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SYSTEMATIC REVIEW AND META-ANALYSIS

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Probiotics for Preventing Late-Onset Sepsis in Preterm Neonates

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

Guo-Qiang Zhang, MSc, Hua-Jian Hu, MD, Chuan-Yang Liu, MSc, Shristi Shakya, MD, and Zhong-Yue Li, MD

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; $I^2 = 26\%$), bacterial sepsis (11 RCTs; RR 0.82, 95% CI 0.71–0.95; $I^2 = 0\%$), and fungal sepsis (6 RCTs; RR 0.57, 95% CI 0.41–0.78; $I^2 = 0\%$). This beneficial effect remains in very low birth weight infants (<1500 g) (19 RCTs; RR 0.86, 95% CI 0.75–0.97; $I^2 = 18\%$), but not in extremely low birth weight infants (<1000 g) (3 RCTs; RR 0.73, 95% CI 0.45–1.19; $I^2 = 53\%$). 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.

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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.[1,2,4] 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.

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From the Department of Gastroenterology (G-QZ, H-JH, SS, Z-YL) and Department of Nephrology (C-YL), Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing International Science and Technology Cooperation Center for Child Development and Disorders, Key Laboratory of Pediatrics in Chongqing, Children's Hospital of Chongqing Medical University, Chongqing, China.
Correspondence: Zhong-Yue Li, MD, Department of Gastroenterology, Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing International Science and Technology Cooperation Center for Child Development and Disorders, Key Laboratory of Pediatrics in Chongqing, Children's Hospital of Chongqing Medical University, No. 136, Zhongshan 2nd Road, Yuzhong District, 400014 Chongqing, China (e-mail: lizhongyue1001@hotmail.com).
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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

