Lack of an Effect of *Lactobacillus reuteri* DSM 17938 in Preventing Nosocomial Diarrhea in Children: A Randomized, Double-Blind, Placebo-Controlled Trial

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Objective To evaluate the efficacy of administering *Lactobacillus reuteri* DSM 17938 for the prevention of nosocomial diarrhea.

Study design Children (n = 106; aged 1-48 months) admitted to the hospital for reasons other than diarrhea were enrolled in a randomized, double-blind, placebo-controlled trial. They received *L reuteri* DSM 17938 at a dose of 10^8 colony-forming units (n = 54) or a placebo (n = 52) orally, once daily, for the duration of the hospital stay.

Results Data from all children were included in the final analysis. *L reuteri* DSM 17938 did not significantly affect the risk of developing nosocomial diarrhea, defined as 3 loose or watery stools per day in a 24-hour period that occurred >72 hours after admission (risk ratio 1.06, 95% CI 0.7-1.5) or rotavirus infection (1.04, 0.6-1.6). There was also no difference between the probiotic and placebo groups for any of the other secondary outcomes (ie, incidence of rotavirus infection, incidence of diarrhea, duration of diarrhea, incidence of recurrent diarrhea, incidence of chronic diarrhea, length of hospital stay in days, and frequency of need for rehydration). No adverse events were reported.

Conclusion In hospitalized children, the administration of *L reuteri* DSM 17938 compared with placebo had no effect on the overall incidence of nosocomial diarrhea, including rotavirus infection. *(J Pediatr 2012;161:40-3)*.

Nosocomial infections, currently referred to as "healthcare-associated infections," "hospital-acquired infections," or "hospital-onset infections," are defined as infections not present and without evidence of incubation at the time of admission to a health care setting.[1] Infections occurring >48 hours after admission are usually considered to be health care–associated infections.[2] In children, rotavirus remains a leading cause of nosocomial gastrointestinal infections.[3] These infections may occur in 27% of hospitalized children.[4] However, the true burden may be underreported due to difficulties in gathering reliable data.[2] Regardless of its site, a nosocomial infection results in a prolonged hospital stay and increased additional medical costs.[5]

There is evidence suggesting that specific probiotics may be antagonistic to pathogens and may enhance immunity, thus contributing to the prevention or treatment of diarrheal diseases. There is currently evidence to recommend the use of *Lactobacillus rhamnosus* GG (LGG),[6-8] as well as some promising evidence to recommend the use of *Bifidobacterium bifidum* (recently renamed *B lactis*) and *Streptococcus thermophilus*,[9] to prevent nosocomial diarrhea. However, there are also studies reporting no preventive effects of probiotics.[10]

Lactobacillus reuteri DSM 17938 is a probiotic strain that is widely available in many countries. The efficacy of *L reuteri* DSM 17938 for preventing or treating gastrointestinal infections has not been studied. However, there is a rationale for expecting positive effects based on the results of 2 trials with a mother strain, *L reuteri* ATCC 55730 (also known as SD2112).[11,12] These studies provided evidence of a moderate beneficial effect of *L reuteri* ATCC 55730 as an adjunct to rehydration therapy in the treatment of acute infectious diarrhea of rotaviral origin in children. Because *L reuteri* ATCC 55730 was found to carry potentially transferable resistance traits for tetracycline and lincomycin, it has been replaced by a new daughter strain, *L reuteri* DSM 17938, with no plasmid-borne resistances.[13] The current study was designed to evaluate the role of *L reuteri* DSM 17938 administration compared with placebo for preventing the development of nosocomial diarrhea in a pediatric hospital setting.

Methods

The standards from the guidelines of the Consolidated Standards of Reporting Trials were followed for this randomized controlled trial (RCT). This trial was registered at ClinicalTrials.gov (NCT01046656). The study was approved by

CFU	Colony-forming units
LGG	*Lactobacillus rhamnosus* GG
RCT	Randomized controlled trial
RR	Risk ratio

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the Ethics Committee of the Medical University of Warsaw. Parents were fully informed about the aims of the study, and informed consent was obtained from at least 1 parent.

This was a prospective, randomized, double-blind, placebo-controlled trial conducted in the Department of Paediatrics of the Medical University of Warsaw, Poland. The study was carried out between December 2009 and May 2011.

