Which is the best probiotic treatment strategy to prevent the necrotizing enterocolitis in premature infants

A network meta-analysis revealing the efficacy and safety

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Abstract

Background: Previous studies have neglected to report the specific action of different probiotic genera in preterm infants. To evaluate the efficacy and safety of specific probiotic genera, we performed a network meta-analysis (NMA) to identify the best prevention strategy for necrotizing enterocolitis in preterm infants.

Methods: MEDLINE, PubMed, EMBASE, and the Cochrane Central Register of Controlled Trials had been searched for randomized control trials reporting the probiotics strategy for premature infants.

Results: We identified 34 eligible studies of 9161 participants. The intervention in the observation group was to add probiotics for feeding: *Lactobacilli* in 6 studies; *Bifidobacterium* in 8 studies; *Bacillus* in 1 study; *Saccharomyces* in 4 studies and probiotic mixture in 15 studies. This NMA showed a significant advantage of probiotic mixture and *Bifidobacterium* to prevent the incidence of necrotizing enterocolitis in preterm infants. A probiotic mixture showed effectiveness in reducing mortality in preterm infants.

Conclusion: The recent literature has reported a total of 5 probiotic strategies, including Bacillus, Bifidobacterium, Lactobacillus, Saccharomyces, and probiotic mixture. Our thorough review and NMA provided a piece of available evidence to choose optimal probiotics prophylactic strategy for premature infants. The results indicated that probiotic mixture and Bifidobacterium showed a stronger advantage to use in preterm infants; the other probiotic genera failed to show an obvious effect to reduce the incidence of NEC, sepsis and all-cause death. More trials need to be performed to determine the optimal probiotic treatment strategy to prevent preterm related complications.

Abbreviations: CIs = confidence intervals, MD = mean difference, NEC = Necrotizing enterocolitis, NICU = neonatal intensive care unit, NMA = network meta-analysis, OR = odds ratios, PRISMA = the Preferred Reporting Items for Systematic Reviews and Meta-Analyses, RCT = randomized controlled trial, SUCRA = Surface under the cumulative ranking curve.

Keywords: necrotizing enterocolitis, preterm infants, probiotics, sepsis

1. Introduction

Necrotizing enterocolitis (NEC) is one of the most common and serious preterm-related complications with high surgical rate and

mortality in premature infants. The morbidity of NEC can be as high as 28% in very low birthweight infants.[1,2] Besides that, although accept medical treatment, patients with NEC tend to suffer from long-term complications which included chronic nutritional intolerance, short bowel syndrome, and growth retardation.[3] Therefore, the prevention of the incidence of NEC seems to be more important and effective than its treatment. Some studies reported that key risk factors for NEC include an overgrowth of pathogenic microflora in premature infants, primarily because of the immature mucosal barrier and immune response in preterm newborns, together with their exposure high-risk hospital milieu with bacterial pathogens.[4–6] Recently, the probiotic product has been reported to be beneficial for decreasing the morbidity of NEC in the literature. Probiotics, as live microbial supplements, might to improve the function of the intestinal mucosal barrier and competitively inhibit the growth of gastrointestinal pathogenic bacteria in preterm infants.[7,8]

Many clinical RCTs have proved probiotics are effective preparations for the prevention of NECs but few studies have examined the effect of different genera.[9] The function of probiotics is species specific, depending on morphological, physiological, and biochemical characteristics of the different probiotic genera.[7] Recent review studies showed that *Bifidobacterium* significantly decreases the incidence of NEC and

mortality rates.[10]*Bifidobacterium breve* may improve intestinal tolerance, which was reported by Kitajima et al.[11] Additionally, in other literature, the effect of a single strain might also differ from combined strains in NEC.[12] Reports showed that supplements of a combination of strains of *Bifidobacterium* species[13] or a combination of *Bifidobacterium* and *Lactobacillus acidophilus*[14] might achieve an earlier total gastrointestinal nutrition. Moreover, other clinical trials considered different strains of probiotics including *Lactobacillus*[15,16] and *Saccharomyces boulardii*.[8] However, the current studies and systematic analysis have failed to recommend an optimal prevention strategy to reduce the incidence of NEC.

