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Original Article

Effect of oral administration of probiotics on intestinal colonization with drug-resistant bacteria in preterm infants

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Abstract

Background *Oral administration of probiotics in newborn preterm infants has been shown to be helpful, especially in reducing the incidence of necrotizing enterocolitis and overall mortality rates.*

Objective *To evaluate the effect of probiotic supplementation on intestinal colonization by antibiotic-resistant microorganisms in preterm infants receiving antibiotics in a neonatal intensive care unit (NICU).*

Methods *The prospective, randomized trial was performed in preterm infants who were hospitalized in the NICU at Baskent University Ankara Hospital between January 2011 and February 2012. A total of 51 infants were enrolled and randomly assigned to one of two groups: Group 1 (n=27) received probiotic therapy and Group 2 (n=24) did not receive probiotics. The probiotic used was Lactobacillus reuteri (Biogaia® AB, Sweden). Subjects underwent weekly nasal swab and stool cultures for a maximum of 6 weeks, and at the time of discharge if this was prior to 6 weeks. All positive cultures were further tested for culture-specific identification and antibiotic suceptibility.*

Results *A total of 607 cultures were evaluated. Positive cultures were found in 37.9% from Group 1 and 35.2% from Group 2. Intestinal colonization by antibiotic-resistant bacteria did not significantly differ between groups (P>0.05).*

Conclusions Oral supplementation with probiotics do not prevent the intestinal colonization of antibiotic-resistant microorganisms in preterm NICU patients who received antibiotic treatment. **[Paediatr Indones. 2017;57:91-8. doi: http://dx.doi.org/10.14238/pi57.2.2017.91-8].**

Keywords: *oral administration of probiotics; intestinal colonization; drug-resistant bacteria; preterm infants*

Preterm newborn infants who require intensive care are at increased risk for nosocomial infections caused by antibiotic-resistant microorganisms. For this reason, in preterm infants who remain in the NICU longer than 48 hours, the prevalence of nosocomial infections ranges from 6% to 22%.[1-5] Several studies have shown that supplementation with probiotics can prevent colonization of the gut by pathogenic microorganisms in preterm newborns.[1,3,4] Probiotics can help regulate enteral feeding, reduce parenteral nutrition dependence, enforce the intestinal mucosal barrier against bacteria, and increase levels of beneficial bacteria in the gut.[2,5-7] At the same time, probiotic therapy is reported to reduce frequencies of sepsis and necrotizing enterocolitis in preterm newborn infants.[2,5,7-10] Normally, the uterus is a sterile environment. As such, the intestinal microbiota starts developing shortly after birth in preterm infants, and the initial source of these colonizing microorganisms is the mother's flora.[1,3,4,11] Development of intestinal microbiota in preterm infants may also be delayed because of the hospital environment that consists of invasive procedures, antibiotic regimens, and

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late enteral feeding in the NICU.[12-14] Treatment with antibiotics can adversely affect the density and diversity of microorganisms in the intestine of the newborn.[15] Various studies have demonstrated that antibiotic-resistant microorganisms colonize preterm newborn infants in the NICU.[1,3,4,16,17]

Our aim in this study was to evaluate the effect of oral probiotic administration on the colonization of the intestine by antibiotic-resistant microorganisms in preterm newborn infants receiving antibiotics in the NICU.

Methods

This prospective study was performed in preterm newborn infants who were hospitalized in the NICU at Baskent University Ankara Hospital between January 2011 and February 2012. The Baskent University Clinical Research Ethics Committee approved the study (project number: KA11/138), and subjects' parents provided informed consent. All infants enrolled were born at ≤36 weeks of gestational age and required antibiotic treatment and/or prophylaxis. Infants with congenital anomalies and those undergoing intestinal surgeries were excluded.

Patients were randomly assigned to two groups, according to the order of NICU admission. A total of 51 patients were enrolled: Group 1 (n=27) received probiotic therapy and Group 2 (n=24) did not receive probiotics. The probiotic used was Lactobacillus reuteri (*Biogaia® AB*, Sweden). Oral probiotics were started on the day of birth. Each newborn in Group 1 received the probiotic directly (not mixed with any other intake) as an oral daily dose (1x108 cfu/day given as 5 drops once daily) during their stay in the NICU.[2]

Nasal swab and stool cultures were collected from all infants. In each case, these specimens were collected immediately upon admission to the NICU (prior to starting antibiotic treatment), at least once weekly throughout the hospital stay, to a maximum of 6 weeks, and at discharge if this was prior to 6 weeks. Each sample was incubated at a microbiology laboratory within 30 minutes of collection (see detailed laboratory methods below).