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

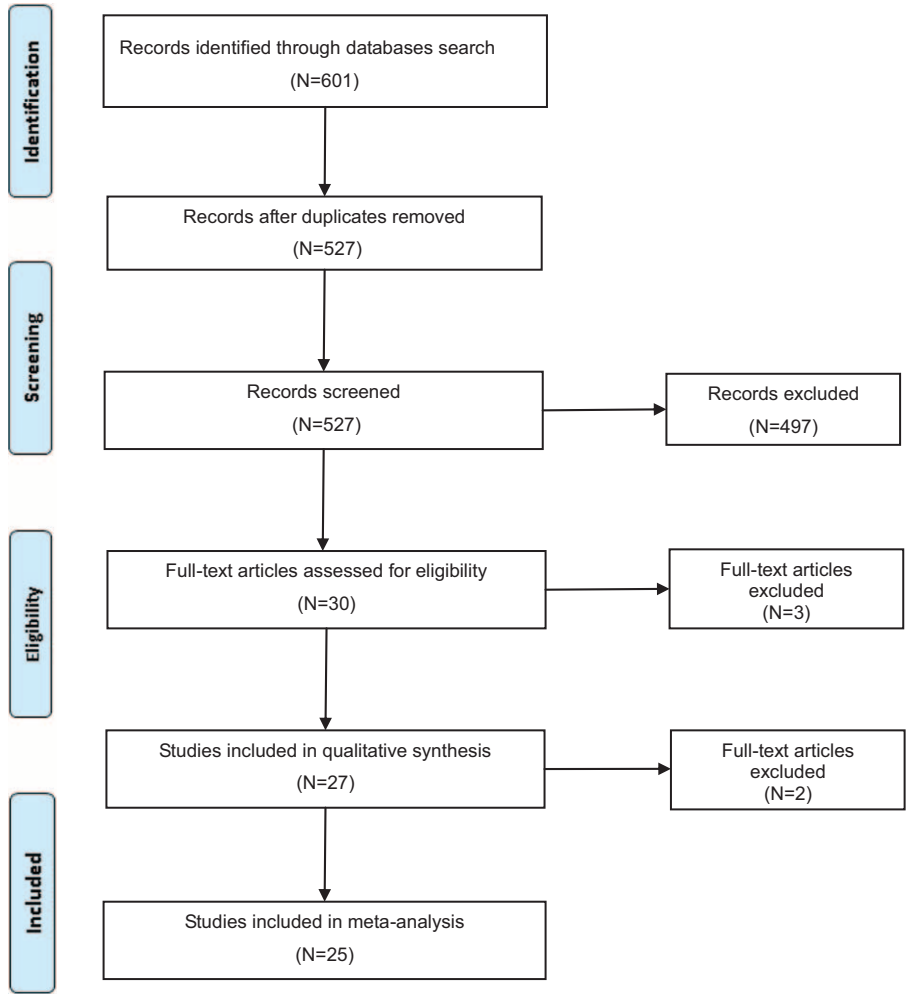

FIGURE 1. Selection process for the studies included in the meta-analysis.

TABLE 2. Characteristics of Randomized Controlled Trials Included in Our Meta-Analysis

			Probiotics Group*		
Source	***N***	**Participants**	**Strains, Doses, and Duration**	**Type of Milk**	**Outcomes of Interest**
AI-Hosni[27]	101	BW 501–1000 g	A mixture of *L. rhamnosus* GG and *B. infantis*, 1×10^9 CFU/d, from first enteral feed to discharge or 34 wk corrected age	FM	Bacterial and/or fungal sepsis (blood culture proven)
Bin-Nun[28]	145	BW ≤ 1500 g	A mixture of *B. bifidus*, *B. infantis*, and *Streptococcus thermophilus*, 1.05×10^9 CFU/d, from first feed to 36 wk corrected age	HM or FM	Any sepsis (blood culture proven)
Braga[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[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[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[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[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-Carrocera[34]	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[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[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[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[38,*]	434	GA < 34 wk and BW < 1500 g	A mixture of *L. acidophilus* and *B. bifidum*, 2×10^9 CFU/d, for 6 wk	HM or FM	Bacterial sepsis (blood culture proven)
Manzoni[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[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[41]	20	GA ≤ 33 wk	*L. rhamnosus* GG, 2×10^8 CFU/d, from initiation of milk feeds for 14 d	HM or FM	Any sepsis (blood culture proven)
Oncel[42]	424	GA ≤ 32 wk and BW ≤ 1500 g	*L. reuteri*, 1×10^8 CFU/d, from first feed to discharge	HM or FM	Bacterial and/or fungal sepsis (blood culture proven)
Patole[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[44]	750	BW ≤ 2000 g	*L. reuteri*, 1×10^8 CFU/d, from first 48 hours of life to discharge	HM or FM	Any sepsis (blood/CSF/urine culture proven)
Romeo[45]	249	GA < 37 wk and BW < 2500 g	*L. reuteri*, 1×10^8 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[46]	94	GA < 32 wk and BW < 1500 g	A mixture of *L. rhamnosus* GG and *B. longum*, 8×10^8 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	Probiotics Group* Strains, Doses, and Duration	Type of Milk	Outcomes of Interest
Roy[47]	112	GA < 37 wk and BW < 2500 g	A mixture of *B. longum*, *B lactis*, *B. bifidum*, and *L. acidophilus*, $1.5–3 \times 10^9$ CFU/d, from first 72 h for 6 wk or to discharge	HM	Bacterial and/or fungal sepsis (blood/urine/CSF culture proven)
Saengtawesin[53]	60	GA ≤ 34 wk and BW ≤ 1500 g	A mixture of *L. acidophilus* and *B. bifidum*, 2×10^9 CFU/d, from first feed for 6 wk or to discharge	HM or FM	Any sepsis (NG)
Samanta[48]	186	GA < 32 wk and BW < 1500 g	A mixture of *B. infantis*, *B. bifidum*, *B. longum*, and *L. acidophilus*, 2.5×10^9 CFU/d, from first enteral feed till discharge	HM	Any sepsis (blood/CSF culture proven)
Sari[49,†]	242	GA < 33 wk or BW < 1500 g	*L. sporogenes*, 3.5×10^8 CFU/d, from first feed to discharge	HM or FM	Bacterial and/or fungal sepsis (blood culture proven)
Serce[50]	208	GA ≤ 32 wk and BW ≤ 1500 g	*S. boulardii*, 2×10^9 CFU/d, from first feed to discharge	HM or FM	Bacterial sepsis (blood culture proven)
Stratiki[51]	77	GA 27–37 wk	*B. lactis*, 2×10^7 CFU/d, from first 48 h to 30 d	FM	Any sepsis (blood culture proven)
Umezaki[52]	208	BW < 1500 g	*B. breve*, 1×10^9 CFU/d, from first several hours after birth to discharge	HM or FM	Any sepsis and/or fungal sepsis (blood culture proven)

B = *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 birth weight between groups, resulting in more infants weighing <750 g in the probiotic group.