To evaluate the efficacy and safety of different genera of probiotics, we sum up available clinical evidence from randomized controlled trials (RCTs) related to this topic and performed a network meta-analysis (NMA) to identify the best prevention strategy for NEC in preterm infants.

2. Materials and methods

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and reported a net-meta analysis of the RCTs.

2.1. Literature searches

Two independent reviewers systematically searched the following electronic databases: PubMed, MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials to identify literature on probiotics for NEC in premature infants before January 2019. We used PubMed medical subject heading terms and free-text words in combination with Boolean operators as comprehensively as possible: (premature birth OR preterm birth OR preterm infants) AND (RCT OR controlled clinical trial OR randomly) AND (probiotics OR probiotic treatment). Besides, we further searched other databases (EBSCO Information Services, Web of Science, and Google Scholars) to identify potentially available studies. The process was completed when no further trials could be determined. The review was limited to RCTs. The ethical approval is not necessary.

2.2. Criteria for study inclusion

All enrolled RCTs met the following inclusion criteria:

1. premature infants with a low birth weight (<2500 g);
2. intervention: probiotics;
3. comparison intervention: placebo or negative control;
4. outcomes, including more than one of the following outcomes; and
5. only clinical studies.

Exclusion criteria were as follows:

1. non-probiotic interventions;
2. non-English language literature;
3. animal studies; and
4. studies including infants who also had other congenital diseases (e.g., intestinal atresia).

The process was completed by two independent investigators. The five eligible probiotic strategies were

1. Lactobacilli included *Lactobacillus rhamnosus* GG (*L casei*), *Lactobacillus reuteri* (*L reuteri*), and *Lactobacillus sporogenes*;

2. Bifidobacterium included *Bifidobacterium longum* (*B longum*), *Bifidobacterium breve* (*B breve*), *Bifidobacterium bifidum* (*B bifidum*), and *Bifidobacterium lactis* (*B lactis*);
3. Bacillus included *Bacillus clausii* (*B clausii*);
4. Saccharomyces included *Saccharomyces boulardii* (*S boulardii*);
5. probiotic mixture included the combination of the different probiotic strains.

Their control treatment included blank or placebo control.

2.3. Primary and secondary outcomes

In this study, the primary outcome is NEC incidence rate (NEC was diagnosed and classified according to the classification of Bell). The secondary outcomes included the incidence of sepsis (which is diagnosed by positive blood culture results) and all-cause mortality.

2.4. Data extraction and risk of bias assessment

Data from all included RCTs was collected: author's name, year of publication, sample size, patient characteristics, probiotic type, intervention time, dose, and outcomes. Any dispute arising from the data collection shall be negotiated by two researchers and determined by the third evaluator. When the specific number is not reported, the relevant incidence rate is extracted from the article and the required data are calculated.

We assessed the quality of randomized controlled studies using the Cochrane Collaboration's Risk of Bias Tool.[17] The main evaluation contents included:

1. the random sequence generation,
2. the allocation sequence concealment,
3. blinding of participants and personnel,
4. the blinding of outcome assessment,
5. the completeness of outcome data,
6. selective reporting,
7. other sources of bias.

Each evaluation of these 7 items was mainly divided into three options of low, high, and unclear. The evaluation process was mainly evaluated by two authors independently.

2.5. Data synthesis and analysis

STATA V13 software was used for the meta-analysis. The Mantel–Haenszel method was used for continuous variables, and the combined odds ratios (ORs) and 95% confidence intervals (CIs) were used for dichotomous variables. The difference was statistically significant when the *P*-value was <.05. The pooled mean difference (MD) was measured in the meta-analysis using the inverse variance method. Inferred heterogeneity was determined according to I^2. When I^2 was <50%, there was no obvious heterogeneity in the analysis, and the fixed effect model was used. When the I^2 was ≥50%, there was significant heterogeneity among the analyses, and the random effect model was selected.

