Prophylactic Probiotics to Prevent Death and Nosocomial Infection in Preterm Infants

WHAT’S KNOWN ON THIS SUBJECT: Several meta-analyses evaluating probiotics in preterm infants suggest a beneficial effect for the prevention of necrotizing enterocolitis and death, but less for nosocomial infection. *Lactobacillus reuteri* may reduce these outcomes because of its immunomodulation and bactericidal properties.

WHAT THIS STUDY ADDS: Although *L reuteri* did not appear to decrease the rate of death or nosocomial infection, the trends suggest a protective role consistent with the literature. Feeding intolerance and duration of hospitalization were significantly decreased in premature infants ≤1500 g.

abstract

BACKGROUND AND OBJECTIVE: It has been suggested that probiotics may decrease infant mortality and nosocomial infections because of their ability to suppress colonization and translocation of bacterial pathogens in the gastrointestinal tract. We designed a large double-blinded placebo-controlled trial using *Lactobacillus reuteri* to test this hypothesis in preterm infants.

METHODS: Eligible infants were randomly assigned during the first 48 hours of life to either daily probiotic administration or placebo. Infants in the intervention group were administered enterally 5 drops of a probiotic preparation containing $10^8$ colony-forming units of *L reuteri* DSM 17938 until death or discharge from the NICU.

RESULTS: A total of 750 infants ≤2000 g were enrolled. The frequency of the primary outcome, death, or nosocomial infection, was similar in the probiotic and placebo groups (relative risk 0.87; 95% confidence interval: 0.63–1.19; *P*= .376). There was a trend toward a lower rate of nosocomial pneumonia in the probiotic group (2.4% vs 5.0%; *P* = .06) and a nonsignificant 40% decrease in necrotizing enterocolitis (2.4% vs 4.0%; *P* = .23). Episodes of feeding intolerance and duration of hospitalization were lower in infants ≤ 1500 g (9.6% vs 16.8% [*P* = .04]; 32.5 days vs 37 days [*P* = .03]).

CONCLUSIONS: Although *L reuteri* did not appear to decrease the rate of the composite outcome, the trends suggest a protective role consistent with what has been observed in the literature. Feeding intolerance and duration of hospitalization were decreased in premature infants ≤1500 g. *Pediatrics* 2012;130:e1113–e1120
In NICUs, nosocomial infection (hospital-acquired bloodstream infection, urinary tract infection, pneumonia, and meningitis) is associated with increased morbidity and mortality.1-4 Preterm infants are especially vulnerable to infections because of their immature skin/mucosal barrier and immune response, as well as their exposure to the hospital milieu that promotes gastrointestinal (GI) colonization with bacterial pathogens.5-8 Translocation of indigenous pathogenic enteric organisms through the intestinal wall results in bloodstream infection, with the potential for infecting the lungs, cerebral spinal fluid (CSF), or other sterile sites in the body.9,10 Aspiration of oropharyngeal secretions containing bacterial pathogens can lead to nosocomial pneumonia in preterm infants who had variable degrees of dysphagia or had been exposed to mechanical ventilation.11 Colonization of the GI tract with probiotics (nonpathogenic anaerobic bacteria) competitively inhibits attachment of bacterial pathogens, decreasing their likelihood for colonization and translocation, therefore preventing life-threatening infections.12-16 Several systematic reviews and meta-analyses of randomized trials evaluating the use of probiotics in preterm infants suggest a beneficial effect for the prevention of necrotizing enterocolitis (NEC) and death but less for nosocomial infection.17-21

The aim of this study was to determine whether prophylactic administration of Lactobacillus reuteri to preterm infants reduces the incidence of the composite outcome of death or nosocomial infection (NI). We chose L. reuteri because it is found naturally in humans and has been studied well with evidence to support its role as a protective organism with immunomodulation and bactericidal properties.22-26

**METHODS**

This multicenter, double-blinded, randomized, placebo-controlled trial was conducted in 9 NICUs from 4 major cities in Colombia: Bogotá, Medellín, Cali, and Bucaramanga, from August 10, 2008 to April 3, 2011. Preterm infants who met the following criteria were enrolled: admission to the NICU, birth weight ≤2000 g, hemodynamically stable (blood pressure not requiring boluses or pressors), and ≤48 hours of age. Infants with evidence or suspicion of congenital intestinal obstruction or perforation, gastroschisis, large omphalocele, congenital diaphragmatic hernia, major congenital heart defects, or anticipated transfer to a NICU not participating in the study were excluded. All preterm infants were screened at birth, and parents of eligible infants were approached to obtain signed consent. Parents could remove their infant from the study at any time.