† 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.

those conducted in children or adolescents. Discrepancies regarding study inclusion between the 2 authors (G-QZ and H-JH) were resolved through discussion with the correspondence author (Z-YL), as required.

Date Extraction and Quality Assessment

Two of the authors (G-QZ and H-JH) independently extracted relevant data from each included trials by using a unified data form. Extracted data were entered into a standardized Word file. The items included in the data form were as follows: source (first author), number of preterm infants enrolled, strains/doses/duration of probiotics administered, type of milk (human milk or formula), and outcomes of interest (any sepsis/bacterial sepsis/fungal sepsis). Discrepancies between authors were resolved by consensus. Authors were contacted in case of inadequate information to clarify or provide additional information. We adopted the Cochrane Risk-of-Bias Tool to assess the risk of bias for each RCT.[20]

Statistical Analysis

To evaluate the effect of probiotics, we calculated relative risks (RRs) for the incidence of LOS between intervention and control groups. Trials with uneven distribution of sepsis-related risk factors between study and control groups were not included in our meta-analysis, such as gestational age, birth weight, Apgar score, prenatal steroids, antimicrobial drugs, and use of invasive devices.[21] When trials investigated 2 separate probiotic groups versus placebo, data on the 2 probiotic groups were combined into a single RR, which we included in the meta-analysis. Heterogeneity across studies was tested by using the I^2 statistic. Studies with an I^2 value of >50% were considered to have significant heterogeneity.[22] The Mantel–Haenszel method with random-effects model was used to calculate pooled RRs and 95% confidence intervals (CIs). Subgroup analyses were conducted according to type of sepsis, birth weight, probiotic organism, probiotic dose, time of initiation, duration of intervention, type of milk, caesarean delivery rate, and risk of bias. We also investigated the influence of a single study on the overall pooled RR by omitting each study in turn. An assessment of publication bias was performed by visually inspecting funnel plot and by using the Begg's and Egger's tests.[23,24] A P value <0.05 was considered as statistically significant, except where otherwise specified. All the statistical analyses were performed using the Stata 12.0 (Stata Corporation, College Station, TX) and RevMan 5.3 (The Nordic Cochrane Centre, Copenhagen, Denmark).

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[14,25,26] were further excluded because incidences of LOS were not reported. Finally, 27 trials were eligible for this review.[27–53] Two trials were not included in meta-analysis because of the uneven distribution of birth

weight[38] and duration of umbilical venous catheter[49] between study and control groups. Hence, 25 trials were statistically analyzed.[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, $I^2 = 37\%$). Further exclusion of any single study did not materially alter the overall combined RR, with a range from 0.81 (95% CI 0.72–0.92) to 0.84 (95% CI 0.75–0.95). 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.

Table 5 reports the pooled RRs for probiotic supplementation in the prevention of LOS in preterm neonates in selected subgroups. Probiotic supplementation was consistently associated with reduced incidence of LOS in most subgroups. Significant differences were observed according to birth weight (<2500, <1500, or <1000 g), probiotic organisms, duration of intervention (<6 weeks or ≥6 weeks), and type of milk (human milk or formula milk).

