Probiotic Supplementation and Late-Onset Sepsis in Preterm Infants: A Meta-analysis

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abstract

CONTEXT: Late-onset sepsis (LOS) is a major cause of mortality and morbidity in preterm infants. Despite various preventive measures, its incidence continues to remain high, hence the urgent need for additional approaches. One such potential strategy is supplementation with probiotics. The updated Cochrane Review (2014) did not find benefits of probiotics in reducing the risk of LOS in preterm infants (19 studies, N = 5338). Currently there are >30 randomized controlled trials (RCTs) of probiotics in preterm infants that have reported on LOS.

OBJECTIVES: To conduct a systematic review including all relevant RCTs.

DATA SOURCES: PubMed, Embase, Cochrane Central Register of Controlled Trials, Cumulative Index of Nursing and Allied Health Literature, and E-abstracts from the Pediatric Academic Society meetings and other pediatric and neonatal conference proceedings were searched in June and August 2015.

STUDY SELECTION: RCTs comparing probiotics versus placebo/no probiotic were included.

DATA EXTRACTION: Relevant data were extracted independently by 3 reviewers.

RESULTS: Pooled results from 37 RCTs (N = 9416) using fixed effects model meta analysis showed that probiotics significantly decreased the risk of LOS (675/4852 [13.9%] vs 744/4564 [16.3%]; relative risk, 0.86; 95% confidence interval, 0.78–0.94; P = .0007; I² = 35%; number needed to treat, 44). The results were significant even after excluding studies with high risk of bias.

CONCLUSIONS: Probiotic supplementation reduces the risk of LOS in preterm infants.

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Late-onset sepsis (LOS) is a major cause of mortality and morbidity, including adverse long-term neurodevelopmental outcomes in preterm infants. The burden of LOS is significant in developed and developing nations of the world. The incidence of LOS varies inversely with gestational age and birth weight. The important risk factors for LOS in preterm infants are intravascular catheters, delayed commencement of enteral feeds, prolonged use of parenteral nutrition, prolonged ventilation, and surgery. Although the predominant organism causing LOS is coagulase-negative staphylococci, other organisms such as Staphylococcus aureus, Gram-negative bacteria, and fungi also are important. The cost-effective strategies for preventing LOS include antimicrobial stewardship, limited steroid use, early enteral feeding, limited use of invasive devices, standardization of catheter care practices, and meticulous hand hygiene. Despite these preventive measures, the incidence of LOS remains high in preterm infants. Therefore, additional approaches to reduce LOS are needed urgently.

One such potential strategy that might reduce LOS is supplementation with probiotics.

Probiotics are defined as live microorganisms that when administered in adequate amounts may confer health benefits on people with specific illnesses. Animal research and in vitro studies have shown that probiotics improve gut barrier function, inhibit gut colonization with pathogenic bacteria, improve colonization with healthy commensals, protect from enteropathogenic infection through production of acetate, enhance innate immunity, and increase maturation of the enteric nervous system. All of which have the potential to decrease the risk of LOS in preterm infants. However, the recent Cochrane Review (2014) concluded that probiotic supplementation did not result in statistically significant reduction of LOS in preterm infants (relative risk [RR] 0.91; 95% confidence interval [CI], 0.80–1.03; 19 studies, N = 5338). Another meta-analysis also reported similar results on LOS (RR, 0.919; 95% CI, 0.823–1.027; P = .137; 17 randomized controlled trials [RCTs], N = 5215).

The meta-analyses done so far have included a maximum of 19 RCTs, whereas currently there are >30 RCTs of probiotic supplementation that have reported on LOS. Therefore, we decided to conduct a systematic review and meta-analysis to evaluate the role of probiotic supplementation in reducing the risk of LOS in preterm infants.

METHODS

Guidelines from the Cochrane Neonatal Review Group (http://neonatal.cochrane.org/resources-review-authors), Centre for Reviews and Dissemination (http://www.york.ac.uk/crd/guidance/), and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement were followed for undertaking and reporting this systematic review and meta-analysis. Ethics approval was not required.

Eligibility Criteria

Types of Studies

Only RCTs were included in the review. Observational studies, narrative reviews, systematic reviews, case reports, letters, editorials, and commentaries were excluded but read to identify potential additional studies.

Types of Participants

Preterm neonates born at a gestational age <37 weeks, low birth weight (<2500 g), or both (same criteria as the Cochrane Review, 2014).

Intervention and Comparison

Enteral administration of probiotic supplement versus placebo or control.

Outcomes

LOS, defined as the presence of positive blood or cerebrospinal fluid culture on a sample collected 48 to 72 hours after birth.

Search Strategy

The databases PubMed (www.ncbi.nlm.nih.gov, 1966–2015), Embase (Excerpta Medica database) via Ovid (http://ovidsp.tx.ovid.com, 1980–2015), Cochrane Central Register of Controlled Trials (www.thecochranelibrary.com, through August 2015), Cumulative Index of Nursing and Allied Health Literature via Ovid (http://ovidsp.tx.ovid.com, 1980–August 2015), and E-abstracts from the Pediatric Academic Society meetings (www.abstracts2view.com/pasall, 2000–August 2015) were searched in August 2015. A similar search was also done in June 2015. Abstracts of other conference proceedings such as Perinatal Society of Australia and New Zealand, European Academy of Pediatric Societies, and the British Maternal and Fetal Medicine Society were searched in Embase. Google Scholar was searched for articles that might not have been cited in the standard medical databases. Gray literature was searched through the national technical information services (http://www.ntis.gov/), Open Grey (http://www.opengrey.eu/), and Trove (http://trove.nla.gov.au/).

The reference lists of eligible studies and review articles were searched to identify additional studies. Reviewers S.C.R., G.K.A.J., and G.C.D. conducted the literature search independently. No language restriction was applied. The non-English studies were identified by reading through the recently published systematic reviews of probiotic supplementation on the incidence of necrotizing enterocolitis in preterm infants.
entocolitis (NEC).\textsuperscript{37,38}

Search of Embase also identified 1 non-English study. Full texts of all the non-English studies were obtained via the library of University of Sydney. A research officer from the University of Sydney translated the articles. Attempts were made to contact the authors for additional data and clarification of methods, but there was no response. Only published data were used for those studies, where available.


