Probiotics for Preventing Late-Onset Sepsis in Preterm Neonates

A PRISMA-Compliant Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Abstract: The effect of probiotics on late-onset sepsis (LOS) in preterm neonates remains controversial. The authors systematically reviewed the literature to investigate whether enteral probiotic supplementation reduced the risk of LOS in preterm neonates in neonatal intensive care units.

PubMed, Embase, and Cochrane Central Register of Controlled Trials were systematically searched for randomized controlled trials (RCTs) regarding the effect of probiotics in preterm neonates. The primary outcome was culture-proven bacterial and/or fungal sepsis. The Mantel–Haenszel method with random-effects model was used to calculate pooled relative risks (RRs) and 95% confidence intervals (CIs).

Twenty-seven trials were included in our review, and 25 trials involving 6104 preterm neonates were statistically analyzed. Pooled analysis indicated that enteral probiotic supplementation significantly reduced the risk of any sepsis (25 RCTs; RR 0.83, 95% CI 0.73–0.94; I² = 26%), bacterial sepsis (11 RCTs; RR 0.82, 95% CI 0.71–0.95; I² = 0%), and fungal sepsis (6 RCTs; RR 0.57, 95% CI 0.41–0.78; I² = 0%). This beneficial effect remains in very low birth weight infants (<1500 g) (19 RCTs; RR 0.86, 95% CI 0.75–0.97; I² = 18%), but not in extremely low birth weight infants (<1000 g) (3 RCTs; RR 0.73, 95% CI 0.45–1.19; I² = 53%). All the included trials reported no systemic infection caused by the supplemental probiotic organisms.

Current evidence indicates that probiotic supplementation is safe, and effective in reducing the risk of LOS in preterm neonates in neonatal intensive care units. Further studies are needed to address the optimal probiotic organism, dosing, timing, and duration. High-quality and adequately powered RCTs regarding the efficacy and safety of the use of probiotics in extremely low birth weight infants are still warranted. (Medicine 95(8):e2581)

Abbreviations: CI = confidence interval, ELBW = extremely low birth weight, Ig = immunoglobulin, LOS = late-onset sepsis, NEC = necrotizing enterocolitis, NICU = neonatal intensive care unit, RCT = randomized controlled trial, RR = relative risk.

INTRODUCTION

In neonatal intensive care units (NICUs), late-onset sepsis (LOS) arising >72 hours after birth is a frequent complication of prematurity, and is associated with increased medical costs, prolonged hospitalization, and significant mortality and morbidity.1–3 Despite the improvements in the quality of neonatal assistance, the reported incidences of LOS are still dramatically high.1,4 Preterm neonates are indeed highly prone to develop bacterial and fungal sepsis because of their immature skin/mucosal barrier and immune response, use of invasive procedures and devices, use of broad-spectrum antimicrobial drugs, and exposure to the hospital milieu, which gives rise to gastrointestinal colonization with pathogens.5–9 Probiotics, defined as live microorganisms, confer health benefits to the host when administered at adequate doses,10 and have been suggested to modify the enteric microflora, suppress the overgrowth and translocation of pathogens in the gut, and therefore prevent life-threatening infections.11–14 Although there is no controversy about probiotics reducing the risk of stage II to III necrotizing enterocolitis (NEC) in preterm neonates,15–17 the effect of probiotics on LOS remains a highly live issue. So far, studies reporting the effect of probiotics on LOS conveyed conflicting results. Furthermore, because of small sample sizes, these studies were not adequately powered to detect the effect of probiotics on LOS in preterm neonates.

Thus, to provide the latest and most convincing evidence, we systematically reviewed the current available literature to investigate whether enteric probiotic supplementation reduced the risk of LOS in preterm neonates in NICUs.

METHODS

This systematic review and meta-analysis was conducted and reported in adherence to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement,18 and the guidelines of the Cochrane Handbook for Systematic Reviews of Interventions.19 Because our study was a review of previous published studies, ethical approval or patient consent was not required.
Literature Search and Selection Criteria

PubMed, Embase, and Cochrane Central Register of Controlled Trials were searched for records that compared enteral probiotics to placebo or no intervention in preterm neonates in NICUs. The language was restricted to English. The search strategy is shown in Table 1. The last search was conducted on August 11, 2015. The cited references of retrieved articles and previous reviews were also manually checked to identify any additional eligible trials. All citations were imported into a bibliographic database (EndNote X7; Thomson Reuters), and 2 of the authors (G-QZ and H-JH) independently screened the candidate articles to check their eligibility for inclusion.