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

TABLE 3. Outcome Data of Included Studies

	Any Sepsis		Bacterial Sepsis		Fungal Sepsis	
Study	**Probiotics**	**Control**	**Probiotics**	**Control**	**Probiotics**	**Control**
AI-Hosni[27]	13/50	16/51	11/50	16/51	2/50	0/51
Bin-Nun[28]	31/72	24/73	NR	NR	NR	NR
Braga[29]	40/119	42/112	NR	NR	NR	NR
Costalos[30]	3/51	3/36	NR	NR	NR	NR
Dani[31,*]	24/295	27/290	24/295	27/290	NR	NR
Demirel[32]	20/135	21/136	20/135	21/136	NR	NR
Dilli[33]	8/100	13/100	8/100	13/100	NR	NR
Fernandez-Carrocera[34]	42/75	44/75	42/75	44/75	NR	NR
Jacobs[35]	72/548	89/551	NR	NR	NR	NR
Kitajima[36]	1/45	0/46	NR	NR	NR	NR
Lin[37]	22/180	36/187	NR	NR	NR	NR
Lin[38,†]	40/217	24/217	40/217	24/217	NR	NR
Manzoni[39]	19/39	22/41	15/39	17/41	4/39	5/41
Mihatsch[40]	28/91	29/89	28/91	29/89	NR	NR
Millar[41]	0/10	0/10	NR	NR	NR	NR
Oncel[42]	13/200	25/200	12/200	22/200	1/200	3/200
Patole[43]	17/77	12/76	NR	NR	NR	NR
Rojas[44]	34/372	40/378	NR	NR	NR	NR
Romeo[45]	3/166	9/83	1/166	5/83	2/166	4/83
Rouge[46]	15/45	13/49	NR	NR	NR	NR
Roy[47,‡]	31/56	42/56	21/56	33/56	23/56	42/56
Saengtawesin[53]	2/31	1/29	NR	NR	NR	NR
Samanta[48]	13/91	28/95	NR	NR	NR	NR
Sari[49,†]	29/110	26/111	26/110	25/111	3/110	1/111
Serce[50]	19/104	25/104	19/104	25/104	NR	NR
Stratiki[51]	0/41	3/36	NR	NR	NR	NR
Umezaki[52]	10/108	22/100	NR	NR	1/108	0/100

NR = not reported.

[*] Data on positive blood culture and positive urine culture were combined because none of the patients with positive urine culture developed positive blood culture.

[†] Studies were not included for meta-analysis because of uneven distribution of birth weight or time of umbilical venous catheter between study and control groups.

[‡] Incidence of fungal sepsis was calculated by subtracting number of preterm neonates without fungal infection from total number of subjects enrolled.

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
AI-Hosni[27]	Unclear	Unclear	Yes	Yes	No	No	No	Unclear
Bin-Nun[28]	Unclear	Unclear	Yes	Yes	Unclear	No	No	Unclear
Braga[29]	Yes	Yes	Yes	Yes	No	No	No	Low
Costalos[30]	Yes	Yes	Yes	Unclear	No	No	No	Unclear
Dani[31]	Unclear	Yes	Yes	Yes	No	No	No	Unclear
Demirel[32]	Yes	Yes	Yes	Yes	No	No	No	Low
Dilli[33]	Yes	Yes	Yes	Yes	No	No	No	Low
Fernandez-Carrocera[34]	Yes	Yes	Yes	Yes	No	No	No	Low
Jacobs[35]	Yes	Yes	Yes	Yes	No	No	No	Low
Kitajima[36]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	High
Lin[37]	Yes	Yes	Yes	Yes	No	No	No	Low
Lin[38]	Yes	Yes	Yes	Yes	No	No	No	Low
Manzoni[39]	Yes	Unclear	Unclear	Unclear	No	No	No	Unclear
Mihatsch[40]	Yes	Yes	Yes	Yes	No	No	No	Low
Millar[41]	Unclear	Unclear	Yes	Unclear	No	Yes	Yes	High
Oncel[42]	Yes	Yes	Yes	Yes	No	No	No	Low
Patole[43]	Yes	Yes	Yes	Yes	No	No	No	Low
Rojas[44]	Yes	Yes	Yes	Yes	No	No	No	Low
Romeo[45]	Yes	Unclear	Unclear	Unclear	No	No	No	Unclear
Rouge[46]	Yes	Unclear	Yes	Yes	No	No	No	Unclear
Roy[47]	Yes	Unclear	Yes	Unclear	No	No	No	Unclear
Saengtawesin[53]	Unclear	Unclear	Unclear	Yes	Unclear	No	Yes	High
Samanta[48]	Unclear	Unclear	Unclear	Unclear	No	Yes	Yes	High
Sari[49]	Yes	Unclear	Unclear	Yes	No	No	No	Unclear
Serce[50]	Yes	Unclear	Unclear	Unclear	No	Yes	Yes	High
Stratiki[51]	Unclear	Unclear	Yes	Yes	No	Yes	Yes	High
Umezaki[52]	Unclear	Yes	Yes	Unclear	No	No	No	Unclear