**Study Selection**

Abstracts of the citations obtained from the initial broad search were read independently by 3 reviewers (S.C.R., G.K.A.J., and G.C.D.) to identify potentially eligible studies. Full-text articles of these studies were obtained and assessed for eligibility by 3 reviewers independently (S.C.R., G.K.A.J., and G.C.D.), under the predefined eligibility criteria. Differences in opinion were resolved by group discussion among all reviewers to reach consensus. Care was taken to ensure that multiple publications of the same study were identified and excluded to avoid duplication of the data.

**Data Extraction**

Reviewers S.C.R., G.K.A.J., and G.C.D. extracted the data independently using a data collection form designed for this review. The number of patients with LOS and the number of patients analyzed in each treatment group of each trial were entered into the form. Information about the study design and outcomes was verified by all reviewers. Discrepancies during the data extraction process were resolved by discussion and consensus among all reviewers. We contacted authors for additional information and clarifications when details on LOS were not available in published manuscripts. Such studies were excluded if there was no response from the authors.

**Assessment of Risk of Bias**

We assessed risk of bias (ROB) by using the Cochrane "Risk of Bias Assessment Tool."\textsuperscript{35} Authors S.C.R. and G.K.A.J. independently assessed the ROB in all domains including random number generation, allocation concealment, blinding of intervention and outcome assessors, completeness of follow-up, selectivity of reporting, and other potential sources of bias. For each domain, the ROB was assessed as low, high, or unclear risk based on the Cochrane Collaboration guidelines.

**Data Synthesis**

Meta-analysis was conducted in Review Manager 5.3 (Cochrane Collaboration, Nordic Cochrane Centre, Copenhagen, Denmark). A fixed-effects model (FEM) (Mantel–Haenszel method) was used. However, analysis using random effects model (REM) was also conducted to ensure that the results and conclusions were not influenced by the type of model used for the meta-analysis. Effect size was expressed as RR and 95% CI.

Statistical heterogeneity was assessed with the $\chi^2$ test and $I^2$ statistic and by visual inspection of the forest plot (overlap of CIs). A $P$ value <.1 on the $\chi^2$ statistic was considered to indicate heterogeneity. $I^2$ statistic values were interpreted according to the guidelines of Cochrane Handbook as follows: 0% to 40%, might not be important; 40% to 60%, may represent moderate heterogeneity; 50% to 90%, may represent substantial heterogeneity; 75% to 100%, considerable heterogeneity.\textsuperscript{35} The risk of publication bias was assessed by visual inspection of the funnel plot.\textsuperscript{39}

**Subgroup Analysis**

Infants <28 weeks’ gestation or <1000 g.

**Sensitivity Analysis**

Considering the importance of random sequence generation and allocation concealment in RCTs,\textsuperscript{40} we conducted sensitivity analyses by excluding studies that had high ROB in these 2 domains separately. Because the risk of LOS is higher in infants born at <32 weeks or <1500 g,\textsuperscript{3,4} we conducted sensitivity analysis by excluding RCTs where the inclusion criteria were ≥32 weeks or ≥1500 g.

Similar analyses were also conducted for studies where *Bifidobacterium* was or was not part of the supplement and studies where *Lactobacillus* was or was not part of the supplement, given the importance of these microorganisms in the neonatal gut flora.\textsuperscript{41} There is some evidence that multistrain probiotics may be more effective than single strains.\textsuperscript{42} We therefore conducted analyses separately for studies that used single-strain supplements and multistrain probiotics. Lastly, we also conducted analyses separately for
studies where LOS was the primary outcome of interest.

**Summary of Findings Table**

The key information about the quality of evidence, the magnitude of effect of the intervention, and the sum of available data on the main outcome was presented in the summary of findings table according to the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) guidelines.43

**RESULTS**

The literature search retrieved 1736 potential relevant citations, of which 1685 were excluded and 51 RCTs were considered eligible for inclusion. Finally, 37 RCTs were included in the systematic review and meta-analysis.44–80 The remaining 14 studies had to be excluded because of lack of information from the published manuscripts.81–94 The flow diagram of the study selection process is given in Fig 1.

Out of the 37 included studies, LOS was the primary outcome of interest in only 9 studies, whereas in the remaining 28 it was a secondary outcome. Single-strain probiotics were used in 23 studies, whereas 14 used multiple strains. *Lactobacillus* was part of the supplementation in 21 studies; *Bifidobacterium* was part of the supplementation in 22 studies. The detailed characteristics of the included studies including the dose and duration of supplementation are given in Table 1.

**ROB of Included Studies**

Of the 37 included studies, 28 (76%) were judged to have low ROB for the domain of “random sequence generation,” and 24 (65%) were considered to have low ROB for “allocation concealment.” Details of the ROB analysis are given in Table 2.

**Outcome of Interest**

The pooled meta-analysis (FEM) of 37 RCTs (*n* = 9416) that compared “probiotics” with “placebo” or “no probiotics” showed that probiotic supplementation resulted in a statistically significant reduction in the incidence of LOS (675/4852 [13.9%] vs 744/4564 [16.3%]; RR 0.86; 95% CI, 0.78–0.94; *P* = .0007; $\chi^2$ statistic for heterogeneity $P = .02$, $I^2 = 35\%$; number needed to treat, 44) (Fig 2). The results were significant even when REM was used (RR 0.85; 95% CI, 0.75–0.95, $P = .007$; $\chi^2$ statistic for heterogeneity $P = .02$, $I^2 = 35\%$). Visual inspection of the funnel plot suggested that there was no publication bias (Fig 3).