**INCLUSION CRITERIA**

**EXCLUSION CRITERIA**

**Stratification and Randomization**

Study participants were randomly assigned to probiotic or placebo by the use of a computer-generated balanced block randomization scheme. Infants were stratified by institution and by birth weight (≤1500 g and 1501-2000 g). Treatment assignment was performed by using sealed, sequentially numbered, opaque envelopes, color-coded for strata, available in each NICU pharmacy. The pharmacist was in charge of assignment to ensure concealment allocation. Both probiotic and placebo were packaged in identical vials of an oil-based suspension labeled with an individual number indicating the randomization sequence. Infants were administered probiotic or placebo regardless of whether enteric feeds were started. Infants in the probiotic group received 5 drops of an oil-based suspension containing 10^9 colony-forming units of L. reuteri DSM 17938 (BioGaia AB, Stockholm, Sweden) once a day. To maintain stability, the oil-based suspension was stored in a specified refrigerator at 2°C to 8°C. Each 5-mL vial was suitable for 25 five-drop doses. For infants without peroral feeds, the 5 drops were administered through a feeding tube followed by a flush of 0.5 mL of sterile water. For infants on oral feeds, 5 drops were placed in the posterior oropharynx after suctioning oral secretions. For infants in the placebo group, an equal number of drops from an identical vial containing only the oil base were administered following the same protocol as that described for the probiotic group. If during the hospitalization enteric feeds were stopped because of feeding intolerance or NEC, the administration of the probiotic or placebo was also stopped and restarted only when the clinician deemed enteric feeds could be reinstated. Assigned treatment was administered daily until death or discharge from the hospital.

In an effort to minimize differences in feeding and nutrition practices among participating institutions, an investigator workshop was held before the initiation of patient recruitment. All investigators agreed to comply with the following practices: (1) if the infant was deemed to be hemodynamically stable, initiate enteric feeds between 24 and 48 hours of life; infants ≤1200 g at 10 mL/kg per day (divided into 8 feedings) with subsequent daily increments of 10 mL/kg per day depending on tolerance; infants >1200 g, at 20 mL/kg per day with subsequent daily increments of 20 mL/kg per day depending on tolerance; (2) remove umbilical lines and place a percutaneous venous catheter once feedings were increased above 20 mL/kg per day; (3) if the infant was deemed to be hemodynamically unstable, start enteric feeds once in a lower concentration and increase at a slower rate; (4) for infants meeting criteria for NEC or death, if the parents consented, the clinician had the option to withhold or discontinue enteric feeds.

**Parental Consent**

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The study was approved by each institution’s ethics committee. The study data were managed by a Robert Wisdom, University of Washington Medical Center, Seattle, WA. The study was supported by a grant from BioGaia AB, Stockholm, Sweden. There are different ways to randomize. Flipping a coin is simple randomization, but doesn’t always give you a 50-50 split. Block means there’s a box of 4, 6 or what ever number tokens - placebo or intervention. You use one box at a time and ensure a 50-50 split.
Every definition clearly spelled out ... great for comparing or replicating

per day; (3) start parenteral nutrition on the second day of life following a standardized protocol; (4) use median chain triglyceride oil (251 kcal/30 ml) or powdered formula to increase the energy content of feedings when needed; and (5) use human milk fortifiers once full enteric feeds were tolerated. Infants of mothers with insufficient production of breast milk were offered premature infant formula.

Outcome Measures

The primary outcome was death or NI. Secondary outcomes included nosocomial pneumonia, NEC, feeding intolerance, and duration of hospitalization.

Definitions

Nosocomial infection: episode occurring after 48 hours of hospitalization, resulting in a positive blood, CSF, or urine culture.

Hospital-acquired bloodstream infection: clinical signs of sepsis occurring after 48 hours of life and followed by a positive blood culture drawn after 48 hours of life. If culture was positive for a coagulase-negative Staphylococcus species, an additional positive culture with the same organism was required for confirmation and treatment.