Other cultures (i.e., cultures of throat swabs, deep tracheal aspirates, endotracheal tube aspirates, blood and urine) were routinely taken from the patients included in the study. The relation of these culture results to the use of probiotic was investigated.

The following data were recorded for each infant during their stay in the NICU: prenatal, natal, and postnatal characteristics, diagnoses, clinical characteristics, surgical therapy and other interventions, prognosis, and complications (such as vomiting, diarrhea, sepsis, etc.) of probiotic treatment (for Group 1).

Cultures were plated and incubated at the *Baskent University Clinical Microbiology and Microbiology Laboratory*, and were evaluated by experts in the *Department of Infectious Diseases*. Specimens were plated on sheep blood agar, chocolate agar, and eosin methylene blue agar. Culture-specific identification and antibiotic susceptibility testing were performed for all microorganisms that grew in culture. The criteria of the *Clinical and Laboratory Standards Institute* were used to assess the antibiotic susceptibility of each microorganism.The methods used were the disc diffusion susceptibility test (Kirby-Bauer method) and determination of minimal inhibitory concentration (MIC).[18,19] The microbes that were cultured included methicillin-resistant *Staphylococcus aureus, Staphylococcus epidermidis*, Enterococcus spp., Klebsiella spp., *Escherichia coli, Pseudomonas aeruginosa*, Enterobacter spp, Acinetobacter spp , Serratia spp, Citrobacter spp., Proteus spp., and Candida spp. The microorganisms detected were classified according to their resistance to antibiotics.[20]

Statistical analysis was performed using the Statistical Package for the Social Sciences (*SPSS version 17.0, SPSS Inc.*, Chicago, IL, USA). The Shapiro-Wilk test was used to assess whether variables were normally distributed. The Mann-Whitney U test was used to compare the group findings. Results for categorical variables were analyzed using the Chi-square test. Within-group comparisons were made to assess weekly increases in quantity of microorganisms isolated from stool and nasal cultures, respectively. These weekly statistical comparisons were made using the Cochran Q test and the Monte Carlo method.

Results

The demographic characteristics of the two groups are summarized in **Table 1**. The groups' clinical characteristics are presented in **Table 2**. In total, 607 cultures were evaluated, with 351 from Group 1 and 256 from Group 2. One hundred thirty-three (37.9%) of the 351 Group 1 cultures were positive. Of the 133 isolates, 79 were Gram-negative microorganisms, 49 were Gram-positive, and 5 were fungi (only blood and catheter cultures). Ninety (35.2%) of the 256 Group 2 cultures were positive. Of the 90 isolates, 53 were Gram-negative and 37 were Gram-positive.

Table 3 shows other cultures were routinely taken from the patients included in the study. However, these culture results were not associated with oral administration of probiotics in both groups. **Table 4** shows the quantities of identified isolates cultured from each group's stool cultures at baseline (admission to NICU) and at weeks 1 through 6. **Table 5** shows the corresponding results for nasal swab cultures. The within-group comparisons of weekly numbers of isolates revealed weekly increases in quantity of microorganisms isolated from nasal cultures.

In this study most microorganisms were antibiotic-resistant (P>0.05). Very few of them were not antibiotic-resistant (**Table 6**).Colonization of intestine with antibiotic-resistant bacteria did not differ between the two groups (P>0.05). Klebsiella spp. isolated from a total of 26 cultures from both groups were positive for extended-spectrum beta-lactamase (ESBL), but *E. coli* isolated from 5 cultures were positive for ESBL from Group 1.

None of the infants in Group 1 developed side effects associated with the use of probiotics, such as diarrhea or vomiting.

Discussion

In this prospective, randomized trial, 607 cultures were evaluated in 51 preterm infants who received antibiotics in the NICU. Group 1 had significantly more antibiotic resistant microorganisms cultured from stool specimens, than Group 2. In Group1, the most common microorganisms isolated from all nasal swab cultures were Staphylococcus 20.0%, while in Group 2, the most common microorganisms isolated from all nasal swab cultures were Staphylococcus 17.6%.