We developed a PICOS (Patient, Intervention, Comparators, Outcome, and Study design) approach as the eligibility criteria: 1) Population: preterm infants <37 weeks or birth weight <2500 g, or both; 2) Intervention: any species/strains/doses regimen of live probiotics administered for >7 days; 3) Comparators: placebo or no probiotics; 4) Outcome: the primary outcome was any sepsis occurring >72 hours after birth, defined as positive blood/urine/cerebrospinal fluid cultures. The secondary outcome was systemic infection caused by supplemented probiotic organisms; 5) Study design: only randomized controlled trials (RCTs) were eligible. We excluded interventions other than live probiotics, administration of probiotics with prebiotics or other agents, and

### TABLE 1. Search Strategy

| Search terms |
|--------------|
| 1. Probiotic, or probiotics, or yogurt, or yoghurt, or lactic acid bacteria, or *acidophilus*, or *Lactobacillus*, or *Lactococcus*, or *Saccharomyces*, or *Streptococcus*, or *Bifidobacterium*, or *Enterococcus*, or *Escherichia coli* |
| 2. Very low birth weight, or VLBW, or low birth weight, or LBW, or extremely low birth weight, or ELBW, or preterm, or premature |
| 3. Clinical trial |
| 4. English |
| 5. 1, 2, 3, and 4 |

FIGURE 1. Selection process for the studies included in the meta-analysis.
TABLE 2. Characteristics of Randomized Controlled Trials Included in Our Meta-Analysis

| Source | N  | Participants   | Strains, Doses, and Duration                                                                 | Type of Milk | Outcomes of Interest                                                                 |
|--------|----|----------------|-----------------------------------------------------------------------------------------------|--------------|--------------------------------------------------------------------------------------|
| Al-Hosni\(^\text{27}\) | 101 | BW 501–1000 g  | A mixture of *L. rhamnosus* GG and *B. infantis*, 1 × 10\(^8\) CFU/d, from first enteral feed to corrected age | FM           | Bacterial and/or fungal sepsis (blood culture proven)                                 |
| Bin-Nun\(^\text{28}\) | 145 | BW ≤ 1500 g    | A mixture of *B. bifidus*, *B. infantis*, and *Streptococcus thermophilus*, 1.05 × 10\(^7\) CFU/d, from first feed to 36 wk corrected age | HM or FM     | Any sepsis (blood culture proven)                                                     |
| Braga\(^\text{29}\) | 243 | BW 750–1499 g  | A mixture of *L. casei* and *B. breve*, 3.5 × 10\(^7\) to 3.5 × 10\(^9\) CFU/d, from second day to 30 d of life or discharge | HM           | Any sepsis (NG)                                                                      |
| Costalos\(^\text{30}\) | 87  | GA 28–32 wk    | *S. boulardii*, 2 × 10\(^9\) CFU/d, from first week for 30 d                                 | FM           | Any sepsis (blood culture proven)                                                     |
| Dani\(^\text{31}\) | 585 | GA < 33 wk or BW < 1500 g | *L. rhamnosus* GG, 6 × 10\(^9\) CFU/d, from first feed to discharge | HM or FM     | Bacterial sepsis (blood/urine culture proven)                                          |
| Demirel\(^\text{32}\) | 278 | GA ≤ 32 wk and BW ≤ 1500 g | *S. boulardii*, 5 × 10\(^9\) CFU/d, from first feed to discharge | HM or FM     | Bacterial sepsis (blood/CSF/urine culture proven)                                      |
| Dilli\(^\text{33}\) | 200 | GA < 32 wk and BW < 1500 g | *B. lactis*, 5 × 10\(^9\) CFU/d, for a maximum of 8 wk or to discharge | HM or FM     | Bacterial sepsis (culture proven)                                                     |
| Fernandez-Carrocer\(^\text{a}\) | 150 | BW < 1500 g    | A mixture of *L. acidophilus*, *L. rhamnosus*, *L. casei*, *L. plantarum*, *B. infantis*, and *Streptococcus thermophilus*, 2.6 × 10\(^9\) CFU/d, from first feed to discharge | HM or FM     | Bacterial sepsis (blood culture proven)                                                |
| Jacobs\(^\text{35}\) | 1099 | GA < 32 wk and BW < 1500 g | A mixture of *B. infantis*, *B. lactis*, and *Streptococcus thermophilus*, 1 × 10\(^9\) CFU/d, to discharge or term corrected age | HM or FM     | Any sepsis (blood/urine/CSF/organ tissue culture proven)                               |
| Kitajima\(^\text{36}\) | 97  | BW < 1500 g    | *B. breve*, 0.5 × 10\(^9\) CFU/d, from first 24 h for 28 d                                  | HM or FM     | Any sepsis (blood culture proven)                                                     |
| Lin\(^\text{37}\) | 367 | BW < 1500 g    | A mixture of *L. acidophilus* and *B. infantis*, 2 × 10\(^9\) CFU/d, from first enteral feed to discharge | HM           | Any sepsis (blood culture proven)                                                     |
| Lin\(^\text{38,9}\) | 434 | GA < 34 wk and BW < 1500 g | A mixture of *L. acidophilus* and *B. bifidum*, 2 × 10\(^9\) CFU/d, for 6 wk or to discharge | HM or FM     | Bacterial sepsis (blood culture proven)                                                |
| Manzoni\(^\text{39}\) | 80  | BW < 1500 g    | *L. rhamnosus* GG, 6 × 10\(^9\) CFU/d, from third day for 6 wk or to discharge               | HM           | Bacterial sepsis and/or IFI (blood culture proven)                                    |
| Mihatsch\(^\text{40}\) | 183 | GA < 30 wk and BW < 1500 g | *B. lactis*, 2 × 10\(^10\) CFU/d, from first milk feed for first 6 wk of life | HM or FM     | Bacterial sepsis (blood culture proven)                                                |
| Millar\(^\text{41}\) | 20  | GA ≤ 33 wk     | *L. rhamnosus* GG, 2 × 10\(^9\) CFU/d, from initiation of milk feeds for 14 d               | HM or FM     | Any sepsis (blood culture proven)                                                     |
| Oncel\(^\text{42}\) | 424 | GA ≤ 32 wk and BW ≤ 1500 g | *L. reuteri*, 1 × 10\(^9\) CFU/d, from first feed to discharge | HM or FM     | Bacterial and/or fungal sepsis (blood culture proven)                                  |
| Patole\(^\text{43}\) | 159 | GA < 33 wk and BW < 1500 g | *B. breve*, 3 × 10\(^9\) CFU/d, from first enteral feed to corrected age 37 wk          | HM or FM     | Any sepsis (blood culture proven)                                                     |
| Rojas\(^\text{44}\) | 750 | BW ≤ 2000 g    | *L. reuteri*, 1 × 10\(^9\) CFU/d, from first 48 hours of life to discharge               | HM or FM     | Any sepsis (blood/CSF/urine culture proven)                                           |
| Romeo\(^\text{45}\) | 249 | GA < 37 wk and BW < 2500 g | *L. reuteri*, 1 × 10\(^9\) CFU/d, or *L. rhamnosus*, 6 × 10\(^9\) CFU/d, from first 48 h for 6 wk or to discharge | HM or FM     | Bacterial and/or fungal sepsis (blood/urine/CSF culture proven)                       |
| Rouge\(^\text{46}\) | 94  | GA < 32 wk and BW < 1500 g | A mixture of *L. rhamnosus* GG and *R. longum*, 8 × 10\(^9\) CFU/d, from first enteral feed to discharge | HM or FM     | Any sepsis (blood culture proven)                                                     |