We performed a Bayesian hierarchical random-effects NMA to assess all probiotic preventions of primary outcomes simultaneously with the use of Markov chain Monte Carlo simulation with a prior distribution. The analyses used generalized linear models with a logit link function with 4 chains and 50,000 iterated simulations discarding the initial 20,000 iterations as burn-in. Convergence was assessed using the Brooks–Gelman–
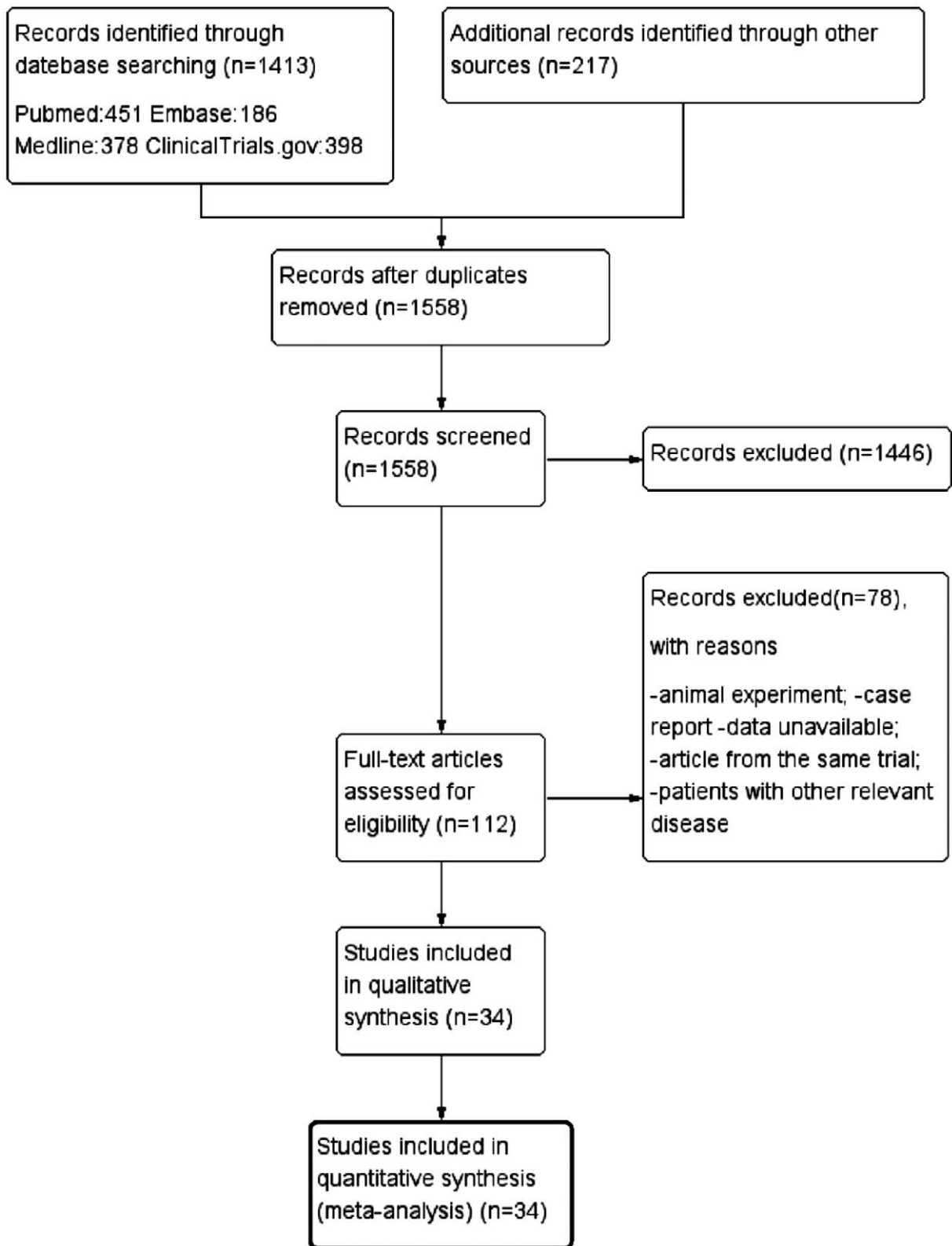
Figure 1. Flow chart showing the search strategy and search results. The relevant number of papers at each point is given.

Table 1

Characteristics of the included RCTs in our network meta-analysis.