* Risk of bias was assessed with use of the Cochrane risk-of-bias tool.

et al[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, $I^2 = 52\%$). In another meta-analysis in 2014[15] 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, $I^2 = 47\%$). 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[27,30,36,45,51,52] for Bernardo et al and 3 trials[34,45,52] for AlFaleh et al, which could potentially lead to publication bias. Second, 1 RCT[54] 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,[55] enhanced mucosal immunoglobulin (Ig) A responses,[56] modulation of the gut barrier function and permeability,[57] production of antimicrobial peptides,[58] and upregulation of immune responses.[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[27,35,42] 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.[39] In summary, probiotics appear promising for use as 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.

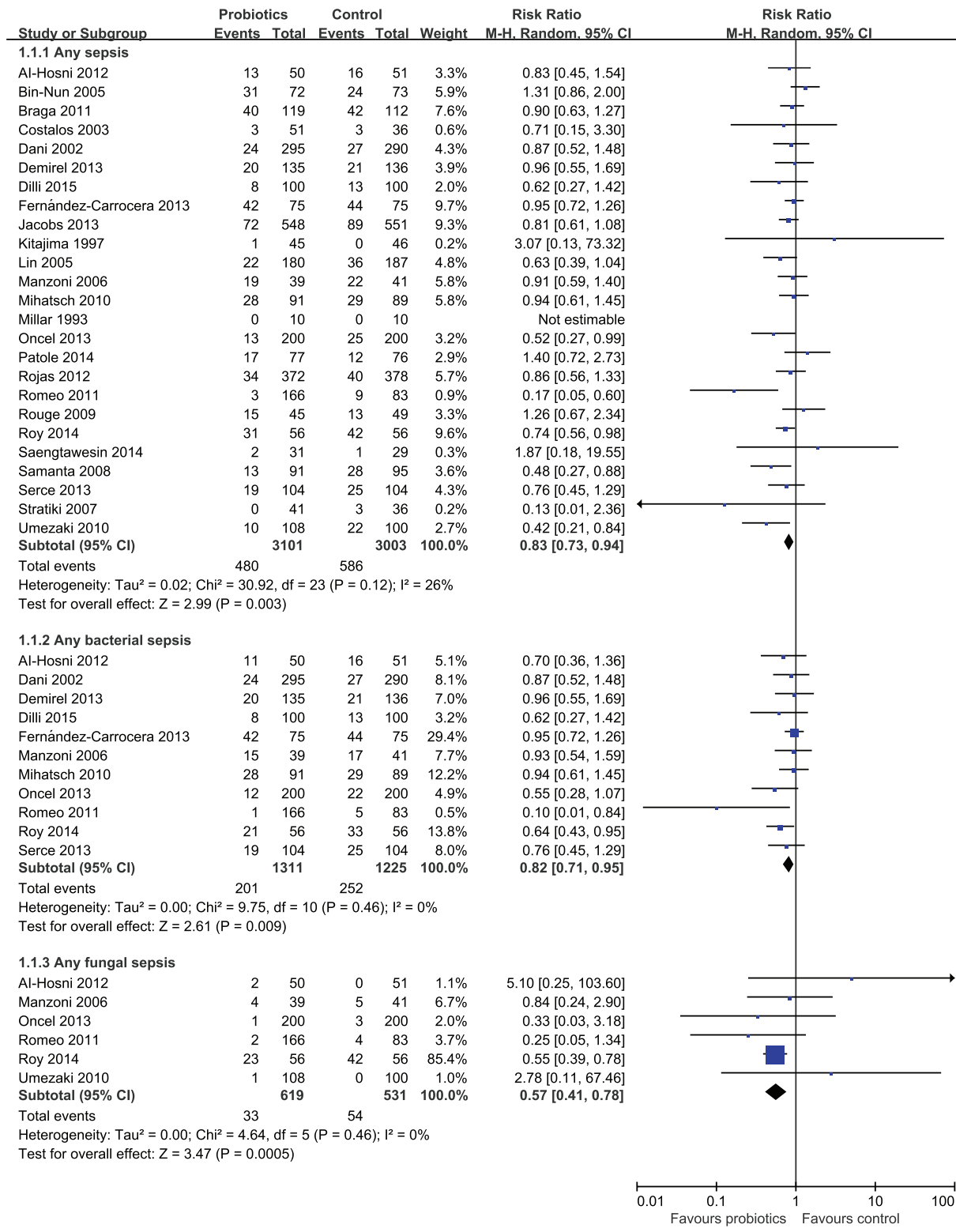

FIGURE 2. Effect of probiotics on late-onset sepsis in preterm neonates.

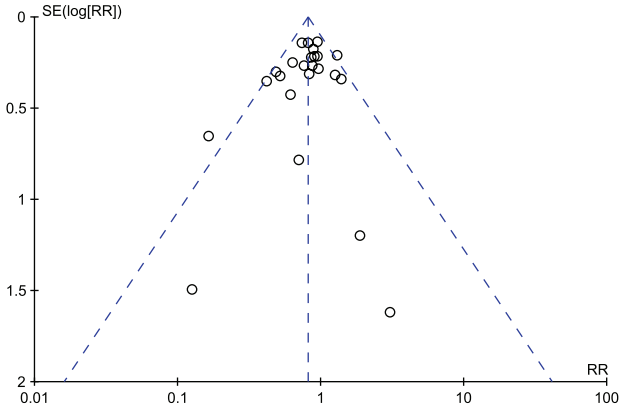

FIGURE 3. Funnel plot of trials on probiotics and prevention of late-onset sepsis.

The reason why there was a lack of effect of probiotics on LOS in the 2 trials,[38,49] 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 (more infants weighing <750 g or longer duration of umbilical venous catheterization in the study group) could probably lead to overturn of the real effects. On the other hand, the pathogens causing sepsis were most often related to catheter-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,[60] 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 *Lactobacillus reuteri* DSM 17938 could significantly reduce the risk of NEC and LOS.[61] To our knowledge, only 1 trial, however, compared the effect of different probiotic strains on LOS in preterm neonates,[45] which showed no difference between groups. Therefore, future experimental and clinical studies are still needed to characterize the mechanisms by which specific probiotic organisms influence the development of LOS.