The intestinal microbiota differs between term and preterm infants. Because preterm infants have immature host defenses, require invasive interventions such as central venous catheter or endotracheal tube insertion, and often have longer antibiotic treatment, they are at high risk for nosocomial and antibiotic-resistant infections. At the same time, colonization by bifidobacteria is delayed in the preterm infants.[15] A previous randomized clinical trial evaluated the effect of *Bifidobacterium lactis Bb12* supplementation on modifying gut microbiota in 69 preterm infants and found that supplementation with *B. lactis Bb12* did not reduce the colonization of antibiotic-resistant organisms.[15]

Other reports suggested that probiotics reduce intestinal inflammation and prevent colonization by pathogenic microorganisms in the gut.[2,3,21] Ren *et al.* reported that the intestinal bacterial colonization rate was lower in the group given probiotics than in the group without probiotics. In their study, *Klebsiella pneumoniae, E.coli*, and *Enterococcus faecium* were found in stool specimens of both the intervention and control groups.[22] In our study, we found that the use of probiotics in preterm infants did not prevent the development of antibiotic-resistant microorganisms. The differences between studies might be due to the diversity of invasive procedures, antibiotic regimens, and other treatments in the NICU. Ren *et al.* also reported that probiotics reduced the risk of sepsis in

Table 1. Demographic characteristics of the study subjects

Characteristics	Group 1 (probiotics) (n=27)	Group 2 (no probiotics) (n=24)	P value
Gender,* n			
Female	15	7	
Male	12	17	
Mode of delivery,** n			
Vaginal	3	2	0.058
Cesarean	24	22	0.739
Early membrane rupture,** n	7	5	
Mode of feeding, n			
Breastfed	22	20	0.699
Formula fed	5	4	0.830

*Chi-square test, **Mann-Whitney U test

Table 2. Clinical features, diagnoses, and interventions in the two study groups

Variables	Group 1 (n=27)	Group 2 (n=24)	P value
Gestational age, weeks			
Mean (SD)	32.5 (0.44)	33.1 (0.40)	0.312
Median (range)	32.7 (27-36)	33.4 (27-35)	
Birth weight, g			
Mean (SD)	1909.6 (111.75)	2048.7 (76.12)	0.242
Median (range)	1870 (840-2880)	2137 (930-2540)	
Apgar 1 minute			0.610
Mean (SD)	6.9 (0.21)	7.0 (0.24)	
Median (range	7.1 (5-9)	7.2 (3-8)	
Apgar 5 minutes			0.254
Mean (SD)	8.1 (0.17)	8.2 (0.20)	
Median (range	8.1 (6-10)	8.4 (5-9)	
Intubated, n	15	13	0.921
Ventilatory support, n	16	14	0.993
Umbilical venous catheter, n	21	14	0.135
Peripheral central catheter, n	3	4	0.565
Surfactant treatment, n	14	12	0.895
Use of antacid, n	8	9	0.552
Respiratory distress syndrome, n	14	12	0.895
Necrotizing enterocolitis, n	5	2	0.291
Sepsis, n	9	4	0.321
Patent ductus arteriosus, n	3	3	0.878
Bronchopulmonary dysplasia, n	3	1	0.357
Intubation duration, days			0.720
Mean (SD)	2.40 (0.84)	1.37 (0.35)	
Median (range	1 (0-19)	1 (0-6)	
Duration of umbilical venous catheter placement, days			0.105
Mean (SD)	6.6 (1.09)	4.0 (0.90)	
Median (range	6 (0-20)	2.5 (0-130	
Duration of peripheral central venous catheter placement, days			0.705
Mean (SD)	2.0 (1.18)	1.6 (0.78)	
Median (range)	0 (0-25)	0 (0-11)	
Duration of nasogastric tube placement, days			0.455
Mean (SD)	15.2 (3.42)	10.3 (1.98)	
Median (range	8 (1-70)	7.5 (1-37)	
Duration of total parenteral nutrition, days			0.638
Mean (SD)	6.9 (1.98)	4.3 (0.98)	
Median (range	5 (0-48)	7.5 (1-37)	
Duration of full enteral feeding, days			0.192
Mean (SD)	13.4 (2.21)	9.6 (1.26)	
Median (range	11 (3-60)	7.5 (0-25)	
Exposure to oxygen, days			0.549
Mean (SD)	9.7 (3.61)	6.3 (1.56)	
Median (range	2 (1-74)	2.5 (1-28)	
Time to first positive culture, days			0.237
Mean (SD)	7.1 (0.67)	6.0 (0.60)	
Median (range	7 (2-17)	5 (2-16)	
Total duration of antibiotic use, days			0.236
Mean (SD)	13.7 (2.35)	9.7 (1.31)	
Median (range	9 (6-45)	8 (3-30)	