Nosocomial pneumonia: development of respiratory distress after 48 hours of hospitalization evidenced by rapid, noisy, or difficult breathing, respiratory rate >60 breaths per minute, chest retractions or grunting, and confirmed with a chest radiograph, a blood culture, or additional blood work. If the chest radiograph was suggestive of pneumonia and the blood culture was negative, clinical signs of sepsis or laboratory tests were required for diagnosis (Duke modification).

Chest radiograph suggestive of pneumonia: presence of nodular or coarse patchy infiltrate, diffuse haziness, or granularity, or lobar or segmental consolidation.

Clinical signs of sepsis: presence of lethargy, recurrent apnea, hypothermia (axillary temperature <37°C) or hyperthermia (>38°C).

Laboratory tests suggestive of sepsis: a leukocyte count out of the reference range (neutropenia <5000 or neutrophilia >25 000), a ratio of immature to total neutrophilic forms >0.2 or an elevated C-reactive protein.

Urinary tract infection: clinical signs of sepsis and a positive urine culture with >106 organisms of a single pathogen obtained by the use of standard sterile technique and urethral catheterization.

Meningitis: clinical signs of sepsis with a CSF white blood cell count >29/mm3 and neutrophil count >60%, or a positive CSF Gram-stain, culture, or polymerase chain reaction for bacterial antigens.

Neonatal lung hypoplasia was defined using the Duke modification of Bells criteria for stage II based clinical and/or radiographic data: (1) pneumatoses or portal vein gas, (2) localized pneumatoses, fixed dilated bowel loops, or pneumoperitoneum AND 2 GI signs/symptoms and 1 systemic sign/symptom, or (3) thickened bowel loops AND an abnormal gas pattern AND 2 GI and 2 systemic signs/symptoms.

GI signs: abdominal distension or tenderness, feeding intolerance, erythema of the abdominal wall, and decreased bowel sounds.

Systemic signs: lethargy, increased frequency or severity of apnea, temperature instability, new-onset metabolic acidosis, hemodynamic instability, and disseminated intravascular coagulation or thrombocytopenia.

Surveillance for NI

All patients in the study were followed closely for signs and symptoms of NI and other secondary outcomes. Radiographs and cultures were obtained at the discretion of the attending physician. Initial evaluation for sepsis included complete blood cell count and differential, Gram-stain, C-reactive protein level, and blood, CSF, and urine cultures. One to 2 ml of blood were inoculated per aerobic culture bottle. The Bactec PEDS Plus microbiology system (Becton Dickinson and Company, Sparks, MD) was used to culture aerobic bacteria. Bloodstream infections and meningitis secondary to L reuteri was suspected if a Gram-stain identified irregular Gram-positive bacilli with rudimentary branching. Negative aerobic blood cultures from patients with clinical evidence of sepsis were forwarded to a central laboratory (Universidad Javeriana, Bogotá, Colombia) to identify L reuteri as a causative organism by culturing on MRS agar and anaerobic incubation at 37°C for 48 hours followed by determination of catalase-negative nature and reuterin production. Typification was done by using the API 50 CHL system. Meningitis was also suspected in cases of culture-negative purulent meningitis. In these cases, CSF inoculation was done on either sheep blood or chocolate agar plates incubated at 37°C in a reduced CO2.
environment. All blood and CSF cultures were followed beyond 48 hours of inoculation.

**Sample Size**

With the use of historical data from a study of the epidemiology of NI in Colombia, we estimated that ~28% of admitted preterm infants ≤2000 g would develop the combined outcome of death or NI before discharge; we estimated a 30% relative risk reduction with the use of probiotics. We chose to do this study in Colombia because of the observed high rates of NI and Gram-negative sepsis. A sample size of 555 infants in each group was calculated to detect this difference at a significance level of 0.05 (2-tailed) with a power of 90%. These results assume that 1 sequential test is made by using the O’Brien-Fleming spending function to determine the test boundaries. A safety-monitoring board was created to follow quarterly mortality and morbidity during the study. An interim analysis was planned with the use of the Wilcoxon rank sum test. Categorical variables were compared by using the χ² test. Relative risks (RRs) with their corresponding 95% confidence intervals (CIs) were calculated to evaluate associations between primary and secondary outcomes and independent variables, initially for each stratum, and subsequently for the total population by the use of the Mantel-Haenszel method. Survival analysis was performed to evaluate differences in time to full feeds. All analyses were based on the principal of “intention to treat” and were conducted by using SAS 9.3 (SAS Institute, Inc, Cary, NC).