On sensitivity analysis (Table 3), the beneficial effects continued to be observed in studies that had low ROB for random sequence generation and also for allocation concealment. The results were also significant in studies that included only infants with gestational age <32 weeks or birth weight<1500 g (24 studies, sample size 7175), studies where *Bifidobacterium* was part of the supplementation (22 studies, sample size 6069), studies where *Lactobacillus* was part of the supplementation (21 studies, sample size 4608), studies where single-strain probiotics were used (23 studies, sample size 5961), and studies where multiple-strain supplements were used (14 studies, sample size 3455); however, on REM, statistical significance was lost for many of these analyses. The overall evidence according to GRADE guidelines is provided as a summary of findings table (Table 4).
<table>
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<tr>
<th>Study ID</th>
<th>Study Characteristics</th>
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<tr>
<td>1. Al Hosni 2012</td>
<td>Participants: Preterm infants 501–1000 g. <strong>Intervention:</strong> (L. rhamnosus GG 0.5 × 10^9 CFU + B. infantis 0.5 × 10^9 CFU) vs no probiotics. <strong>Duration of supplementation:</strong> Once daily from the time of initiation of enteral feeds, until discharge or 34 wk PMA. <strong>Sample size:</strong> 101 (probiotics 50, controls 51). <strong>Type of milk:</strong> EBM. <strong>Type of delivery:</strong> CD 44% vs 59%. <strong>Primary outcome:</strong> % age of infants &lt;10th centile at 34 wk PMA. <strong>LOS:</strong> 13/50 (26%) vs 16/51 (31%).</td>
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<td>2. Awad 2010</td>
<td>Participants: All neonates admitted to nursery, 28–41 wk and wt 1.1–4.3 kg. <strong>Intervention:</strong> KP (L. acidophilus, 6 × 10^9 CFU) vs LP (L. acidophilus, 6 × 10^9 CFU) vs. placebo. <strong>Duration of supplementation:</strong> Commenced on d1, duration NA. <strong>Sample size:</strong> 150 (60 vs 60 vs 30), preterm 89 (37 vs 36 vs 16). <strong>Type of milk:</strong> Details NA. <strong>Type of delivery:</strong> Preterm CD: KP (57%) vs LP (56%) vs placebo (75%). <strong>Primary outcome:</strong> Incidence of neonatal sepsis and NEC in neonates and evaluating whether a KP would be equally efficacious. <strong>LOS:</strong> Preterm: LP (18/36, 50%) vs KP (25/37, 68%) vs placebo (12/16, 75%).</td>
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<td>3. Bin-Nun 2005</td>
<td>Participants: Preterm infants &lt;1500 g. <strong>Intervention:</strong> (B. infantis 0.35 × 10^9 CFU + S. thermophilus 0.35 × 10^9 CFU + B. bifidus 0.35 × 10^9 CFU) vs no probiotic. <strong>Duration of supplementation:</strong> Once daily from the day of commencement of enteral feeds until 36 wk PMA. <strong>Sample size:</strong> = 145 (probiotics 72, controls 73). <strong>Type of milk:</strong> EBM/formula. <strong>Type of delivery:</strong> 78% vs 78%. <strong>Primary outcome:</strong> Stage ≥II NEC. <strong>LOS:</strong> 31/72 (43%) vs 24/73 (33%).</td>
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<td>4. Braga 2010</td>
<td>Participants: Preterm infants 750–1499 g. <strong>Intervention:</strong> (L. casei + B. breve vs 3.5 × 10^7 to 3.5 × 10^9 CFU once daily until 30 d of life) vs no probiotic. <strong>Duration of supplementation:</strong> Once daily from the second day of life until d30. <strong>Sample size:</strong> 231 (probiotics 119, controls 112). <strong>Type of milk:</strong> EBM/PDHM. <strong>Type of delivery:</strong> CD 53.8% vs 49.1%. <strong>Primary outcome:</strong> ≥Stage II NEC. <strong>LOS:</strong> 40/119 (33.6%) vs 42/112 (37.5%).</td>
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<td>5. Chrzanowska-Liszewska 2012</td>
<td>Participants: Preterm infants &lt;32 wk and birth wt &gt;1000 g. <strong>Intervention:</strong> L. rhamnosus 6 × 10^9 CFU vs placebo (maltodextrin). <strong>Duration of supplementation:</strong> Once daily from 0–3 d of life until d42. <strong>Sample size:</strong> 47 (probiotics 21, placebo 26). <strong>Type of milk:</strong> Formula. <strong>Type of delivery:</strong> Spontaneous VD 23% vs 34%. <strong>Primary outcome:</strong> Bacterial colonization of the gut. <strong>LOS:</strong> 2/21 (8.5%) vs 3/26 (11.5%).</td>
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<td>6. Costalos 2003</td>
<td>Participants: Preterm infants 28–32 wk. <strong>Intervention:</strong> S. boulardii (1 × 10^9 CFU) vs placebo (maltodextrin). <strong>Duration of supplementation:</strong> Twice daily for a median duration of 30 d. <strong>Sample size:</strong> 87 (probiotics 51, placebo 36). <strong>Type of milk:</strong> Formula. <strong>Type of delivery:</strong> CD 49% vs 38%. <strong>Primary outcome:</strong> Tolerance to S. boulardii supplemented formula, fecal flora analysis, intestinal D xylose absorption, and fecal lipid excretion. <strong>LOS:</strong> 3/51 (5.8%) vs 3/36 (8.3%).</td>
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<td>7. Dani 2002</td>
<td>Participants: Infants &lt;33 wk or birth wt &lt;1500 g. <strong>Intervention:</strong> Lactobacillus GG (6 × 10^9 CFU) vs placebo. <strong>Duration of supplementation:</strong> Once a day until discharge, starting with the first feed. <strong>Sample size:</strong> 585 (probiotics 295, placebo 290). <strong>Type of milk:</strong> Breast milk, formula. <strong>Type of delivery:</strong> CD 76.3% vs 82.4%. <strong>Primary outcome:</strong> Urinary tract infection, bacterial sepsis, NEC. <strong>LOS:</strong> 14/295 (4.7%) vs 12/290 (4.1%).</td>