All children aged 1-48 months who were admitted to the hospital for reasons other than diarrhea were eligible for entry into the study. Children with a history of probiotics and/or prebiotic use within 7 days before admission, acute gastroenteritis within 3 days before admission, symptoms other than diarrhea suggestive of gastroenteritis (eg, vomiting), underlying intestinal disease, or the presence of visible blood in the stool were excluded, as well as those infants who were being breastfed.

The tested probiotic, *L reuteri* DSM 17938, was administered orally at a dose of 10^8 colony-forming units (CFU) in 5 drops. Under supervision, patients received either the *L reuteri* preparation or placebo once daily during their hospitalization according to the randomization list. Both *L reuteri* DSM 17938 and the placebo were manufactured and supplied by BioGaia AB (Lund, Sweden) as a fluid in identical bottles and kept refrigerated until use. The manufacturer had no role in the conception, design, or conduct of the study, or in the analysis or interpretation of the data. Upon enrollment in the study, initial microbiological testing was performed in all children. As the study products were administered in the hospital by the hospital personnel who were informed about the study, no further measures to assess compliance were taken.

Investigators at the Medical University of Warsaw used computers to generate independent allocation sequences and a randomization list (StatsDirect Ltd, StatsDirect statistical software, http://www.statsdirect.com). To avoid disproportionate numbers of patients, randomization was performed in blocks of 6 patients (3 receiving the probiotic product and 3 receiving the placebo). To ensure allocation concealment, an independent person prepared the randomization schedule and oversaw the packaging and labeling of the study products. All study personnel, parents, and guardians were unaware of the group assignments. Randomization codes were secured until all data were analyzed.

All participants and investigators were blinded to the assigned treatment throughout the study. The 2 products, *L reuteri* DSM 17938 as well as the placebo, were packed in identical packages. The unblinding was done when all data were analyzed.

The *primary* outcome measure was the incidence of nosocomial diarrhea, defined as the passage of ≥3 loose or watery stools in a 24-hour period that occurred >72 hours after admission. The *secondary* outcomes were as follows: incidence of rotavirus infection (ie, the detection of rotavirus or antigen in the stools), incidence of diarrhea (ie, the passage of ≥3 loose or watery stools in a 24-hour period), duration of diarrhea (ie, time until the last loose watery stools from the onset of diarrhea measured in days), incidence of recurrent diarrhea (ie, recurrence of diarrhea after 48 hours of normal stools), incidence of chronic diarrhea (ie, diarrhea lasting >14 days), length of hospital stay in days, and frequency of need for rehydration. Patients were evaluated daily for stool number and consistency. All data regarding the number of stools per day, the number of vomiting episodes per day, and the need for parenteral rehydration were collected on a daily basis. Stool samples obtained on admission and during an episode of diarrhea were analyzed for bacteria with standard stool cultures and rotavirus antigen. No tests for parasites or protozoa such as *Giardia lamblia* were performed, as these microorganisms are not a common causes of acute diarrhea in our setting.

For the primary outcome measure, we assumed the proportion of children who have had diarrhea. Based on data from the literature, the incidence of diarrhea in hospitalized children is 33%.[7] To achieve a clinically significant difference in efficacy between the groups, the incidence of diarrhea needed to be reduced by 50%. With parameters $\alpha = 5\%$ and $\beta = 20\%$ and control subjects per case = 1, we calculated the minimum total sample size to be 88 patients. After taking into account that about 20% of participants could not complete the study as planned, it was found that the group size should be 106 (53 subjects per group). The sample size was calculated with computer software StatsDirect version 2.3.8 (StatsDirect Ltd).

Statistical Analysis

The computer software StatsDirect was used to calculate the risk ratio (RR) and mean difference, all with a 95% CI. The difference between study groups was considered significant when the P value was <.05 or when the 95% CI for RR did not exceed 1.0 and the mean difference did not exceed 0 (equivalent to $P < .05$). All statistical tests were 2-tailed and performed at the 5% level of significance. All analyses were conducted on an intention-to-treat basis, including all patients in the groups to which they were randomized for whom outcomes were available.