(Continued on next page)
TABLE 2. Continued

| Source          | N   | Participants | Strains, Doses, and Duration | Type of Milk | Outcomes of Interest                  |
|-----------------|-----|--------------|-----------------------------|--------------|---------------------------------------|
| Roy             | 112 | GA < 37 wk and BW < 2500 g | A mixture of B. longum, B. lactis, B. bifidum, and L. acidophilus, 1.5–3 × 10⁹ CFU/d, from first 72 h for 6 wk or to discharge | HM | Bacterial and/or fungal sepsis (blood/urine/CSF culture proven) |
| Saengtawesin    | 60  | GA < 34 wk and BW < 1500 g | A mixture of L. acidophilus and B. bifidum, 2 × 10⁹ CFU/d, from first feed for 6 wk or to discharge | HM or FM | Any sepsis (NG) |
| Samanta         | 186 | GA < 32 wk and BW < 1500 g | A mixture of B. infantis, B. bifidum, B. longum, and L. acidophilus, 2.5 × 10⁹ CFU/d, from first enteral feed till discharge | HM | Any sepsis (blood/CSF culture proven) |
| Sart            | 242 | GA < 33 wk or BW < 1500 g | L. sporogenes, 3.5 × 10⁹ CFU/d, from first feed to discharge | HM or FM | Bacterial and/or fungal sepsis (blood culture proven) |
| Serce           | 208 | GA < 32 wk and BW < 1500 g | S. boullardii, 2 × 10⁹ CFU/d, from first feed to discharge | HM or FM | Bacterial sepsis (blood culture proven) |
| Stratiki        | 77  | GA 27–37 wk   | B. lactis, 2 × 10⁹ CFU/d, from first 48 h to 30 d | FM | Any sepsis (blood culture proven) |
| Umezaki         | 208 | BW < 1500 g   | B. breve, 1 × 10⁹ CFU/d, from first several hours after birth to discharge | HM or FM | Any sepsis and/or fungal sepsis (blood culture proven) |

R = Bifidobacterium, BW = birth weight, CSF = cerebrospinal fluid, FM = formula milk, GA = gestational age, HM = human milk (mother’s milk and/or donor milk), IFI = invasive fungal infection, L = Lactobacillus, NG = not given, S = Saccharomyces.

