Prophylactic Probiotics to Prevent Death and Nosocomial Infection in Preterm Infants

AUTHORS: Mario A. Rojas, MD, MPH,[a] Juan M. Lozano, MD, MSc,[b] Maria X. Rojas, RN, MSc,[c] Viviana A. Rodriguez, MSc (Cand),[c] Martin A. Rondon, MSc,[c] Jaime A. Bastidas, MD,[d] Luis A. Perez, MD,[e] Catherine Rojas, MD,[f] Oscar Ovalle, MD,[g] Jorge E. Garcia-Harker, MD,[h] Maria E. Tamayo, MD, MSc,[i] Gloria C. Ruiz, MD,[j] Adriana Ballesteros, MD,[k] Maria M. Archila, MD,[g] and Mauricio Arevalo, MD[d]

[a]Department of Pediatrics, Wake Forest School of Medicine, Winston-Salem, North Carolina; [b]Division of Research, College of Medicine, Florida International University, Miami, Florida; [c]Department of Clinical Epidemiology and Biostatistics, Pontificia Universidad Javeriana, Bogotá, Colombia; [d]Clínica Los Farallones/Remedios, Universidad del Valle, Cali, Colombia; [e]Departmento de Pediatría, Hospital Universitario de Santander, Bucaramanga, Colombia; [f]Policlínico del Olaya, Bogotá, Colombia; [g]SaludCoop, Bogotá, Colombia; [h]Clínica San Luis, Bucaramanga, Colombia; [i]Departamento de Pediatría, Universidad de Antioquia, Hospital Universitario San Vicente Fundación, Medellín, Colombia; [j]Departamento de Pediatría, Universidad Javeriana, Bogotá, Colombia; and [k]Fundación Valle de Lili, Cali, Colombia

KEY WORDS
probiotics, preterm infants, nosocomial infection, *Lactobacillus reuteri*

ABBREVIATIONS
CSF—cerebral spinal fluid
CI—confidence interval
GI—gastrointestinal
NEC—necrotizing enterocolitis
NI—nosocomial infection
RR—relative risk

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 have 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]

yes... tell us why L reuteri and not some other lactobacillus like GG, etc...

METHODS

This multicenter, double-blinded, randomized, placebo-controlled trial was conducted in 9 NICUs from 4 major cities in Colombia: Bogotá, Medellin, 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 10^8 colony-forming

How often have we seen such excellent accounting of the randomization process in RCTs? These folks do it right.

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 per oral 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.

yada, yada, yada but good for replication

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[27]; (2) remove umbilical lines and place a percutaneous venous catheter once feedings were increased above 20 mL/kg

a problem in multicenter trials but they had a plan to address

there are different ways to randomize. Flipping a coin is simple randomization, but doesn't always get you a 50-50 split. Block means there's a box of 4, 6 or whatever 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 (231 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.

which is a
stronger
outcome?

Outcome Measures

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

very, very, very nice
every outcome explicitly defined
do you agree with definitions?

Definitions

Nosocomial infection: episode occurring after 48 hours of hospitalization, resulting in a positive blood, CSF, or urine culture.[4]

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.

not all
coag neg
staph
+ cx's
qualified

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[28] modified definition).

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 >10^4 organisms of a single pathogen obtained by the use of standard sterile technique and urethral catheterization.[4]

Meningitis: clinical signs of sepsis with a CSF white blood cell count >29/mm^3 and neutrophil count >60%, or a positive CSF Gram-stain, culture, or polymerase chain reaction for bacterial antigens.[4]

Feeding intolerance: any of the following: recurring emesis, gastric residuals with 50% or more of the previous feed volume, abdominal distension, or the presence of macroscopic blood in stools.

Necrotizing enterocolitis: modification of Bells criteria for stage II[29] based clinical and/or radiographic data: (1) pneumatosis or portal vein gas, (2) localized pneumatosis, 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 (bifid) 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.[30] 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 CO_2

local

not
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environment.[31] All blood and CSF cultures were followed beyond 48 hours of inoculation.

[handwritten note: a key component of a RCT is sample size. To calculate, need expected difference which they said was 30% plus p-value and power]

Sample Size

With the use of historical data from a study of the epidemiology of NI in Colombia,[32] 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.[32] A sample size of 555 infants in each group was calculated to detect this difference at a significance level of .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 first 500 patients enrolled in the study. It was agreed that the study would be stopped if mortality was significant at $P = .05$, and adverse events were significant at a $P = .01$. The interim analysis did not show excess mortality or morbidity; however, it indicated that an additional 302 infants in each group (857 infants per group) were required to have the power to detect a 30% difference in the primary outcome (19.3%–13.5%) with a power of 90% and an α-error of 5%. The study protocol was approved by the institutional review boards of all participating centers before patient recruitment. The trial was registered through ClinicalTrials.gov (identifier NCT00727363).

Data Analysis

Demographic and clinical data were collected prospectively. Continuous variables were evaluated for normality of distribution with the use of the Kolmogorov-Smirnov Z tests, and in cases where normality of distribution was confirmed, results were compared by using t tests. In cases where normality was not confirmed, the Wilcoxon rank sum test was used. Categorical variables were compared by using the χ^2 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 (Table 2)

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

[handwritten note: Great table one. How was the block randomization?]