Table 2. Clinical features, diagnoses, and interventions in the two study groups (continued)

Variables	Group 1 (n=27)	Group 2 (n=24)	P value
Hospital stay, days			
Mean (SD)	22.1 (3.52)	15.0 (1.82)	0.121
Median (range	15 (6.-74)	13 (6-43)	
Weight at discharge, g			0.278
Mean (SD)	2085.9	2148.3 (57.93)	
Median (range)	2050 (1620-2900)	2150 (1740-2700)	
Deaths, n	0	1	0.284

Table 3. Diagnostic value of IT ratio and procalcitonin as compared to blood cultures

	Culture type						
	Throat swab	DTA	ETA	Central catheter tip	Blood	Urine	Total
Group 1 (probiotics)							
Negative cultures	7	13	3	17	53	11	104
Enterococcus spp	0	0	0	1	0	2	3
Staphylococcus epidermidis	0	0	0	0	1	1	2
Stenotrophomonas maltophilia	0	3	1	0	0	0	4
E. coli	0	1	0	0	0	2	3
Klebsiella pneumoniae	0	0	0	0	0	2	2
Burgholderia spp	0	0	0	0	1	0	1
Candida parapsilosis	0	0	0	1	3	0	4
Group 2 (no probiotics)							
Negative cultures	7	11	2	8	41	7	76
S. epidermidis	0	0	1	1	0	0	2
Serratia marcescens	0	0	0	0	0	1	1
Streptococcus spp.	0	0	0	0	1	1	2
Total	14	28	7	28	100	27	204

DTA=deep tracheal aspirate; ETA=endotracheal tube aspirate

the preterm newborn infants.[22] In contrast, we found that intestinal bacterial colonization was higher in Group 1 than in Group 2, but the risk of sepsis did not increase in either group (**Table 4** and **5**).

Another study on very low-birth weight infants (VLBW, <1500 g), reported that colonization in stool samples were Lactobacillus sp. 71% and Klebsiella sp. 0%, within the first week of life without oral administration of probiotics.[23] Jacquot *et al.* reported that the most common bacteria in stool specimens found at 3 - 4 weeks postnatally was Clostridium.[24] The same study showed that Enterobacteriaceae accounted for less than < 10% and 44.4% in stool cultures at 6 and 8 weeks of life, respectively, and Bifidobacterium was < 10% at 8 weeks.[24] Rougé *et al.* investigated intestinal microbiota in 10 preterm infants. They reported that *Lactobacillus rhamnosus* and *Bifidobacterium longum* were in the intestinal flora of preterm infants who received probiotics. However, in preterm infants not receiving probiotics, Staphylococci was the first isolated bacteria in the intestinal flora.[25] Unlike these three reports, our study revealed higher Klebsiella spp in stool cultures in three weeks in both intervention and control groups. These differences may be explained by the blockage of saprophytic flora formation due to the use of antibiotics in both of our study groups. So, oral probiotic administration did not enhance the development of saprophytic flora. Vidal *et al.* investigated the impact of probiotics on the intestinal colonization of vancomycin-resistant enterococci (VRE) in mice receiving oral vancomycin. Administration of probiotics did not affect the density

Table 4. Microorganisms isolated from the weekly stool cultures for the two groups

	Admission to NICU	1 wk	2 wks	3 wks	4 wks	5 wks	6 wks
Group 1* (probiotics)							
Negative cultures	24	10	1	0	1	0	0
Klebsiella spp	1	8	13	5	3	0	0
Enterococcus	0	1	5	1	1	1	2
E. coli	1	3	4	2	0	1	0
Enterobacter	0	1	4	4	1	0	0
Staphylococcus epidermidis	1	3	1	2	1	0	0
Proteus spp	0	0	1	1	1	0	1
Stenotrophomonas maltophilia	0	1	1	1	0	0	0
Acinetobacter baumannii	0	1	0	1	0	0	0
Serratia marcescens	0	1	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0	1	0
Citrobacter spp	0	1	0	0	0	0	0
Total cultures**	27	30	30	17	8	3	3
Microorganism isolated, n(%)	3 (11.1)	20 (66.6	29 (96.6)	17 (100)	7 (87.5)	3 (1001)	3 (100)
Group 2* (no probiotics)							
Negative cultures	22	7	0	0	1	0	0
Klebsiella spp	0	6	13	8	1	1	1
Enterococcus spp	0	2	2	2	0	1	1
S. epidermidis	2	4	0	1	0	0	0
E. coli	0	0	2	1	1	0	1
Enterobacter spp	0	4	0	0	0	0	0
Proteus spp	0	1	1	1	0	0	0
Serratia SPP	0	0	1	1	0	0	0
A. baumannii	0	1	0	0	0	0	0
Total cultures**	24	25	19	14	3	2	3
Microorganisms isolated. n(%)	2 (8.3)	18 (72.0)	19 (100)	14 (100)	2 (66.7)	2 (100)	3 (100)