In our study, we observed that preterm neonates fed exclusively human milk benefit more from probiotics. It is well known that human milk contains several substances with putative anti-infective actions, such as lactoferrin, IgA, IgG, and IgM, etc.[21,62] The feeding of human milk was also associated with decreased gut permeability,[63] which might result in less translocations of pathogens from the gut and ultimately less infections.[62] On the other hand, human milk promotes the establishment of beneficial microorganism in the infant gut by providing several substances, such as oligosaccharides, which act as favorable substrates for probiotic organisms.[38,64] Also in a European cohort, probiotics were reported to prevent NEC only in preterm neonates fed breast milk not formula.[65] Therefore, probiotics and human milk may have synergistic effects to prevent LOS development. Still, further studies investigating the influence of feeding formula or breast milk on the effect of probiotics are needed.

Although none of our included studies reported septicemia caused by probiotic organisms, several cases of systemic infections caused by supplemental probiotics have been reported.[66–69] Jenke et al[70] also reported *Bifidobacterium septicemia* 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.[71] So, more studies are needed to establish the safety of probiotics in preterm neonates, especially in ELBW neonates.[71]

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

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

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)	0
Mixture	10	0.85 (0.73, 1.00)	29
Probiotic dose*			
$\leq 1 \times 10^9$	10	0.73 (0.55, 0.98)	38
$>1 \times 10^9$	15	0.85 (0.73, 0.99)	20
Time of initiation			
≤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
≥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			
<median (69%)	11	0.80 (0.69, 0.94)	0
≥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

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* (1×10^8 CFU/d) with *Lactobacillus rhamnosus* (6×10^9 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).

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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