillin derivates such as amoxicillin (16,17). However, amoxicillin is efficiently absorbed in the ileum and the proportion that reaches the colon is not well known. As a result, it is possible that *in vitro* antibiotic susceptibility tests do not reflect what really happens in the lumen of the colon, with its multiplicity of bacteria, some of them probably with the ability to degrade this xenobiotic. Interestingly, Goldin *et al.* (18) showed that therapeutic doses of ampicillin had little effect on *Lactobacillus rhamnosus* GG despite the fact that this strain is sensitive *in vitro* to this antibiotic.

The main finding of our study is that 1 wk after the antibiotic treatment, infants fed a milk formula supplemented with prebiotics had higher counts of bifidobacteria than those fed the same formula but without the prebiotics. A similar tendency was also observed for the *Lactobacillus* population, although without reaching statistical significance. At the same time, the total fecal bacterial count as well as the *E. coli* population returned to the levels before amoxicillin administration without differences between the groups. These results confirm that prebiotics do not influence the recovery of the total flora, which is very rapid, but that they function as bifidogenic factors for the endogenous bifidobacteria. The milk formula used in this study contained a mixture of oligofructose and inulin; it has been suggested that the short-chain FOS oligofructose is preferentially fermented in the proximal colon and fermentation of the longer chain inulin would take place in the distal colon. Providing both types of FOS could stimulate higher counts of bifidobacteria along the colon. A similar alteration of the response of the intestinal microbiota to antibiotics was also observed in patients who received probiotics (*L. acidophilus* and *B. bifidum*) after an antibiotic treatment for *Helicobacter pylori* (19). From a clinical standpoint, the bifidogenic effect of FOS may not appear as too relevant; however, in children who are malnourished or who are immunosuppressed or subjected to longer treatments with other antibiotics, these effects may become important. Evidently, this is an aspect that deserves further exploration.

The number of episodes of diarrhea detected throughout the follow-up of the patients was small and agrees with the considerable improvements in sanitation that Santiago has experienced in the past decades. However, it is interesting to note that the asymptomatic shedding of enteropathogens (17.5%) remained rather frequent. No seasonal variation in presence of enteropathogens was observed, and these were cultured in more than one of the four samples, without preference as to which of these was the one associated with this detection. The incidence of asymptomatic shedding of *C. jejuni* was high, but this had been observed in other studies carried out in the southern Santiago (20,21). The *C. histolyticum* and *C. lituseburense* groups were evaluated in our study because they respectively include *C. perfringens* and *C. difficile*, two opportunist pathogens frequently implicated in antibiotic-associated diarrhea; however, no changes were observed in these bacterial populations.

In conclusion, amoxicillin administration to infants for 1 wk resulted in quantitative and qualitative alterations of their fecal flora. Feeding a milk formula supplemented with prebiotics increased the levels of bifidobacteria early after the antibiotic therapy and may contribute to the reestablishment of the homeostasis of the gut microbiota.

Acknowledgments. The authors thank M. M. Gonzalez, M.D., resident physician at the Health Center, M. Figueroa, R.N., and P. Mondaca, R.N., for help with the follow-up of children in the Field Station and sample collection. P. Torres is thanked for her excellent technical assistance in stool sample processing for FISH and R. Montalva for the flow-cytometry analysis.

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