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<td>8. Demirel, Erdeve 2013A</td>
<td>Participants: Preterm infants ≤32 wk and ≤1500 g. <strong>Intervention:</strong> S. boulardii 5 × 10^9 CFU vs no probiotic. <strong>Sample size:</strong> 211 (probiotic 135, controls 136). <strong>Type of milk:</strong> EBM/formula. <strong>Type of delivery:</strong> CD 77.7% vs 83.0%. <strong>Primary outcome:</strong> NEC ≥ stage 2. <strong>LOS:</strong> 20/135 (14.9%) vs 21/138 (15.4%).</td>
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| 9. Dilli 2015 | Participants: VLBW infants with a gestation of <32 wk and birth wt <1500 g  
Duration of supplementation: From d 8 of life, once daily until discharge or a maximum of 8 wk  
Sample size: 200 (probiotic 100, placebo 100)  
Type of milk: EBM/formula  
Primary outcome: NEC (≥Stage 2)  
LOS: 8/100 (8%) vs 13/100 (13%) |
| 10. Dutta 2015 | Participants: Preterm infants 27–33 wk gestation  
Intervention: High dose (10 billion CFU: L. acidophilus, L. rhamnosus, B. longum, S. boulardii) vs low dose (1 billion CFU: L. acidophilus, L. rhamnosus, B. longum, S. boulardii) vs placebo (potato starch, maltodextrin)  
Duration of supplementation: High-dose long-course group + low-dose long-course group (21 d) vs high-dose short-course group (d1–d14 and d15–d21)  
Sample size: 149 (probiotic 114, placebo 35)  
Type of milk: EBM/formula; Type of delivery: Probiotic group vs placebo: Spontaneous VD (69% vs. 60%), CD: data NA  
Primary outcome: Stool colonization rates on d14, d21, and d28 with 3 different regimens of probiotic  
LOS: 10/114 (8.8%) vs 6/35 (17.1%) |
| 11. Fernandez-Carrocera 2013 | Participants: Preterm infants <1500 g  
Intervention: Multispecies probiotic product (L. acidophilus + L. rhamnosus + L. casei + L. plantarum + B. infantis + S. thermophilus) vs no probiotic  
Duration of supplementation: From the day of commencement of enteral feeds, once daily. Duration of supplementation: not clear  
Sample size: 150 (probiotics 75, controls 75)  
Type of milk: EBM/formula; Type of delivery: Data NA  
Primary outcome: ≥Stage 2 NEC  
LOS: 42/75 (56%) vs 44/75 (58.7%) |
| 12. Hays 2015 | Participants: Preterm infants <32 wk and <1500 g  
Intervention: Probiotic group (3 subgroups receiving B. lactis only vs B. longum only vs B. lactis + B. longum 10⁹ CFU of each strain) vs placebo (maltodextrin)  
Duration of supplementation: 4 wk if ≥29 wk and 6 wk if ≤28 wk gestation  
Sample size: 199  
Type of milk: EBM/PDHM/formula; Type of delivery: Probiotic group vs placebo group: CD (79.3% vs 75%)  
Primary outcome: Effect of probiotic supplementation on short-term postnatal growth and body composition  
LOS: 17/145 (11.7%) vs 19/52 (37%) |
| 13. Hikaru 2012 | Participants: Extremely low birth weight and VLBW infants  
Intervention: B. breve (0.5 x 10⁹ CFU twice daily) vs no supplementation  
Duration of supplementation: From the day of birth until discharge from the NICU  
Sample size: 208 (probiotics 108, controls 100)  
Type of milk: EBM/formula; Type of delivery. NA  
Primary outcome: CD 55.5% vs 64.5%  
LOS: 2/119 (1.7%) vs 8/138 (6.0%) |
| 14. Hua 2014 | Participants: Preterm <37 wk, admitted to NICU  
Intervention: Probiotic Jin Shuang Qi (L. acidophilus, S. thermophilus, Bifidobacterium) 5 x 10⁹ CFU/d vs no probiotic  
Duration of supplementation: From the day of commencement of enteral feeds, once daily. Duration of supplementation: not clear  
Sample size: 257 (probiotics 119, controls 138)  
Type of milk: EBM/formula; Type of delivery. CD 55.5% vs 64.5%  
Primary outcome: Incidence and severity of NEC  
LOS: 2/119 (1.7%) vs 8/138 (5.8%) |
| 15. Kitajima 1997 | Participants: VLBW infants  
Intervention: B. breve YIT4010 (0.5 x 10⁹ CFU) vs distilled water  
Duration of supplementation: From the day of birth until d 28  
Sample size = 97 randomized, 91 analyzed (1 (probiotics 45, controls 46)  
Type of milk: EBM/formula; Type of delivery: Data NA  
Primary outcome: Gut colonization with B. breve BBG  
LOS: 1/45 (2.2%) vs 0/48 (0%) |
| 16. Lin 2005 | Participants: VLBW infants  
Intervention: L. acidophilus and B. infantis (minimum of 1 004 356 and 1 015 697 organisms, respectively), twice daily  
Duration of supplementation: After d 7 of life, from the time of commencement of enteral feeds  
Sample size = (probiotics 180, controls 187)  
Type of milk: EBM/PDHM; Type of delivery: CD 57.8% vs 53.5%  
Primary outcome: Incidence and severity of NEC  
LOS: 22/180 (12.2%) vs 36/187 (19.3%) |
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| 17. Lin 2008 | Participants: VLBW preterm infants <34 wk gestation  
Intervention: *L. acidophilus* and *B. bifidum* (1 × 10⁹ CFU each, twice daily) vs no probiotic  
Duration of supplementation: From the time of commencement of enteral feeds, for 6 wk  
Sample size = 434 (probiotics 217, controls 217)  
Type of milk: EBM/formula; Type of delivery: CD 69.6% vs 63.3%  
Primary outcomes: Death or NEC ≥Stage 2  
LOS: 40/217 (19.8%) vs 24/217 (11.5%) |
| 18. Manzoni 2006 | Participants: Preterm VLBW infants  
Intervention: *L. rhamnosus* GG (6 × 10⁹ CFU once daily) vs no probiotic  
Duration of supplementation: From d 3 of life, for 6 wk or until discharge, if discharge occurred <6 wk  