Results

The **Figure** (available at www.jpeds.com) is a flow diagram showing the subjects' progression through the study. Of the 106 children who underwent randomization, 54 were assigned to the probiotic group and 52 were assigned to the placebo group. Baseline demographic and clinical characteristics did not differ between the 2 groups (**Table I**). The outcome measures are summarized in **Table II**. We found no difference between the study groups with respect to the incidence of nosocomial diarrhea. Of the 54 children in the probiotic group, 18 (33%) had diarrhea compared with 16 (31%) of the 52 children in the placebo group (RR 1.06, 95% CI 0.7-1.5). In 19 patients, rotavirus was detected, with no significant difference between the study groups with respect to the incidence of rotavirus infection (RR 1.04, 95% CI 0.6-1.6). In 3 patients, adenovirus was detected (all in the probiotic group), and in 11 patients, the etiology of the diarrhea was unknown. There was no difference between the

Table I. Baseline characteristics of the study groups

Variable	Probiotic group (n = 54)	Placebo group (n = 52)
Age, mean ± SD mo	11.5 ± 9.2	11.1 ± 9.2
Female sex, n (%)	22 (41)	20 (38)
Respiratory infections, n (%)	13 (24)	21 (40)
Ear, nose, and throat disorders, n (%)	17 (32)	14 (27)
Urinary tract infections, n (%)	12 (22)	8 (15)
Skin disorders, n (%)	2 (4)	3 (6)
Others, n (%)	6 (11)	2 (4)

study groups for any other secondary outcome (ie, incidence of rotavirus infection, incidence of diarrhea, duration of diarrhea in days, incidence of recurrent diarrhea, incidence of chronic diarrhea, length of hospital stay in days, and frequency of need for rehydration). Both the probiotic and placebo were well tolerated, and no adverse events were reported; however, the design did not include the collection of any information on harms unless spontaneously reported by parents.

Discussion

This study provides evidence that in hospitalized children, the administration of *L reuteri* DSM 17938 at a dose 10^8 CFU per day compared with placebo had no effect on the overall incidence of nosocomial diarrhea, including rotavirus infection.

The strengths of this study include adequate randomization, the use of a double-blind design, comprehensive follow-up, and the use of intention-to-treat analysis, all of which minimize the risk of bias. Considering the negative findings, one limitation of the current study is the lack of analysis of fecal *L reuteri* DSM 17938 in order to confirm compliance with administration. However, we used the same study product as in one of the studies with positive results in which infants who were given the probiotic were found to have fecal *L reuteri* DSM 17938 but not those who received the placebo.[14]

We adopted a more stringent definition of health care–associated diarrhea than the current definition, defining it as an infection that occurs after >48 hours of hospital treatment in a patient admitted for a problem likely not related to the microbial pathogen. Such a stringent definition allowed us to differentiate between clinically relevant conditions and clinically less important changes in the consistency of stools. Of note, post hoc analysis, using the current definition, did not reveal a difference in the rate of nosocomial diarrhea between groups (RR 1.26, 95% CI 0.75-2.14).

In our study in children, the initial negative microbiological stool tests on the first day of hospitalization confirm nosocomial acquisition. However, in practice, in many settings patients are not routinely screened; this does not reflect everyday practice, and such screening would incur additional costs.

In our trial, antibiotic use was not reported nor taken into account in the data analysis, so that it was not possible to distinguish between diarrheal episodes of infectious vs noninfectious origin. However, for clinical practice, this is not important as, in principle, the management of diarrhea is the same regardless of its etiology and the focus is on rehydration. Similar to other pediatric studies investigating the prevention of nosocomial diarrhea, no routine testing was performed for *Clostridium difficile*; however, it is unlikely that this pathogen was relevant in our study population considering that none of the children required antibiotic treatment due to nosocomial diarrhea.

The lack of an effect may be explained by several factors, mainly related to the probiotic itself. First, the wrong selection of the probiotic strain for a given clinical situation may lead to the lack of an effect. Documented efficacy in one condition, ie colic in infants, as in the case of *L reuteri* DSM 17938,[14-16] does not guarantee efficacy in another condition. Second, an inadequate probiotic dose may have led to the lack of an effect. We chose a daily intake of 10^8 CFU, as recommended by the manufacturer and as used in previous studies with *L reuteri* DSM 17938[15] that yielded positive findings. However, the optimal dose has not been clearly established for either this or other probiotics.[17] One could not exclude the possibility that a higher dose may be needed for preventing diarrheal diseases.