**RESULTS**

**Study Participants**

Of the 2055 preterm infants screened, 1446 (70%) met inclusion criteria. Parental consent was given for 58% of eligible infants. One eligible infant was randomly assigned before parental consent and was removed from the study after the parents refused consent. The study was terminated before the completion of the targeted sample because of a substantial drop in patient recruitment among participating institutions as well as funding restrictions that limited our ability to recruit the required additional subjects. A total of 750 infants were enrolled; 372 were randomly assigned to the probiotic group and 378 to the placebo group. No significant demographic or clinical differences were observed between groups at baseline (Table 1).

**Infection-Related Outcomes**

The frequency of the primary outcome, death or NI, was similar between groups (RR 0.87; 95% CI: 0.63–1.19; P = .376), as were the frequencies of each of these considered individually (death, RR 0.80; 95% CI: 0.47–1.37; NI, RR 0.88; 95% CI: 0.61–1.28). The lower rate of nosocomial pneumonia in the probiotic group was not quite statistically significant (2.4% vs 5.0%; P = .06). No differences were observed in the rates of meningitis or urinary tract infections.

**Great table one. How was the block randomization?**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Probiotic, n = 372</th>
<th>Placebo, n = 378</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal noninstrumented, n (%)</td>
<td>61 (16)</td>
<td>66 (17)</td>
</tr>
<tr>
<td>Vaginal instrumented, n (%)</td>
<td>0 (0.0)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>Elective cesarean delivery, n (%)</td>
<td>69 (18)</td>
<td>64 (17)</td>
</tr>
<tr>
<td>Nonelective cesarean delivery, n (%)</td>
<td>245 (65)</td>
<td>246 (65)</td>
</tr>
<tr>
<td>Confirmed chorioamnionitis, n (%)</td>
<td>40 (11)</td>
<td>33 (89)</td>
</tr>
<tr>
<td>Prenatal steroids, n (%)</td>
<td>270 (72)</td>
<td>277 (73)</td>
</tr>
<tr>
<td>Fetal tachycardia, n (%)</td>
<td>18 (5)</td>
<td>25 (6)</td>
</tr>
<tr>
<td>GA, wk, median±</td>
<td>32 (30–33)</td>
<td>32 (29–33)</td>
</tr>
<tr>
<td>Birth weight, g, median±</td>
<td>1530 (1253–1750)</td>
<td>1516 (1129–1750)</td>
</tr>
<tr>
<td>Gender (male), n (%)</td>
<td>186 (50)</td>
<td>185 (49)</td>
</tr>
<tr>
<td>Appgar 5 min, median±</td>
<td>9 (8–9)</td>
<td>9 (8–9)</td>
</tr>
<tr>
<td>Small for GA, n (%)</td>
<td>106 (28)</td>
<td>111 (29)</td>
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<tr>
<td>Nutrition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal breast milk alone, n (%)</td>
<td>12 (7)</td>
<td>9 (4)</td>
</tr>
<tr>
<td>Cow-based formula, n (%)</td>
<td>143 (80)</td>
<td>161 (81)</td>
</tr>
<tr>
<td>MBM and formula, n (%)</td>
<td>24 (13)</td>
<td>28 (14)</td>
</tr>
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</table>

MBM, maternal breast milk; GA, gestational age.
± (Interquartile range).
tract infection. A total of 104 episodes of NI were observed, 47 (12.6%) in the probiotic group and 57 (15.1%) in the placebo group. The etiology for NI was observed, 47 (12.6%) in the probiotic group and 57 (15.1%) in the placebo group. The etiology for NI were observed, 47 (12.6%) in the probiotic group and 57 (15.1%) in the placebo group. The etiology for NI were observed, 47 (12.6%) in the probiotic group and 57 (15.1%) in the placebo group. The etiology for NI were observed, 47 (12.6%) in the probiotic group and 57 (15.1%) in the placebo group. The etiology for NI were observed, 47 (12.6%) in the probiotic group and 57 (15.1%) in the placebo group.