Sample size: 80 (probiotics 39, controls 41)  
Type of milk: EBM/PDHM; Type of delivery: VD 30% vs 35%  
Primary outcome: Enteric fungal colonization  
LOS: 19 (47.9%) vs 22 (54.7%) |
| 19. Mihatsch 2010 | Participants: VLBW infants <30 wk gestation  
Intervention: *B. lactis* BB12 (2 × 10⁹ CFU/kg, 6 times a day) vs placebo (HMF)  
Duration of supplementation: From the time of commencement of enteral feeds, for 6 wk  
Sample size: 183 (probiotics 93, placebo: 90)  
Type of milk: EBM/formula; Type of delivery: VD 30% vs 31%  
Primary outcome: Incidence density of nosocomial infections, defined as periods of elevated C-reactive protein from 7 to 42 d after initiation of enteral feeding  
LOS: 28/91 (30.7%) vs 29/89 (32.6%) |
| 20. Millar 1993 | Participants: Preterm infants <33 wk  
Intervention: *Lactobacillus* GG 1 × 10⁸ CFU twice daily  
Duration of supplementation: From the time of commencement of enteral feeds until discharge  
Sample size: 20 (probiotics 10, control 10)  
Type of milk: EBM/preterm formula  
Primary outcome: Gut colonization  
LOS: 0/10 vs 0/10 |
| 21. Oncel and Sari 2014 | Participants: Preterm infants ≤32 wk and <1500 g  
Intervention: *L. reuteri* DSM 17938 in oil-based suspension, 1 × 10⁸ CFU/day vs placebo (oil-based suspension without probiotics)  
Duration of supplementation: From the time of first enteral feeds until discharge  
Sample size: 400 (probiotics 200, placebo 200)  
Type of milk: EBM/preterm formula; Type of delivery: CD 75% vs 76%  
Primary outcome: ≥Stage 2 NEC  
LOS: 25/200 (12.5%) vs 20/200 (10%) |
| 22. Partty 2013 | Participants: Preterm infants (32–36 wk)  
Intervention: *L. rhamnosus* GG 1 × 10⁹ CFU vs placebo (microcrystalline cellulose and dextrose anhydrate)  
Duration of supplementation: Once daily until d 30 and twice daily from d 31–60  
Sample size: 63 (probiotic 31, placebo 32)  
Type of milk: BM/formula; Type of delivery: VD 63% vs 81%  
Primary outcomes: Gut microbiota, fussing, and crying  
LOS: 0/31 vs 0/32 |
| 23. Patole 2014 | Participants: Preterm infants <33 wk  
Intervention: *B. breve* M16V, 3 × 10⁹/d vs placebo (Dextrin)  
Duration of supplementation: Ready to commence or on enteral feeds for <12 h  
Sample size: 159 (probiotics 79, placebo 80)  
Type of milk: EBM/PDHM; Type of delivery: CD 75% vs 65%  
Primary outcome: *B. breve* fecal counts  
LOS: 17/74 (23%) vs 12/66 (18%) |
| 24. PiPS 2014 | Participants: Preterm infants <31 wk  
Intervention: *B. breve* BBG-001 (2.1 to 5.3 × 10⁸ CFU) once daily vs placebo (freeze-dried cornstarch)  
Duration of supplementation: Commenced within 48 h of birth, until 36 wk PMA  
Sample size: 1310 (probiotics 650, placebo 650)  
Type of milk: NA; Type of delivery: NA  
Primary outcome: ≥Stage 2 NEC, LOS, death  
LOS: 73 (11.2%) vs 77 (11.7%) |
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<tr>
<td>25. ProPrems 2013</td>
<td>Participants: Preterm infants &lt;32 wk gestation and &lt;1500 g</td>
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<td></td>
<td>Intervention: <em>B. infantis</em> + <em>S. thermophilus</em> + <em>B. lactis</em> (1 × 10^9 organisms in total) vs placebo (maltodextrin)</td>
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<td>Duration of supplementation: until discharge from hospital or till term corrected age</td>
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<td>Sample size: 1099 (probiotic 548, placebo 551)</td>
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<td></td>
<td>Type of milk: EBM/formula; Type of delivery: CD 65.5% vs 68.4%</td>
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<td>Primary outcome: LOS</td>
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<td>LOS: 72 (13.1%) vs 89 (16.5%)</td>
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<td>26. Ren B 2010</td>
<td>Participants: Preterm infants (exact gestation unclear)</td>
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<td>Intervention: <em>Bacillus clausii</em> (1 × 10^7 CFU) and <em>Clostridium (butyricum)</em> San Chang Le Kang (1 × 10^6 CFU) (Shandong Kexing Biological Products) 250 mg twice daily vs no probiotics</td>
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<td>Duration of supplementation: Until discharge</td>
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<td>Sample size: 70 (probiotic 35, controls 35)</td>
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<td>Type of milk: NA; Type of delivery: NA</td>
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<td>Primary outcome: Intestinal bacterial colonization rate</td>
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<td>LOS: 2 (6%) vs 9 (26%)</td>
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<td>27. Rojas 2012</td>
<td>Participants: Preterm infants ≤ 2000 g</td>
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<td></td>
<td>Intervention: <em>L. reuteri</em> DSM 17938, 1 × 10^8 CFU, once daily vs placebo (oil-based suspension without probiotics)</td>
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<td>Duration of supplementation: Commenced within 48 h of life. Duration: NA</td>
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<td>Sample size: 750 (probiotics 372, placebo 378)</td>
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<td>Type of milk: EBM/formula; Type of delivery: VD noninstrumental 16% (probiotics) vs 17% (placebo), VD instrumental 0% (probiotics) vs 0.5% (placebo)</td>