Study	Design	Simple size (P/C)	Patients	Probiotic strain (s)	Probiotic duration	Outcomes
Braga et al[13]	RCT	119/112	Preterm; BW 750–1499 g; admission to hospital	L casei (3.5 × 10⁹ CFU OD) and B breve (3.5 × 10⁷ CFU OD)	From day 2 to day 30 or the occurrence of primary outcomes	NEC, sepsis, death
Lin et al[8]	RCT	180/187	Preterm; BW < 1500 g enteral nutrition; age > 7 d	L acidophilus and B infantis ≥10⁶ CFU each probiotic, BD)	From enteral feeding to discharge	NEC
Al-Hosni et al[23]	RCT	50/51	Preterm; BW 501–1000g, appropriate for GA, and ≤14 d of age at the time of feeding initiation	L rhamnosus GG and B infantis (0.5 × 10⁹ CFU, OD)	From first enteral feeding to discharging or PMA > 34 wk	Weight gain CLD, ROP, NEC, sepsis, mortality, mean volume of feeding
Chou et al[24]	RCT	153/148	Preterm very low birth weight infants; enteral feeding; age > 7 d	L acidophilus and B infantis ≥10⁶ CFU each probiotic (=125 mg/kg), BD)	From enteral feeding to discharge	Growth: height, weight, HC, neurodevelopmental and sensory; outcomes: NEC, death, CLD, sepsis, hospitalization; visual impairment, deafness, cerebral palsy
Lin et al[25]	RCT	217/217	Preterm; GA < 34 wk; BW ≤ 1500 g; enteral feeding	L acidophilus NCDO 1748 and B bifidum NCDO 1453 (1 × 10⁹ CFU; ==125 mg/kg BD)	From day 2 of age; duration 6 wk	NEC, sepsis, death, IVH, CLD, NICU stay, weight gain
Hays et al[3]	RCT	P1/P2/P3/C:50/48/47/52	700–1600 g; GA 25–31 wk	B lactis (1 × 10⁹ CFU OD); B longum (1 × 10⁹ CFU OD); B lactis + B longum;	From age <7 d to 4 wk (GA 29W)/6 wk (GA 28 W)	Weight, length, HC, NEC, sepsis, body composition, bacterial count
Jacobs et al[26] 2013	RCT	548/551	Preterm; GA < 32 wk BW < 1500 g	B infantis BB-02 (300 CFU ×10⁶), Streptococcus thermophilus Th-4 (350 × 10⁶ CFU); B lactis BB-12 (350 CFU ×106)	From enteral feed ≥ 1 mL/4 h to discharge or term corrected age	Sepsis, death, hospitalization, duration of parenteral nutrition, days to full enteral feeds, weight at 28 d; ROP, CLD, IVH
Patole et al[27]	RCT	77/76	Preterm; GA < 33 wk; BW < 1500 g; enteral feeds for <12 h	B breve (3 × 10⁹ CFU OD)	From enteral feed to corrected age of 37 wk	Discharge weight, death, hospitalization; NEC
Costalos et al[15]	RCT	51/36	GA 28–32 wk; no major GI problem; not receiving antibiotics; not receiving breast milk	Saccharomyces boulardii (1 × 10⁹ CFU BD)	Non-specified of the start time; Median duration of probiotic supplementation: 30 d	Weight gain and loss, daily milk in taking, NEC, sepsis, bacterial counts
Roy et al[28]	RCT	56/56	Preterm; GA < 37 wk; BW < 2500 g; enteral feeding; age < 72 h	L acidophilus (1.25 × 10⁹ CFU/g); B bifidum (0.125 × 10⁹ CFU/g × 1g); B lactis (1 × 10⁹ CFU ×1 g)	From 72 h of life, duration 6 wk or at discharge	Full feed establishment, candida, death, NEC, hospitalization
Sari et al[29]	RCT	86/88	Preterm; GA < 33 wk; or BW < 1500 g	L sporogenes (0.35 × 10⁹ CFU OD)	From first feed to discharge	Weight gain, length, HC, CLD, hospitalization, NEC, sepsis, feeding intolerance, oxygen days, full-feeding days