**TABLE 3**

<table>
<thead>
<tr>
<th>Death or NI, n (%)</th>
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<th>RR</th>
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<tr>
<td>Death, n (%)</td>
<td>57 (15.3)</td>
<td>67 (17.7)</td>
<td>0.87</td>
<td>0.63–1.19</td>
<td>.38</td>
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<tr>
<td>BSI, n (%)</td>
<td>47 (12.6)</td>
<td>57 (15.1)</td>
<td>0.88</td>
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<td>.35</td>
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<tr>
<td>Positive culture, n (%)</td>
<td>24 (6.5)</td>
<td>17 (4.5)</td>
<td>1.44</td>
<td>0.78–2.63</td>
<td>.24</td>
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<td>NEC, n (%)</td>
<td>34 (9.1)</td>
<td>40 (10.9)</td>
<td>0.86</td>
<td>0.56–1.33</td>
<td>.51</td>
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<tr>
<td>Nosocomial pneumonia, n (%)</td>
<td>9 (2.4)</td>
<td>19 (5.0)</td>
<td>0.48</td>
<td>0.22–1.05</td>
<td>.06</td>
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<td>Nosocomial UTI, n (%)</td>
<td>7 (1.9)</td>
<td>5 (0.8)</td>
<td>2.37</td>
<td>0.62–9.10</td>
<td>.19</td>
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<td>Nosocomial meningitis, n (%)</td>
<td>1 (0.3)</td>
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<td>1.02</td>
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<td>.99</td>
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<td>Necrotizing enterocolitis, n (%)</td>
<td>9 (2.4)</td>
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<td>Elevated C-reactive protein, n (%)</td>
<td>37 (10.0)</td>
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<td>Leukocytosis, n (%)</td>
<td>54 (14.5)</td>
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**BSI,** bloodstream infection; **UTI,** urinary tract infection.

**Secondary Outcomes (Table 4)**

Although not significant, a 40% decrease in the rate of NEC was observed in the probiotic group. Infants exposed to *L reuteri* had fewer episodes of feeding intolerance in comparison with placebo-exposed infants, but the difference was not statistically significant (7% vs 10.6%; *P* = .08). Feeding intolerance episodes and duration of hospitalization were significantly lower in preterm infants ≤1500 g exposed to *L reuteri* in comparison with placebo-exposed infants: 9.6% vs 16.8%; *P* = .04 and 32.5 days vs 37 days; *P* = .03, respectively. No differences were observed when time to full feeds was compared between groups (*P* = .134). A total of 373 (49.7%) infants received *L reuteri* before the first feeding, and 355 (95.2%) of these were initially formula fed.

**DISCUSSION**

Our trial of probiotics, the largest to date, failed to detect a significant reduction in the combined outcome of death or NI, although the observed trends in main outcomes are consistent with published meta-analyses. There are several potential explanations for our results. The first and most likely is insufficient statistical power owing to the observed drop in the rate of the primary outcome in the placebo group in comparison with historical data; this may be explained in part by an unanticipated improvement in survival and infection control protocols in NICUs, as a result of their participation in this and 3 previous multicenter studies.32–34 The observed shift to a predominance of Gram-positive organisms responsible for NI support this explanation. Inclusion of more mature infants and early termination of the study were also contributing factors. Second, it is possible that the dose of *L reuteri* that we used was not sufficient to colonize the gut of premature infants or that treatment with antibiotics, which frequently are given to premature infants, did not allow sufficient growth of *L reuteri*. Smith et al35 assessed colonization and persistence of *L reuteri* DSM 17938 after daily or alternate-day dosing (10^9 colony-forming units) and demonstrated fecal recovery of *L reuteri* was achieved within 4 days and was detected in the feces ∼1 and 2 weeks after supplementation was discontinued. Hakalehto et al36 demonstrated that *L reuteri* is resistant to the acid media of the stomach. These studies support adequate colonization of the GI tract by *L reuteri*, but there are no published studies that evaluate the effect of frequent exposure to antibiotics on colonization. To overcome this potential problem, we administered *L reuteri* on a daily basis from the day of randomization until discharge. We did not test random samples of stools to evaluate colonization, and this is a limitation of our study. Mechanical ventilation has been associated with a 6- to 21-fold increased risk of developing