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<td></td>
<td>Primary outcome: Nosocomial infection and mortality</td>
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<td></td>
<td>LOS: 24 (6.5%) vs 17 (4.5%)</td>
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<tr>
<td>28. Romeo 2011</td>
<td>Participants: Preterm infants &lt;37 wk and &lt;2500 g</td>
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<td>Intervention: <em>L. reuteri</em> (1 × 10^9 CFU) vs <em>L. rhamnosus</em> (6 × 10^9) vs no probiotics</td>
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<tr>
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<td>Duration of supplementation: From within 72 h of life until discharge or for 6 wk</td>
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<td>Sample size: 249 (<em>L. reuteri</em> 83, <em>L. rhamnosus</em> 83, controls 83)</td>
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<td>Type of milk: EBM/formula; Type of delivery: CD 94% (<em>L. reuteri</em> group) vs 86% (<em>L. rhamnosus</em> group) vs 93% (controls)</td>
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<td>Primary outcome: Enteric colonization by Candida; LOS, neurologic outcome at 12 mo corrected gestational age</td>
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<td>LOS: <em>L. reuteri</em> 1/83 (1.2%), <em>L. rhamnosus</em> 2/83 (2.4%), controls 9/83 (3.6%)</td>
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<td>29. Rougé 2009</td>
<td>Participants: Preterm infants &lt;32 wk and &lt;1500 g</td>
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<td>Intervention: <em>L. reuteri</em> DSM 17938, 1 × 10^9 CFU, once daily vs placebo (oil-based suspension without probiotics)</td>
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<td>Duration of supplementation: From commencement of enteral feeds until discharge</td>
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<td></td>
<td>Sample size: 94 (probiotics 45, placebo 49)</td>
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<td></td>
<td>Type of milk: EBM/formula; Type of delivery: CD 62.2% vs 71.4%</td>
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<td>Primary outcome: Percentage of infants receiving &gt;50% of feeds via enteral route at d 14 of life</td>
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<td>LOS: 15 (33.3%) vs 13 (26.5%)</td>
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<tr>
<td>30. Roy 2014</td>
<td>Participants: Preterm infants &lt;37 wk and birth wt &lt;2500 g</td>
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<td>Interventions: Half of the 1-g sachet that contained <em>L. acidophilus</em> 1.25 × 10^9 + <em>B. longum</em> 0.125 × 10^9 + <em>B. bifidum</em> 0.125 × 10^9 + <em>B. lactis</em> 1 × 10^9 vs sterile water</td>
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<td></td>
<td>Duration of supplementation: Commenced within 72 h of birth for 6 wk or until discharge</td>
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<td>Sample size: 112 (probiotics: 56, placebo: 56)</td>
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<td></td>
<td>Type of milk: EBM; Type of delivery: CD 83.9% vs 76.8%</td>
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<td>Primary outcome: Enteric fungal colonization</td>
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<td>LOS: 55.4% vs 70%</td>
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<td>31. Saengtawesin 2014</td>
<td>Participants: Preterm infants &lt;34 wk and birth wt ≤1500 g</td>
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<td>Intervention: Inforan (<em>L. acidophilus</em> and <em>B. bifidum</em>, 1 × 10^8 CFU each) 125 mg/kg/dose twice daily vs no probiotic</td>
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<td>Duration of supplementation: From commencement of feeds until 6 wk or discharge</td>
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<td>Sample size: 60 (probiotics 31, controls 29)</td>
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<td></td>
<td>Type of milk: EBM/preterm formula; Type of delivery: CS 67.7% vs 62%</td>
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<td>Primary outcome: NEC</td>
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<td>LOS: 2 (6.45%) vs 1 (3.44%)</td>
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<tr>
<td>32. Samanta 2009</td>
<td>Participants: Preterm (&lt;32 wk) and VLBW (&lt;1500 g) infants</td>
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<td></td>
<td>Interventions: Probiotic mixture (<em>B. infantis</em> + <em>B. bifidum</em> + <em>B. longum</em> + <em>L. acidophilus</em>, each 2.5 × 10^9 CFU), administered twice daily vs no probiotic</td>
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<td>Duration of supplementation: NA</td>
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<td>Sample size: 186 (probiotics 91, controls 95)</td>
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<td></td>
<td>Type of milk: EBM; Type of delivery: CD 46.15% vs 49.47%</td>
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<td>Primary outcomes: NEC, death due to NEC, feed tolerance</td>
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<td>LOS: 13 (14.3%) vs 28 (29.5%)</td>
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</table>
Subgroup analysis of infants born at <28 weeks' gestation or <1000 g revealed no significant benefits of probiotic supplementation in reducing LOS (Fig 4).

