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