TABLE 1 Baseline Demographic and Clinical Characteristics of the Study Population

Variable	Probiotic, $n = 372$	Placebo, $n = 378$
Vaginal noninstrumented, n (%)	61 (16)	66 (17)
Vaginal instrumented, n (%)	0 (0.0)	2 (0.5)
Elective cesarean delivery, n (%)	69 (18)	64 (17)
Nonelective cesarean delivery, n (%)	243 (65)	246 (65)
Confirmed chorioamnionitis, n (%)	40 (11)	33 (89)
Prenatal steroids, n (%)	270 (72)	277 (73)
Fetal tachycardia, n (%)	18 (5)	23 (6)
GA, wk, median[a]	32 (30–33)	32 (29–33)
Birth weight, g, median[a]	1530 (1253–1750)	1516 (1129–1750)
Gender (male), n (%)	186 (50)	185 (49)
Apgar 5 min, median[a]	9 (8–9)	9 (8–9)
Small for GA, n (%)	106 (28)	111 (29)
Nutrition		
Maternal breast milk alone, n (%)	12 (7)	9 (4)
Cow-based formula, n (%)	143 (80)	161 (81)
MBM and formula, n (%)	24 (13)	28 (14)

MBM, maternal breast milk; GA, gestational age.
[a] (Interquartile range).

Primary outcomes here...

TABLE 2 Nosocomial Infection–Related Outcomes of the Study Population

Primary Outcomes	Probiotic, n = 372	Placebo, n= 378	RR	95% CI	P
Death or NI, n (%)	57 (15.3)	67 (17.7)	0.87	0.63–1.19	.38
Death, n (%)	22 (5.9)	28 (7.4)	0.80	0.47–1.37	.41
NI, n (%)	47 (12.6)	57 (15.1)	0.88	0.61–1.28	.33
BSI, n (%)	24 (6.5)	17 (4.5)	1.44	0.78–2.63	.24
Positive culture, n (%)	34 (9.1)	40 (10.6)	0.86	0.56–1.33	.51
Nosocomial pneumonia, n (%)	9 (2.4)	19 (5.0)	0.48	0.22–1.05	.06
Nosocomial UTI, n (%)	7 (1.9)	3 (0.8)	2.37	0.62–9.10	.19
Nosocomial meningitis, n (%)	1 (0.3)	1(0.3)	1.02	0.06–16.20	.99
Necrotizing enterocolitis, n (%)	9 (2.4)	15 (4.0)	0.61	0.27–1.38	.23
Elevated C-reactive protein, n (%)	37 (10.0)	48 (12.7)	0.78	0.52–1.17	.24
Leukocytosis, n (%)	54 (14.5)	71 (18.8)	0.77	0.56–1.07	.12

BSI, bloodstream infection; UTI, urinary tract infection.

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 culture-positive sepsis was a Gram-positive pathogen in 68% of cases and a Gram-negative pathogen in 29% of cases. Within strata defined by birth weight, no differences were observed for infection-related outcomes (Table 3). We did not observe any Gram-stain suspicious for probiotic infection, and no suspicious cultures were sent to the central laboratory for identification of *L reuteri*.

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 consis-tent 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 al[35] 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 al[36] 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

TABLE 3 Nosocomial Infection–Related Outcomes According to Weight

	Probiotic	Placebo	RR	95% CI	P
≤1500 g	n = 176	n = 184			
Death or NI, n (%)	48(27.3)	54 (29.4)	0.93	0.67–1.29	.66
BSI, n (%)	19 (10.8)	16 (8.7)	1.24	0.66–2.33	.50
Positive culture, n (%)	28 (15.9)	33(17.9)	0.89	0.56–1.40	.61
Nosocomial pneumonia, n (%)	9 (5.1)	16 (8.7)	0.59	0.27–1.30	.18
NEC, n (%)	6 (3.4)	10 (5.4)	0.63	0.23–1.70	.35
>1500 g	n = 196	n = 194			
Death or NI, n (%)	9(4.6)	13 (6.7)	0.69	0.30–1.60	.37
BSI, n (%)	5 (2.6)	1 (0.5)	4.95	0.58–42.0	.10
Positive culture n (%)	6 (3.1)	7 (3.6)	0.85	0.29–2.48	.76
Nosocomial pneumonia, n (%)	0 (0.0)	3 (1.5)			.08
NEC, n (%)	3 (1.5)	5 (2.6)	0.59	0.14-2.50	.47

BSI, blood stream infection; NEC necrotizing enterocolitis; NI, nosocomial infection.

Stratified analysis is when you analyze to see if the outcome is different in different group, hence Table 2

TABLE 4 Secondary Outcomes

	Probiotics	Placebo	P
Total	N = 372	N = 378	
Episodes of feeding intolerance, n (%)	26 (7.0)	40 (10.6)	.08
Duration of hospitalization, d, median[a]	20.0 (11–33)	20.0 (11–38)	.53
≤1500 g	n = 176	n = 184	
Episodes of feeding intolerance, n (%)	17 (9.6)	31 (16.8)	.04
Duration of hospitalization, d, median[a]	32.5 (21–44)	37.0 (23–51)	.03
>1500 g	n = 196	n = 194	
Episodes of feeding intolerance, n (%)	9 (4.5)	9 (4.6)	.98
Duration of hospitalization, d, median[a]	14.0 (8–19)	13.0 (7–19)	.33

[a] (Interquartile range).

nosocomial pneumonia.[37] Wu et al,[38] in a randomized controlled study of infants receiving mechanical ventilation, demonstrated a protective effect against ventilator-associated pneumonia with exposure to *Bifidobacterium*. The explanation for the expected benefit of probiotics is theorized through its potential to prevent oropharyngeal and gastric colonization of pathogens.[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.[17–21,40–42]

Finally, we found that exposure to *L reuteri* significantly decreased the number of infants ≤1500 g who experienced feeding intolerance episodes. Two recent studies, one using *L reuteri*,[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 *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[19] and Alfaleh et al.[20]

CONCLUSIONS

Although *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.

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