* This study had methodological misstep that caused uneven distribution of the time of umbilical venous catheter between groups, 7 days in probiotic group and 3 days in control group.

RESULTS

The selection process is detailed in Figure 1. A total of 601 potentially relevant records were identified by our search strategy. Seventy-four records were excluded for duplicates and an additional 497 records were excluded based on the titles and abstracts. The remaining 30 full-text articles were assessed for eligibility, 3 of which were further excluded because incidences of LOS were not reported. Finally, 27 trials were eligible for this review. Two trials were not included in meta-analysis because of the uneven distribution of birth to discharge.
weight\textsuperscript{38} and duration of umbilical venous catheter\textsuperscript{49} between study and control groups. Hence, 25 trials were statistically analyzed\textsuperscript{27–37,39–48,50–53}. Characteristics of the 27 trials are summarized in Table 2 and the outcome data of each included study are presented in Table 3. The quality of the trials assessed by the Cochrane Risk-of-Bias Tool is summarized in Table 4.

Figure 2 shows the results from each trial and overall, using a random-effects model, for probiotics in the prevention of LOS in preterm neonates. Of the 25 estimates, 20 were <1.0. The summary of RR of LOS was 0.83 (95% CI 0.73–0.94). Results of the studies were homogeneous ($I^2 = 26\%$). Furthermore, including the 2 trials with uneven distribution of sepsis-related risk factors between intervention and control groups, the RR was consistent with the main analysis (RR 0.86, 95% CI 0.76–0.98, $I^2 = 37\%$). There was no evidence of significant publication bias by inspection of the funnel plot and formal statistical tests (Egger’s test, $P = 0.269$; Begg’s test, $P = 0.264$; Figure 3). None of the included trials reported any systemic infection caused by the supplemented probiotic organisms.

**DISCUSSION**

The results of our meta-analysis indicated that administration of prophylactic probiotics could significantly reduce the incidence of LOS in preterm neonates in NICUs. Low heterogeneity, influence analysis, lack of publication bias, and the consistency of results in most subgroups added robustness to our main findings. Our study also provided robust safety data of probiotics utilization in preterm neonates.

**Comparison with Previous Studies**

Differences between the current meta-analysis and 2 recent meta-analyses should be noted. A meta-analysis by Bernardo...
et al\textsuperscript{16} in 2013 evaluated the effect of probiotics on sepsis in preterm neonates (gestational age <34 weeks or birth weight <1500 g). The authors included 12 RCTs involving 2907 subjects and concluded that enteral administration of probiotics reduced the incidence of sepsis in preterm neonates, although with no significant difference between groups (RD = -0.03, 95\% CI = -0.05 to -0.00, $I^2 = 47\%$). In another meta-analysis in 2014\textsuperscript{17} focusing on preterm neonates (gestational age <37 weeks or birth weight <2500 g), AlFaleh et al included 19 RCTs involving 5338 subjects and concluded that there was no evidence of probiotic supplementation reducing the risk of nosocomial sepsis (RR 0.91, 95\% CI 0.80–1.03, $I^2 = 37\%$). Several limitations, however, should be noted in the 2 meta-analyses. First, not all trials that met their specific eligibility criteria were included, for example, 6 trials\textsuperscript{27,30,36,45,51,52} for Bernardo et al and 3 trials\textsuperscript{28,45,52} for AlFaleh et al, which could potentially lead to publication bias. Second, 1 RCT\textsuperscript{34} should not be included because of ineligible intervention (probiotics administered with bovine lactoferrin). Third, these pooled results were based on an improper model of fixed effects model because of significant clinical/statistical heterogeneity. Overall, both previous meta-analyses had obvious flaws that might threaten the authenticity of their findings. After the 2 meta-analyses, several studies investigating the effect of probiotics in preterm neonates were published. Our updated meta-analysis included 25 RCTs with a total of 6104 subjects. In contrast with the previous meta-analyses, the current 1 suggested that enteral probiotic supplementation significantly reduced the incidence of LOS in preterm neonates in NICUs. Moreover, low heterogeneity, influence analysis, lack of publication bias, and the consistency of results in most subgroups added robustness to our main findings.