**DISCUSSION**

Our systematic review of 37 RCTs (N = 9416) showed that probiotic supplementation leads to a statistically significant decrease in the risk of LOS in preterm infants born at <37 weeks or <2500 g. To our knowledge, this is the largest meta-analysis of probiotic supplementation in preterm neonates (4078 more than the previous ones). It is also the largest meta-analysis of RCTs for any intervention in neonatal medicine so far.

Our results are in contrast to those of the latest meta-analyses (Alfaleh 2014 Cochrane review, 19 studies, N = 5338; Lau 2015, 17 studies, N = 5215) that did not find statistically significant benefit of probiotic supplementation in reducing LOS in preterm infants. The most likely reason for the difference between our meta-analysis and the previous ones is the sample size. The latest Cochrane Review found a “trend” toward reduction in LOS with probiotic supplementation (RR 0.91; 95% CI, 0.80–1.03), but probably the sample size was inadequate to detect a small but significant beneficial effect. Our systematic review has 4078 more preterm infants than the previous ones.

Our results are also in contrast to the recently concluded 2 large multicenter trials (ProPrems, N = 1099; PIPS, N = 1310). In the ProPrems trial, there was a significant decrease in LOS in infants born at ≥28 weeks’ gestation (probiotics, 5.5%; placebo, 10.8%; P = .01); however, in the overall group born at <32 weeks’ gestation, there was no such benefit (probiotics, 13.1%; placebo, 16.2%; RR 0.81; 95% CI, 0.61–1.08; P = .16). The probable reason for nonsignificant results

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Study Characteristics</th>
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</table>
| 33. Sari 2011 | Participants: Preterm infants <33 wk or birth wt <1500 g  
Intervention: L. sporogenes, 0.35 × 10⁹ CFU, once a day vs no probiotic  
Duration of supplementation: From first enteral feeds until discharge  
Sample size: 221 (probiotics 110, controls 111)  
Type of milk: EBM/formula  
Type of delivery: CD 67.3% vs 75.7%  
Primary outcomes: NEC ≥Stage II  
LOS: 29 (26.4%) vs 26 (23.4%) |
| 34. Serce 2013 | Participants: Preterm infants <32 wk and <1500 g  
Intervention: S. boulardii 0.5 × 10⁹ CFU twice daily vs placebo (distilled water)  
Duration of supplementation: From the first enteral feed until discharge  
Sample size: 208 (probiotic 104, placebo 104)  
Type of milk: EBM/formula  
Type of delivery: CD 80.8% vs 88.5%  
Primary outcomes: Stage ≥2 NEC  
LOS: 19 (18.3%) vs 25 (24.3%) |
| 35. Stratiki 2007 | Participants: Preterm infants of 27–37 wk gestation  
Intervention: Preterm formula supplemented with B. lactis (2 × 10⁹/g formula) vs preterm formula  
Duration of supplementation: Until discharge  
Sample size: 75 (study 41, controls 34)  
Type of milk: Preterm formula  
Type of delivery: CD 36.5% vs 35%  
Primary outcomes: Intestinal permeability  
LOS: 0 vs 3 (8.8%) |
| 36. Tewari 2015 | Participants: Preterm infants <34 wk (2 groups: extremely preterm, 27–30 + 6 wk, and very preterm, 31–33 + 6 wk)  
Intervention: Bacillus clausii (2.4 × 10⁹ spores/d) vs placebo  
Duration of supplementation: Commenced d5 in asymptomatic and d10 in symptomatic neonates and continued for 6 wk, discharge, death, or occurrence of LOS, whichever was earlier  
Sample size: 244 (study: extremely preterm 61, very preterm 62) vs (placebo: 121)  
Type of milk: EBM/PDHM  
Type of delivery: CD: extremely preterm, 86% vs 59% and very preterm, 58% vs 80%  
Primary outcome: Incidence of definite and probable LOS in neonates <34 wk  
LOS: extremely preterm 6/61 (9.8%) vs 8/59 (13.6%) and very preterm 2/62 (3%) vs 3/62 (4.8%) |
| 37. Totsu 2014 | Participants: VLBW infants (<1500 g)  
Intervention: B. bifidum 1.25 × 10⁹ CFU twice daily vs placebo (dextrin)  
Duration of supplementation: Within 48 h of birth and continued until discharge  
Sample size: 283 (probiotic 153, placebo 130)  
Type of milk: EBM/formula  
Type of delivery: CD 50.5% vs 79.2%  
Primary outcome: Postnatal day when enteral feed exceeding 100 mL/kg/d  
LOS: 6/153 (3.9%) vs 13/130 (10%) |

For all outcomes, results in the probiotic group are given first. B, Bifidobacterium; CD, cesarean delivery; EBM, expressed breast milk; GG, Gorbach and Goldin; HMF, human milk fortifier; KP, killed probiotic; L, Lactobacillus; LB, live probiotic; NA, not available; PDHM, pasteurized donor human milk; PMA, postmenstrual age; S, Saccharomyces; VD, vaginal delivery; VLBW, very low birth weight.
is the small sample size, because to detect a statistically significant benefit for an RR reduction of 20%, a sample size of ~4500 (2250 in each arm) would be needed. The PiPS trial (of infants born at <31 weeks) also found no significant reduction in LOS in the probiotic group compared with the placebo group (11.2% vs 11.7%; adjusted RR 0.97; 95% CI, 0.73–1.29). The ProPrems trial used a multistrain probiotic supplement at a dosage of 1.0 × 10⁹ colony-forming units (1 billion CFUs), whereas the PiPS trial used a single-strain probiotic at a dosage of 2.1 to 5.3 × 10⁸ CFUs (0.2–0.53 billion CFUs) daily.

In our review, it was reassuring to note that for the main analysis of LOS in preterm infants, the benefits continued to remain significant even when REM was used (FEM \(P = .0007\); REM \(P = .007\)). However, for many of the sensitivity analyses, statistical significance was lost when REM was used (Table 3). There is ongoing debate about the pros and cons of FEM and REM. In a detailed analysis of the Cochrane Reviews in perinatal medicine, Villar et al found that the REM estimates showed wider CIs, particularly in those meta-analyses showing heterogeneity in the trial results. Schmidt et al compared the results of 68 meta-analyses in psychological medicine using REM and FEM. They reported that the published FE CIs around mean effect sizes were on average 52% narrower than their actual width, compared with the REM methods. They concluded that because most meta-analyses in the literature use FEM, the precision of findings in the literature has often been substantially overstated, with important consequences for research and practice. The Cochrane Neonatal Review Group recommends

<table>
<thead>
<tr>
<th>Study</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Blinding of Participants and Personnel</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Reporting</th>
<th>Other Bias</th>
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the use of FEM (http://neonatal.cochrane.org/resources-review-authors, accessed August 10, 2015). Considering these issues, it is prudent to check the results with both FEM and REM to increase their reliability.

We conducted sensitivity analysis after excluding RCTs with high ROB because such studies are known to overestimate the effect size (by up to 30%), which can lead to spuriously optimistic results. It was reassuring to note that the results were significant with both FEM and REM even after we excluded studies that had high ROB on random sequence generation and allocation concealment separately.

Subgroup analysis of extremely preterm infants (born at <28 weeks' gestation or <1000 g) revealed no significant benefits of probiotic supplementation in reducing LOS, but the sample size was small. On the other hand, sensitivity analysis of 24 studies (n = 7175) where the inclusion criteria were more mature preterm infants (born at <32 weeks or <1500 g) found probiotic supplementation to be beneficial in reducing LOS (FEM RR 0.88; 95% CI, 0.80–0.98; P = .02; REM RR 0.89; 95% CI, 0.79–1.00; P = .06). Unlike the NICUs of the developed world, where the focus of attention is extremely preterm infants (born at <28 weeks or <1000 g), the majority of NICUs around the world cater to the needs of more mature infants (born at <32 weeks or <1500 g). Therefore, the positive results of probiotic supplementation for more mature infants could have global implications.