Costeloe et al[30]	RCT	650/660	GA 23–30 wk	B breve (1.6 × 10⁹ CFU OD)	Until corrected age of 36 wk	ROP, death, NEC, sepsis
Fernández-Carrocera et al[16]	RCT	75/75	Preterm; BW < 1500 g infants with NEC IA and IB were excluded	S thermophilus (6.6 × 10⁵CFU/g); B infantis (2.76 × 10⁷CFU/g); L plantarum (1.76 × 10⁸ CFU/g); L casei (1 × 10⁹ CFU/g); L rhamnosus (4.4 × 10⁸ CFU/g), L acidophilus (1CFU/g)	From enteral feeding, non-specified end time	Weight, death, NEC
Manzoni et al[31]	RCT	39/41	BW < 1500 g, age > 3 d, not receive any form of antifungal prophylaxis other than LGG	L rhamnosus LGG (6 × 10⁹ CFU/d)	From the third day of life to the age of sixth week or discharge from the NICU	Hospitalization, time of achievement of full feedings, death, NEC
Mihatsch et al[32]	RCT	91/89	Preterm; GA < 30 wk; BW ≤ 1500 g	B lactis (2 × 10⁹ CFU/kg 6 times/d)	From enteral feeding, non-specified end time	NEC, death, nosocomial infections
Oncel et al[33]	RCT	200/200	Preterm; GA ≤ 32 wk; BW ≤ 1500 g, enteral feeding	L reuteri (1 × 10⁸ CFU OD)	From first feed to death or discharge	NEC, sepsis, death, hospitalization, feeding intolerance
Rojas et al[34]	RCT	372/378	Preterm; BW ≥ 2000 g, age ≤ 48 h; HS; enteral feeding	L reuteri (1 × 10⁸ CFU OD)	From age ≤48 h to death or discharge	Death, duration of a hospital, NEC, sepsis
Rougé et al[35]	RCT	45/49	Preterm; GA < 32 wk; BW < 1500 g, age ≤ 2 wk, excluded non-preterm birth related diseases; enteral feeding	B longum BB536 and L rhamnosus GG (1 × 10⁸ CFU/d)	From enteral feeding to discharge	Nutrition–total calories delivered enterally, duration of hospital stays, death, oxygen therapy duration
Samanta et al[14]	RCT	91/95	Preterm; GA < 32 wk; BW < 1500 g; enteral feeding; age > 48 h	L acidophilus; B bifidum and B infantis (2.5 × 10⁹ CFU, BD)	From enteral feeding to discharging.	NEC, death, hospitalization
Stratiki et al[36]	RCT	41/34	Preterm; GA 27–32 wk, formula-fed, without major congenital anomalies	B lactis (2 × 10⁷ CFU/g of milk power)	From enteral feeding, non-specified end time	Weight, length, NEC, time to full enteral feed
Chowdhury et al[37]	RCT	52/50	Preterm; GA < 33 wk; BW < 1500 g; enteral feeding; age > 48 h	Capsule TS6 probiotic+ containing Bifidobacterium spp, and Lactobacillus (6 × 10⁹ CFU/d)	From first feeding to discharged	NEC, hospital stay
Demirel et al[38]	RCT	91/90	GA < 32 wk (lowest GA 24 wk); BW < 1500 g (lowest BW 500 g)	S boulardii (5 × 10⁹ CFU OD)	From first feed to discharge.	Feeding amount, full feeding day, weight, NEC, sepsis
Dani et al[39]	RCT	295/290	GA < 33 wk; or BW < 1500 g	L rhamnosus GG (6 × 10⁹ CFU OD)	From first feed to discharge	antibiotic treatment; UTI; sepsis; NEC
Sari et al[40]	RCT	110/111	GA < 32 wk or BW < 1500 g; enteral feeding	L sporogenes (0.35 × 10⁹ CFU OD)	From first feed to discharge	