Potential underlying mechanisms by which probiotics might prevent sepsis include competitively colonizing the gut, competitive exclusion of potentially pathogenic luminal bacteria and fungi,\textsuperscript{55} enhanced mucosal immunoglobulin (Ig) A responses,\textsuperscript{56} modulation of the gut barrier function and permeability,\textsuperscript{57} production of antimicrobial peptides,\textsuperscript{58} and upregulation of immune responses.\textsuperscript{59} We, however, saw a lack of effect of probiotics in extremely low birth weight infants (ELBW; <1000 g). One probable reason was that our study was not adequately powered to detect its beneficial effect, because only 3 studies involving 771 neonates were included in this subgroup analysis. But, we still cannot exclude the possibility that probiotics may have a lesser effect in ELBW infants, compared with neonates with a birth weight of <1500 g, because of even greater increase in the overall risk of infection.\textsuperscript{39} In summary, probiotics appear promising as a prevention strategy for LOS, but there are still insufficient data about the efficacy and safety of the use of probiotics in ELBW infants. Hence, high-quality and adequately powered RCTs in ELBW infants are warranted.

### TABLE 4. Risk-of-Bias Assessment of the Included Randomized Controlled Trials

| Study                      | Adequate Sequence Generation? | Allocation Concealment? | Blinding of Participants and Personnel | Blinding of Outcome Assessment | Incomplete Outcome Data? | Selective Reporting? | Other Bias? | Overall Risk of Bias |
|----------------------------|--------------------------------|-------------------------|----------------------------------------|-------------------------------|--------------------------|----------------------|-------------|---------------------|
| Al-Hosni\textsuperscript{27} | Unclear                        | Unclear                 | Yes                                    | Yes                           | No                       | No                   | No          | Unclear             |
| Bin-Nun\textsuperscript{28}  | Unclear                        | Unclear                 | Yes                                    | Yes                           | Unclear                  | No                   | No          | Unclear             |
| Braga\textsuperscript{29}    | Yes                            | Yes                     | Yes                                    | Yes                           | Yes                      | No                   | No          | Low                 |
| Costalos\textsuperscript{30} | Yes                            | Yes                     | Yes                                    | Yes                           | No                       | No                   | No          | Low                 |
| Dani\textsuperscript{31,39}  | Unclear                        | Unclear                 | Yes                                    | Yes                           | Yes                      | No                   | No          | Unclear             |
| Demirel\textsuperscript{32}  | Yes                            | Yes                     | Yes                                    | Yes                           | Yes                      | No                   | No          | Low                 |
| Dilli\textsuperscript{33}     | Yes                            | Yes                     | Yes                                    | Yes                           | No                       | No                   | No          | Unclear             |
| Fernandez-Carroccera\textsuperscript{34} | Yes                 | Yes                     | Yes                                    | Yes                           | No                       | No                   | No          | Low                 |
| Jacobs\textsuperscript{35}    | Yes                            | Yes                     | Yes                                    | Yes                           | Yes                      | No                   | No          | Low                 |
| Kitajima\textsuperscript{36}  | Unclear                        | Unclear                 | Unclear                                | Yes                           | Yes                      | No                   | No          | High                |
| Lin\textsuperscript{37}       | Yes                            | Yes                     | Yes                                    | Yes                           | Yes                      | No                   | No          | Low                 |
| Lin\textsuperscript{38}       | Yes                            | Yes                     | Yes                                    | Yes                           | Yes                      | No                   | No          | Low                 |
| Manzoni\textsuperscript{39}   | Yes                            | Unclear                 | Unclear                                | Unclear                       | No                       | No                   | No          | Unclear             |
| Mihatsch\textsuperscript{40}  | Yes                            | Yes                     | Yes                                    | Yes                           | No                       | No                   | No          | Low                 |
| Millar\textsuperscript{41}    | Unclear                        | Unclear                 | Yes                                    | Unclear                       | No                       | Yes                  | Yes         | High                |
| Oncel\textsuperscript{42}     | Yes                            | Yes                     | Yes                                    | Yes                           | Yes                      | No                   | No          | Low                 |
| Patole\textsuperscript{43}    | Yes                            | Yes                     | Yes                                    | Yes                           | No                       | No                   | No          | Low                 |
| Rojas\textsuperscript{44}     | Yes                            | Yes                     | Yes                                    | Yes                           | Yes                      | No                   | No          | Low                 |
| Romeo\textsuperscript{45}     | Yes                            | Unclear                 | Unclear                                | Unclear                       | No                       | No                   | No          | Unclear             |
| Rouge\textsuperscript{46}     | Yes                            | Unclear                 | Yes                                    | Yes                           | No                       | No                   | No          | Low                 |
| Roy\textsuperscript{47}       | Yes                            | Unclear                 | Yes                                    | Unclear                       | No                       | No                   | No          | Unclear             |
| Saengtawesin\textsuperscript{53} | Unclear                        | Unclear                 | Yes                                    | Unclear                       | No                       | Yes                  | No          | High                |
| Samanta\textsuperscript{48}   | Unclear                        | Unclear                 | Unclear                                | Unclear                       | No                       | Yes                  | Yes         | High                |
| Sar\textsuperscript{49}       | Yes                            | Unclear                 | Unclear                                | Unclear                       | Yes                      | No                   | No          | Unclear             |
| Sere\textsuperscript{50}      | Yes                            | Unclear                 | Unclear                                | Unclear                       | No                       | Yes                  | Yes         | High                |
| Stratik\textsuperscript{51}   | Unclear                        | Unclear                 | Yes                                    | Yes                           | No                       | Yes                  | Yes         | High                |
| Umezaki\textsuperscript{52}   | Unclear                        | Unclear                 | Yes                                    | Unclear                       | No                       | No                   | No          | Unclear             |