The main strength of our systematic review is the large sample size and its exclusive focus on LOS (unlike the previous meta-analyses where the main attention was on NEC).

The limitations of our systematic review include the fact that LOS was a secondary outcome of interest in majority of the studies, we lacked information from 14 RCTs, and minimal information was available on extremely preterm or extremely low birth weight infants. Another limitation was the fact that we could not objectively assess the effect of variables such as dosage and duration of supplementation on LOS in this review. These highly important questions are best addressed by head-to-head comparisons of...
different doses or durations in future RCTs.

Now that our meta-analysis has shown that probiotic supplementation results in statistically significant benefits in reducing LOS, it is up to the individual units and clinicians to decide whether a 14% RR reduction or an absolute risk reduction of 2.4% is enough to warrant routine supplementation. If the evidence is considered sufficient, this intervention can be adopted after the safety and quality of the probiotic product are ensured.99,100 If clinicians and researchers are not convinced that the evidence is strong enough, the other option is to conduct a multicenter RCT. If one were to do a megatrial, to detect a statistically significant difference of ~14% RR reduction in the incidence of LOS (from 16.3% to 13.9%), with a power of 80% and an α error of 0.05, a sample size of ~7152 preterm infants born at <37 weeks (3576 in each group) would be needed. To our knowledge, trials involving such large sample size have not been conducted in neonatal medicine so far. For the extremely preterm infants, the incidence of LOS is higher, and therefore the necessary sample size will be lower (to show a reduction in the incidence from 21% in the placebo to 17% in the probiotic group [20% RR reduction], with a power of 80% and an α error of 0.05, the total sample size needed is ~3000). Because the number of extremely preterm infants is also low, such an RCT will also need multicenter coordination.

### TABLE 3 Results of the Sensitivity Analyses

<table>
<thead>
<tr>
<th>Item</th>
<th>Number of Studies</th>
<th>Sample Size</th>
<th>RR (95% CI) FEM</th>
<th>RR (95% CI) REM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studies with low ROB on random sequence generation</td>
<td>28</td>
<td>7820</td>
<td>0.87 (0.79–0.96); P = .005</td>
<td>0.86 (0.76–0.96); P = .02</td>
</tr>
<tr>
<td>Studies with low ROB on allocation concealment</td>
<td>24</td>
<td>7576</td>
<td>0.89 (0.80–0.98); P = .02</td>
<td>0.87 (0.78–0.98); P = .02</td>
</tr>
<tr>
<td>Studies that included gestational age &lt;32 wk or birth wt &lt;1500 g</td>
<td>24</td>
<td>7175</td>
<td>0.88 (0.80–0.98); P = .02</td>
<td>0.89 (0.79–1.00); P = .06</td>
</tr>
<tr>
<td>Studies where <em>Bifidobacterium</em> was part of the supplementation</td>
<td>22</td>
<td>6069</td>
<td>0.87 (0.78–0.96); P = .007</td>
<td>0.86 (0.75–1.00); P = .004</td>
</tr>
<tr>
<td>Studies where <em>Bifidobacterium</em> was not part of the supplementation</td>
<td>15</td>
<td>3347</td>
<td>0.82 (0.69–0.99); P = .04</td>
<td>0.80 (0.64–1.02); P = .07</td>
</tr>
<tr>
<td>Studies where <em>Lactobacillus</em> was part of the supplementation</td>
<td>21</td>
<td>4608</td>
<td>0.86 (0.76–0.97); P = .01</td>
<td>0.84 (0.70–1.00); P = .05</td>
</tr>
<tr>
<td>Studies where <em>Lactobacillus</em> was not part of the supplementation</td>
<td>16</td>
<td>4808</td>
<td>0.85 (0.74–0.97); P = .02</td>
<td>0.86 (0.73–1.01); P = .07</td>
</tr>
<tr>
<td>Multiple-strain supplementation</td>
<td>14</td>
<td>3455</td>
<td>0.86 (0.76–0.97); P = .02</td>
<td>0.86 (0.71–1.04); P = .12</td>
</tr>
<tr>
<td>Single-strain supplementation</td>
<td>23</td>
<td>5561</td>
<td>0.85 (0.74–0.97); P = .02</td>
<td>0.84 (0.71–0.98); P = .03</td>
</tr>
<tr>
<td>Studies where LOS was the primary outcome</td>
<td>9</td>
<td>4677</td>
<td>0.85 (0.74–0.99); P = .04</td>
<td>0.81 (0.63–1.03); P = .08</td>
</tr>
</tbody>
</table>

### TABLE 4 Summary of Findings According to GRADE Guidelines43

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Absolute Risk</th>
<th>Relative Effect, RR (95% CI)</th>
<th>Number of Participants</th>
<th>Quality of Evidence GRADE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOS</td>
<td>744/4564 (16.3%)</td>
<td>875/4852 (15.9%)</td>
<td>0.86 (0.78–0.94); P = .0007</td>
<td>High</td>
</tr>
</tbody>
</table>

The evidence was deemed high in view of the large sample size, low risk of bias in majority of the included studies, narrow CIs around the effect size estimate, very low P value for effect size estimate, and mild statistical heterogeneity.

FIGURE 4

Probiotic supplementation in infants born at <28 weeks or <1000 g. M-H, Mantel–Haenszel.
CONCLUSIONS
Given the serious consequences of LOS in preterm infants, we believe that a strategy that has been shown by this largest neonatal meta-analysis to date is worth consideration by health care policymakers, clinicians, and, most importantly, the parents of preterm infants. Another important factor that must be considered is the fact that probiotic supplementation has been shown to reduce the risk of NEC in preterm infants. If a simple intervention such as probiotic supplementation can reduce the risk of 2 of the most devastating conditions that affect preterm infants, it is worth paying attention.

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