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**BSI,** bloodstream infection; **NEC,** necrotizing enterocolitis; **NI,** nosocomial infection.
nosocomial pneumonia.\textsuperscript{37} Wu et al,\textsuperscript{38} in a randomized controlled study of infants receiving mechanical ventilation, demonstrated a protective effect against ventilator-associated pneumonia with exposure to \textit{Bifidobacterium}. The explanation for the expected benefit of probiotics is theorized through its potential to prevent oropharyngeal and gastric colonization of pathogens.\textsuperscript{39} Post hoc analysis revealed that 85.7\% of infants diagnosed with nosocomial pneumonia in our study were also exposed to mechanical ventilation, but we lacked adequate power to test this hypothesis. Although we found a decrease of 40\% in the incidence of NEC in the probiotic group, this difference was not statistically significant, but it may be clinically relevant and is consistent with previous studies that chose NEC as the primary outcome.\textsuperscript{17–21,40–42}

Finally, we found that exposure to \textit{L reuteri} significantly decreased the number of infants $\leq$1500 g who experienced feeding intolerance episodes. Two recent studies, one using \textit{L reuteri},\textsuperscript{42,43} also found a significant decrease in feeding intolerance in probiotic exposed preterm infants of similar weight. The observed decrease in duration of hospitalization in our study may be explained in part by better feeding tolerance. The absence of episodes of NI secondary to \textit{L reuteri} with the use of a strict protocol for identification of this microorganism supports the safety of this probiotic in the population studied. Our study differs from previously published trials because of its unique design. Because colonization of the GI tract begins immediately after birth, our study design required infants to receive the probiotic within the first 48 hours after birth. Our study was designed with the premise that not all probiotics behave the same. The probiotic chosen was adequately studied, easy to administer and blind; we also made an effort to minimize differences in feeding and nutrition practices among participating institutions before initiating the study. The dramatic drop in patient recruitment observed during the conduct of this trial occurred after the publication of the meta-analyses by Deshpande et al\textsuperscript{18} and Alfaleh et al.\textsuperscript{20}

### CONCLUSIONS

Although \textit{L reuteri} did not appear to decrease the rate of the composite outcome, the trends suggest a protective role for mortality, NI, and NEC consistent with what has been observed in previous systematic reviews and meta-analyses. Our results increase the precision of the pooled estimate for these outcomes while expanding the generalizability of these findings by rigorously studying the same question with a different organism.

### ACKNOWLEDGMENTS

We thank Pilar A. Lopez, Mauricio Gamboa, and Juliana Guzmán for helping coordinate this study. We thank Drs Michael O’Shea and Paula M. Sisk for the critical review of this manuscript. We thank all the research assistants who were invaluable to the conduct of this trial. Special thanks to Dr Travis Honeycutt, Angela Hoyos, Juan G. Piñeros, Guillermo Robayo, and Astrid Celis for their initial contributions to the design of the study. Maria X. Rojas is a PhD student in the Department of Pediatrics and Preventive Medicine, School of Medicine, Universitat Autónoma de Barcelona, Spain.

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40. Lin HC, Su BH, Chen AG, et al. Oral probiotics reduce the incidence and severity

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Dr Rojas developed the original idea, study design, and research methodology; he was also involved in obtaining funding and resources for the project and supervised the conduct of the trial as research director, he was involved in data analysis and interpretation, and was responsible for writing and final approval of the version of the manuscript to be published; Dr Lozano, Ms Rojas, Ms Rodriguez and Mr Rondon as faculty members of the Department of Epidemiology and Biostatistics at the Pontificia Universidad Javeriana were responsible for the coordination of this multicenter trial; their responsibilities included organization of the principal investigators workshop on probiotics, research design, and sample size calculations for the trial; identification of participating centers; Spanish translation of the protocol, development of the manual of operations, data collections sheets and computerized database; training of research assistants, oversight of participating centers; data entry, statistical analysis, data interpretation and review of the final manuscript; Dr Lozano and Ms Rojas were responsible for submitting the protocol to COLCIENCIAS, Drs Bastidas, Perez, Rojas, Ovalle, Garcia-Harker, Tamayo, Ruiz, Ballesteros, Archila, and Arevalo, as principal investigators for each participating center, were actively involved in research design (principal investigators workshop), implementation, conduct, local oversight of the trial, interpretation of data, and manuscript revision; and all authors take responsibility for the integrity of the data and accuracy of the data analysis.

This trial has been registered at www.clinicaltrials.gov (identifier NCT00727363).

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