*Risk of bias was assessed with use of the Cochrane risk-of-bias tool.*
## FIGURE 2. Effect of probiotics on late-onset sepsis in preterm neonates.

| Study or Subgroup | Events | Probiotics Events | Control Events | Risk Ratio | Risk Ratio |
|-------------------|--------|-------------------|----------------|------------|------------|
|                   | Total  | Total             | Total          | M-H       | Random     |
|                   |        |                   |                | 95% CI     | 95% CI     |
| 1.1.1 Any sepsis  |        |                   |                |            |            |
| Al-Hosni 2012     | 13     | 50                | 16             | 3.3%       | 0.83 [0.45, 1.54] |
| Bin-Nun 2005      | 31     | 72                | 24             | 5.9%       | 0.82 [0.86, 2.00] |
| Braga 2011        | 40     | 119               | 42             | 7.6%       | 0.90 [0.63, 1.27] |
| Costalos 2003     | 3      | 51                | 3              | 0.6%       | 0.71 [0.15, 3.30] |
| Dani 2002         | 24     | 295               | 27             | 4.3%       | 0.87 [0.52, 1.48] |
| Demiril 2013      | 20     | 135               | 21             | 3.9%       | 0.96 [0.55, 1.69] |
| Dilli 2015        | 8      | 100               | 13             | 2.0%       | 0.62 [0.27, 1.42] |
| Fernández-Carrocer 2013 | 42  | 75                | 44             | 9.7%       | 0.95 [0.72, 1.26] |
| Jacobs 2013       | 72     | 546               | 89             | 9.3%       | 0.81 [0.61, 1.08] |
| Ktajima 1997      | 1      | 45                | 0              | 0.2%       | 3.07 [0.13, 73.32] |
| Lin 2005          | 22     | 180               | 36             | 4.6%       | 0.63 [0.39, 1.04] |
| Manzoni 2006      | 19     | 39                | 22             | 5.8%       | 0.91 [0.59, 1.40] |
| Mihatsch 2010     | 28     | 91                | 29             | 5.8%       | 0.94 [0.61, 1.45] |
| Millar 1993       | 0      | 10                | 0              | Not estimable |            |
| Oncel 2013        | 13     | 200               | 25             | 3.2%       | 0.52 [0.27, 0.99] |
| Pailole 2012      | 17     | 77                | 12             | 12.9%      | 1.40 [0.72, 2.73] |
| Roes 2012         | 34     | 372               | 40             | 5.7%       | 0.86 [0.56, 1.33] |
| Romeo 2011        | 3      | 166               | 9              | 0.9%       | 0.17 [0.05, 0.60] |
| Rouge 2009        | 15     | 45                | 13             | 3.3%       | 1.26 [0.67, 2.34] |
| Roy 2014          | 31     | 56                | 42             | 9.6%       | 0.74 [0.56, 0.98] |
| Saengtawesin 2004 | 2      | 31                | 1              | 29%        | 1.87 [0.18, 19.55] |
| Samanta 2008      | 13     | 91                | 28             | 9.5%       | 0.48 [0.27, 0.88] |
| Serce 2013        | 19     | 104               | 25             | 4.3%       | 0.76 [0.46, 1.29] |
| Strilzi 2007      | 0      | 41                | 3              | 0.2%       | 0.13 [0.01, 1.36] |
| Umezaki 2010      | 10     | 108               | 22             | 2.7%       | 0.42 [0.21, 0.84] |
| Subtotal (95% CI) | 3101   | 3003              | 100.0%         | 0.83 [0.73, 0.94] |

Total events: 480
Heterogeneity: Tau² = 0.02; Chi² = 30.92, df = 23 (P = 0.12); I² = 26%
Test for overall effect: Z = 2.99 (P = 0.003)