(continued)

Table 1

(continued).

Study	Design	Simple size (P/C)	Patients	Probiotic strain (s)	Probiotic duration	Outcomes
						NICU stay; NEC; death; weight gain; feeding intolerance
Demirel et al[41]	RCT	135/136	GA ≤ 32 w; BW ≤ 1500 g, enteral feeding	S boulardii (5 × 10^9 CFU OD)	From first feed to discharge	Feeding amount, full feeding day, weight, NEC, sepsis
Dilli et al[42]	RCT	100/100	GA < 32 w BW < 1500 g, admitted to the NICU within the first week of life; fed enterally before inclusion	B lactis (5 × 10^9 CFU)	From beyond d 7 after birth to death or discharge (max 8 w)	Height, weight, HC, NEC, sepsis, feeding intolerance, RDS, stay at NICU, mortality
Fujii et al[43]	RCT	11/8	GA < 34 wk	B breve M-16V (1 × 10^9 CFU BD)	Non-specified start, until discharge	Duration of hospital stay, NEC, CLD, ROP, infection
Kanic et al[44]	RCT	40/40	GA < 33 wk; BW < 1500 g	L acidophilus, Enterococcus faecium and B infantum (0.6 × 10^7 CFU BD)	From enteral feeding to discharge	Hospitalization, late-onset sepsis, pneumonia, NEC, death, meningitis, UTI, and omphalitis
Saengtawesin et al[45]	RCT	31/29	GA ≤ 34 wk BW ≤ 1500 g	L acidophilus (1 × 10^9 CFU); B bifidum (1 × 10^9 CFU)	From feeding to 6 w of age or discharge	Feeding amount; weight gain; length of stay; NEC; sepsis; ROP; PVL; IVH
Serce et al[46]	RCT	104/104	GA ≤ 32 wk; BW ≤ 1500 g, enteral feeding	S boulardii (0.5 × 10^9 CFU/kg BD)	Non-specified start time and end time.	NEC, weight gain, death, hospitalization, sepsis, time to reach 100 mL/kg/d of oral feeding
Tewari et al[47]	RCT	123/121	GA < 34 wk; admitted to the NICU	B clausii (8 × 10^8 CFU, TD)	From age 24 h to postnatal age of 6 wk, discharge or death	Sepsis; NEC; feed intolerance; death
Totsu et al[48]	RCT	153/130	BW < 1500 g	B bifidum (2.5 × 10^9 CFU, divided in two doses)	Start within 48 h after birth, until body weight 2000 g	Bodyweight, hospitalization, sepsis, HC, accelerated the establishment of enteral feeding, death, NEC, CLD
Kitajima et al[11]	RCT	45/46	BW < 1500 g	B breve YIT4010 (0.5 × 10^9 CFU OD)	Start within 24 h of life, duration of probiotic supplementation 28 days	Weekly change of aspirated air volume from stomach, frequency of vomiting and apnoea; Duration of antibiotics; NEC
Bin-Nun et al[22]	RCT	72/73	Preterm; BW < 1500 g; enteral feeding	S thermophilus; B bifidus and B infantis (0.35 × 10^9 CFU, OD)	From enteral feeding to 36 wk postconceptual age.	Weight gain NEC, TPN

BW = birth weight, CLD = chronic lung disease, GA = gestational age, GI = gastrointestinal, HC = head circumference, HS = hemodynamically stable, IVH = intraventricular hemorrhage, NCDO = National Collection of Dairy Organisms, NEC = necrotizing enterocolitis, NICU = neonatal intensive care unit, NR = no reported, PMA = postmenstrual age, PVL = periventricular Leuko-malacia, RCT = randomized controlled trial, RDS = respiratory distress syndrome, ROP = retinopathy of prematurity, TPN = total parenteral nutrition, UTI = urinary tract infection.

Rubin method. We calculated the ORs and 95% CI to compare the pairwise relative treatment efficacy of the competing interventions. In addition, we ranked all interesting treatments with each endpoint and assessed the probabilities. The surface under the cumulative ranking curve (SUCRA) was used. SUCRA is a simple summary index indicating the degree to which an intervention is better or worse than others, taking a value between 0 (certainly the worst intervention) and 1 (certainly the best intervention).[18] We planned to use the node-splitting to evaluate the incoherence between direct and indirect comparison. Finally, the funnel plots were used to assess the publication bias. We produced summary results for all outcomes. We performed an NMA using Stata version 13.1 and WinBUGS 1.4.3.

2.6. Quality-of-evidence assessment

We used the GRADE method to assess the quality of the evidence of direct, indirect and network results.[19,20] The contents of the evaluation included the following five factors: risk of bias; indirectness; inconsistency; imprecision; publication bias. As for indirect results, we chose the optimal indirect comparison approach and assessed separately the quality of the evidence of each group in the approach. The lower level of evidence is used as the level of evidence for the indirect comparison. If only indirect evidence existed, the level of evidence for indirect comparison represented the level of evidence. If the direct and indirect evidence both existed, the higher level of the evidence is used as the level of evidence for the NMA results. According to established guidelines, we finally assessed the strength of evidence as high, moderate, low, or insufficient.