Notes: *Between the two groups weekly positive culture status in stool cultures was found significant by Cochran Q test (P=0.009). Positive culturing rates were higher in the probiotic group than in the no probiotic group. **Total cultures performed for the group. All patients had samples cultured on admission to the NICU. In following weeks, the numbers of cultures dropped as patients were discharged from hospital.

of VRE colonization in the gut.[26] Similarly, we found that the use of probiotics did not prevent development of resistant microorganisms in preterm infants.

The limitations of our study were the small sample size and the lack of ability to culture anaerobic microorganisms. In our study, rates of antibiotic-resistant microorganisms were found to be high in both groups. Clearly, our study shows that the use of probiotics does not prevent the colonization of antibiotic-resistant pathogens. We suggest that the antibiotic regimens and NICU conditions play the greatest role in the development of the intestinal microbiota and microbes cultured.

In conclusion, our study revealed that the use of probiotics do not prevent development of antibiotic resistant microorganisms in preterm infants receiving antibiotics in the NICU. Further studies may investigate the potential of oral supplementation of other probiotic strains in preventing antibiotic-resistant bacteria.

Conflict of Interest

None declared.

Table 5. Microorganisms isolated from the weekly nasal swab cultures for the two groups

	Admission to NICU	1 wk	2 wks	3 wks	4 wks	5 wks	6 wks
Group 1* (probiotics)							
Negative cultures	26	20	15	8	5	1	3
Staphylococcus epidermidis	0	6	8	5	1	2	0
Streptococcus pneumonia	0	1	1	0	0	0	0
Klebsiella spp	0	0	2	1	1	0	0
Enterococcus spp	2	0	0	0	0	0	0
E. coli	0	0	0	1	0	0	0
Serratia marcescens	0	0	1	0	0	0	0
Total cultures**	28	27	27	15	7	3	3
Microorganism isolated, n(%)	2 (7.1)	7 (25.9)	12 (44.4)	7 (46.6)	2 (28.5)	2 (66.6)	0
Group 2* (no probiotics)							
Negative cultures	24	17	12	5	2	0	0
S. epidermidis	0	4	4	5	0	2	0
S. pneumoniae	0	0	0	0	0	0	0
Enterobacter cloacae	0	0	1	0	0	0	0
Staphylococcus aureus	0	0	0	1	0	0	0
Klebsiella pneumoniae	0	0	0	1	0	0	0
Enterococcus faecalis	0	1	0	0	0	0	0
Stenotrophomonas maltophilia	0	0	1	0	0	0	0
S. marcescens	0	0	0	1	0	0	0
Acinobacter baumannii	0	0	0	0	0	0	1
P. aeruginosa	0	0	0	0	1	0	0
Total cultures**	24	24	18	13	3	2	1
Microorganisms isolated. n(%)	0	7 (29.1)	6 (33.3)	8 (61.5)	1 (33.3)	2 (100)	1 (100)

Notes: *Weekly positive culture status in nasal swab cultures was not significantly different between the two groups by Cochran Q test (P=0.097). **Total number of cultures performed for the group. In following weeks, the numbers of cultures dropped as patients were discharged from hospital.

Table 6. Distribution of microorganisms isolated from the two groups listed according to categories of antibiotic resistance

Category of antibiotic resistance	Group 1	Group 2	P value*
Not resistant	8 (6.5)	8 (8.5)	> 0.05
Resistant to one drug	13 (10.5)	7 (7.4)	> 0.05
Resistant to two drugs	5 (4.0)	6 (6.3)	> 0.05
Resistant to three or more drugs	105 (85.3)	73 (77.6)	> 0.05
Total	123 (100)	94 (100)	> 0.05

*Chi-square test

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