1.1.2 Any bacterial sepsis

| Study or Subgroup | Events | Probiotics Events | Control Events | Risk Ratio | Risk Ratio |
|-------------------|--------|-------------------|----------------|------------|------------|
|                   | Total  | Total             | Total          | M-H       | Random     |
|                   |        |                   |                | 95% CI     | 95% CI     |
| Al-Hosni 2012     | 11     | 50                | 16             | 5.1%       | 0.70 [0.36, 1.36] |
| Dani 2002         | 24     | 295               | 27             | 8.1%       | 0.87 [0.52, 1.48] |
| Demiril 2013      | 20     | 135               | 21             | 7.0%       | 0.96 [0.55, 1.69] |
| Dilli 2015        | 8      | 100               | 13             | 0.2%       | 0.62 [0.27, 1.42] |
| Fernández-Carrocer 2013 | 42  | 75                | 44             | 29.4%      | 0.95 [0.72, 1.26] |
| Manzoni 2006      | 15     | 39                | 17             | 4.1%       | 0.93 [0.54, 1.59] |
| Mihatsch 2010     | 28     | 91                | 29             | 12.2%      | 1.94 [0.61, 1.45] |
| Oncel 2013        | 12     | 200               | 22             | 4.9%       | 0.55 [0.28, 1.07] |
| Romeo 2011        | 1      | 166               | 5              | 0.5%       | 0.10 [0.01, 0.84] |
| Roy 2014          | 21     | 56                | 33             | 13.8%      | 0.64 [0.43, 0.95] |
| Serce 2013        | 19     | 104               | 25             | 8.0%       | 0.76 [0.45, 1.29] |
| Subtotal (95% CI) | 1311   | 1225              | 100.0%         | 0.82 [0.71, 0.95] |

Total events: 201
Heterogeneity: Tau² = 0.00; Chi² = 9.75, df = 10 (P = 0.46); I² = 0%
Test for overall effect: Z = 2.61 (P = 0.009)

1.1.3 Any fungal sepsis

| Study or Subgroup | Events | Probiotics Events | Control Events | Risk Ratio | Risk Ratio |
|-------------------|--------|-------------------|----------------|------------|------------|
|                   | Total  | Total             | Total          | M-H       | Random     |
|                   |        |                   |                | 95% CI     | 95% CI     |
| Al-Hosni 2012     | 2      | 50                | 0              | 1.1%       | 5.10 [0.25, 103.60] |
| Manzoni 2006      | 4      | 39                | 5              | 6.7%       | 0.84 [0.24, 2.90] |
| Oncel 2013        | 1      | 200               | 3              | 2.0%       | 0.33 [0.03, 3.18] |
| Romeo 2011        | 2      | 166               | 4              | 3.7%       | 0.25 [0.05, 1.34] |
| Roy 2014          | 23     | 56                | 42             | 85.4%      | 0.55 [0.39, 0.78] |
| Umezaki 2010      | 1      | 108               | 0              | 1.0%       | 2.78 [0.11, 67.46] |
| Subtotal (95% CI) | 619    | 531               | 100.0%         | 0.57 [0.41, 0.78] |

Total events: 33
Heterogeneity: Tau² = 0.00; Chi² = 4.64, df = 5 (P = 0.46); I² = 0%
Test for overall effect: Z = 3.47 (P = 0.0005)

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The reason why there was a lack of effect of probiotics on NEC in the 2 trials, which were excluded from our meta-analysis, should be discussed. Of note, there was uneven distribution of infection-related risk factors between study and control groups. This uneven baseline characteristics between groups could probably lead to overturn of the real effects. On the other hand, the pathogens causing NEC were most often related to cathereter-related infections in the 2 trials. It is tempting to speculate that probiotics alone are not capable of preventing the invasive procedures inducing infections, because the effects of orally administered probiotics are primarily in the gastrointestinal tract.

Because different probiotic organisms probably have distinct regulatory effects on the host, caution is needed in interpreting our results. Our study indicated that Lactobacillus species or a mixture of 2 or 3 species of probiotics may be more effective in reducing the risk of LOS. A meta-analysis conducted in 2015 also found that effective in reducing the risk of LOS. A meta-analysis conducted in 2015 also found that