RCTs are subject to type I ("false positive") and type II ("false negative") statistical errors. In our opinion, the latter could not explain the negative results in the current study. The sample size calculation was calculated based on the results of a similar study conducted earlier in the same location.[7] In line with our previous results, and also those reported by Saavedra et al,[9] the incidence of nosocomial diarrhea in the control group was approximately 30%.

Table II. Primary and secondary outcome measures

Outcome	Probiotic group (n = 54)	Placebo group (n = 52)	RR (95% CI)	Mean difference (95% CI)
Primary, n (%)				
Nosocomial diarrhea	18 (33)	16 (31)	1.06 (0.7 to 1.5)	
Secondary				
Rotavirus infection, n (%)	10 (18)	9 (17)	1.04 (0.6 to 1.6)	
Diarrhea, n (%)	21 (39)	16 (31)	1.2 (0.8 to 1.7)	
Duration of diarrhea, days ± SD	3.9 ± 1.1	4.1 ± 1.1		0.2 (−0.6 to 0.2)
Recurrent diarrhea, n (%)	-	-		
Chronic diarrhea, n (%)	-	-		
Need for rehydration, n (%)	6 (11)	10 (19)	0.7 (0.3 to 1.2)	
Length of hospital stay, days ± SD	7.7 ± 2.7	7.3 ± 2.7		0.4 (−0.65 to 1.45)

The finding is the opposite of that in a previous study conducted in the same setting that showed that another probiotic, LGG, reduced the risk of nosocomial diarrhea (≥3 loose or watery stools/24 h) in comparison with placebo (33.3% vs 6.7%; RR 0.2, 95% CI 0.06-0.6).[7] The choice of probiotic, or dose (10^{10} vs 10^{8} CFU), may explain the difference.

Despite evidence of the therapeutic benefits of probiotics in treating diarrhea in children, the evidence that probiotics can actually prevent infectious diarrhea is still scant. So far, only a limited number of probiotic microorganisms have been studied. A meta-analysis of 3 RCTs documented that LGG appears to be an effective strategy for preventing or reducing the risk of nosocomial diarrhea, including that of rotavirus origin, in the pediatric setting.[8] Also, B *bifidum* and *Str thermophilus* were shown to be effective in the prevention of nosocomial diarrhea in infants who were admitted to a chronic care hospital (relatively long stay).[9]

Whether other probiotic strains or higher doses of *L reuteri* DSM 17938 have such effects needs to be substantiated in further randomized trials. ■

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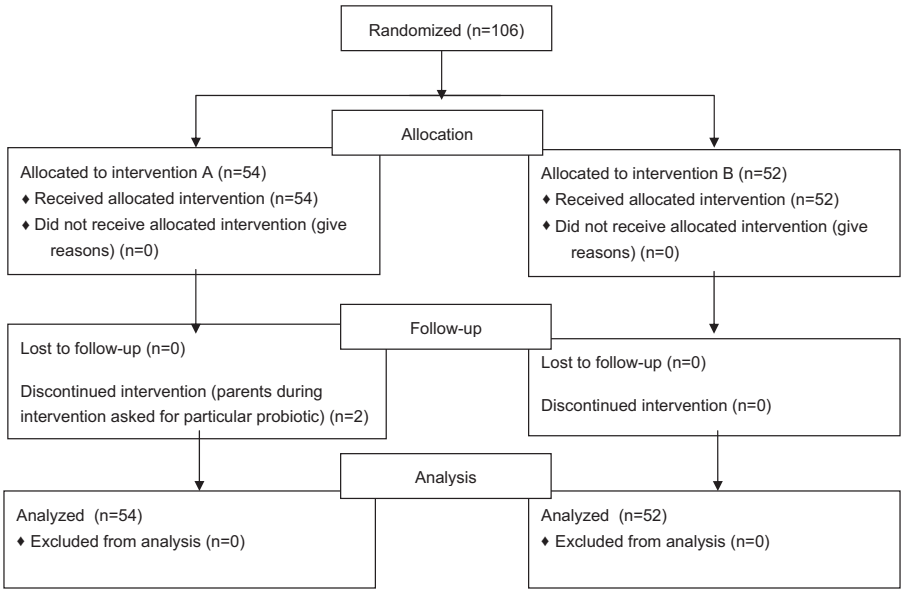

Figure. Flow diagram of the subjects' progression through the study.