| Subgroup | Number of Studies | RR (95% CI) | $I^2$ (%) |
|----------|------------------|-------------|-----------|
| Any sepsis | 25 | 0.83 (0.73, 0.94) | 26 |
| Any bacterial sepsis | 11 | 0.82 (0.71, 0.95) | 0 |
| Any fungal sepsis | 6 | 0.57 (0.41, 0.78) | 0 |
| Birth weight, g | | | |
| <2500 | 25 | 0.83 (0.73, 0.94) | 26 |
| <1500 | 19 | 0.86 (0.75, 0.97) | 18 |
| <1000 | 3 | 0.73 (0.45, 1.19) | 53 |
| Probiotic organism | | | |
| Lactobacillus species | 6 | 0.72 (0.50, 1.03) | 51 |
| Bifidobacterium species | 6 | 0.78 (0.48, 1.25) | 46 |
| Saccharomyces boulardii | 3 | 0.84 (0.58, 1.22) | 29 |
| Mixture | 10 | 0.85 (0.73, 1.00) | 29 |
| Probiotic dose* | | | |
| $\leq 1 \times 10^8$ | 10 | 0.73 (0.55, 0.98) | 38 |
| $>1 \times 10^8$ | 15 | 0.85 (0.73, 0.99) | 20 |
| Time of initiation | | | |
| $\leq 72$ h of age | 8 | 0.73 (0.56, 0.95) | 44 |
| At the time of first feed | 14 | 0.89 (0.75, 1.05) | 25 |
| When feeds were tolerated | 3 | 0.79 (0.60, 1.03) | 0 |
| Duration of intervention | | | |
| $<6$ wk | 5 | 0.88 (0.63, 1.22) | 0 |
| $\geq 6$ wk or to discharge | 17 | 0.79 (0.69, 0.90) | 23 |
| Type of milk | | | |
| HM | 5 | 0.76 (0.63, 0.91) | 9 |
| FM | 3 | 0.76 (0.43, 1.33) | 0 |
| HM or FM | 17 | 0.87 (0.73, 1.03) | 35 |
| Caesarean delivery rate | | | |
| $<\text{median (69%)}$ | 11 | 0.80 (0.69, 0.94) | 0 |
| $\geq \text{median}$ | 10 | 0.85 (0.69, 1.06) | 45 |
| Not reported | 4 | 0.73 (0.34, 1.57) | 65 |
| Risk of bias | | | |
| Low | 10 | 0.86 (0.75, 0.98) | 0 |
| Unclear or high | 15 | 0.78 (0.62, 0.98) | 43 |

TABLE 5. Subgroup Analyses for Probiotic Supplementation in the Prevention of Late-Onset Sepsis

All RRs were calculated using random-effects models. CI = confidence interval, FM = formula milk, HM = human milk (mother’s milk and/or donor milk).

One trial (Romeo et al) compared Lactobacillus reuteri (1 $\times 10^9$ CFU/d) with Lactobacillus rhamnosus (6 $\times 10^9$ CFU/d) in separate groups, and 1 trial (Braga et al) did not report definite probiotic doses. Duration of intervention ranged from $<6$ wk to $>6$ wk in 3 trials (Bin-Nun et al, Dilli et al, and Patole et al).

Several potential limitations should be taken into consideration when interpreting the results. First, although no statistical heterogeneity was found for the primary outcome, population characteristics, probiotic regimens (various organisms, daily doses, time of initiation, and length of intervention), and type of milk differed across the included studies. We adopted random-effects model to try to account infections caused by supplemental probiotics have been reported. Jenke et al also reported Bifidobacterium septicaemia in an ELBW infant under probiotic therapy. Owing to concerns about the safety issues, studies regarding the efficacy and safety of probiotics in ELBW infants are scant. So, more studies are needed to establish the safety of probiotics in preterm neonates, especially in ELBW neonates.
for this variability. Second, to examine the influence of these clinical factors on the overall pooled estimate and to verify the robustness of our findings, subgroup analyses were conducted and the results were consistent in most selected subgroups. We, however, can only analyze covariates that are available to us from the original articles. Moreover, subgroup analyses were susceptible to type II errors because of relatively small sample sizes. Third, our search language was restricted to only English, which could potentially lead to publication bias. We, however, used a very thorough and comprehensive search strategy yielding 27 RCTs, which made our study the largest review to date, and the funnel plot and formal statistical tests also did not show any publication bias. Finally, our results should be viewed with caution because 15 of 25 trials included in our meta-analysis were of low methodological quality, that is, unclear or high risk of bias. We tried to verify the robustness of our findings by subgroup analyses (Table 5). When stratified by risk of bias, the beneficial effects of probiotics remained in the 2 strata, especially with no statistical heterogeneity among the 10 studies with low risk of bias ($I^2 = 0\%$).

**CONCLUSIONS**

Current evidence indicates that probiotic supplementation is safe, and effective in reducing the risk of LOS in preterm neonates in NICUs. Further studies are needed to address the optimal probiotic organism, dosing, timing, and duration. High-quality and adequately powered RCTs regarding the efficacy and safety of the use of probiotics in ELBW infants are still warranted.

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