3. Results

3.1. The results of the literature search

A total of 1630 related literature were obtained by a preliminary literature search. 1558 trials were identified by scanning the titles

and abstracts, and 112 were identified after reading the full text. 78 trials were excluded because the data were not available, the same trial article or the patient had other related conditions. After literature retrieval and total text examination, 34 RCTs were finally included in our analysis (Fig. 1).[3,8,11,13–16,21–48]

3.2. Characteristics of included studies

All the 34 studies were RCTs with a total of 9171 objects. The intervention in the observation group was to add probiotics for feeding: *Lactobacilli* in 6 studies; *Bifidobacterium* in 8 studies; *Bacillus* in 1 study; *Saccharomyces* in 4 studies; and probiotic mixture in 15 studies. In all studies, there are 443 cases of NEC, 1304 cases of sepsis, and 544 deaths. More details of the included RCTs are shown in Tables 1 and 2. The network structure of evidence reporting on the probiotic strategy to prevent NEC in preterm infants is illustrated using network plots in Figure 2.

3.3. Risk of bias of included studies

The results showed that most of the included trials followed a strict blind procedure for the researchers, outcome evaluators, and intervention participants, but one of the trials had a high risk of randomization and blindness.[43] The risk assessment of all included RCTs bias is shown in Figure 3.

3.4. Meta-analysis results for NEC incidence, gut associated sepsis and mortality

The result showed that the risk of incidence of NEC (OR = 0.38, 95%CI: 0.27–0.54), gut associated sepsis (OR = 0.82, 95%CI: 0.69–0.98) and mortality (OR = 0.54, 95%CI: 0.42–0.71) were significantly reduced after the administration of probiotic mixture. In addition, *Lactobacillius* (OR = 0.58, 95%CI: 0.37–0.91) and *Bifidobacterium* (OR = 0.68, 95%CI: 0.50–0.94) both reduced the risk of incidence of NEC compared with the placebo.

Table 2

The incidence of NEC (a), sepsis (b) and all-cause mortality (c).

NEC	Studies	Participants	Probiotics events	Probiotics total	Placebo events	Placebo total
Probiotic mixture	15	3561	44	1776	113	1785
Lactobacillus	6	2210	31	1102	53	1108
Bifidobacterium	9	2513	68	1266	98	1247
Bacillus	1	244	2	123	2	121
Saccharomyces	4	747	20	381	22	366
(a)						

Sepsis	Studies	Participants	Probiotics events	Probiotics total	Placebo events	Placebo total
Probiotic mixture	12	3197	293	1593	342	1604
Lactobacillus	5	1460	99	730	104	730
Bifidobacterium	8	2422	163	1221	187	1201
Bacillus	1	244	20	123	25	121
Saccharomyces	3	566	42	290	49	276
(b)						

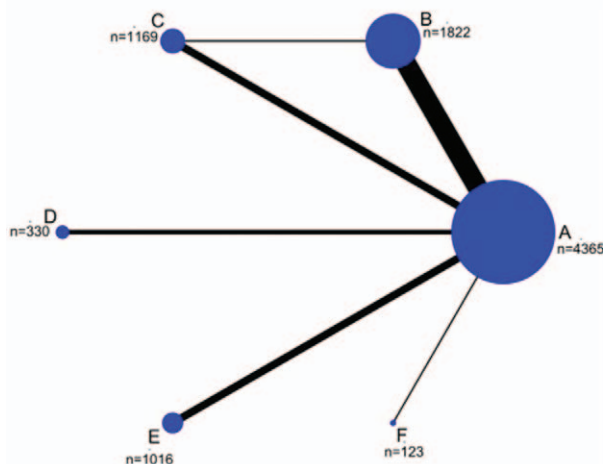| Mortality	Studies	Participants	Probiotics events	Probiotics total	Placebo events	Placebo total
Probiotic mixture	14	3557	98	1822	165	1735
Lactobacillus	5	2036	45	1016	59	1020
Bifidobacterium	6	2328	64	1169	75	1159
Bacillus	1	244	12	123	14	121
Saccharomyces	3	660	12	330	9	330
(c)						
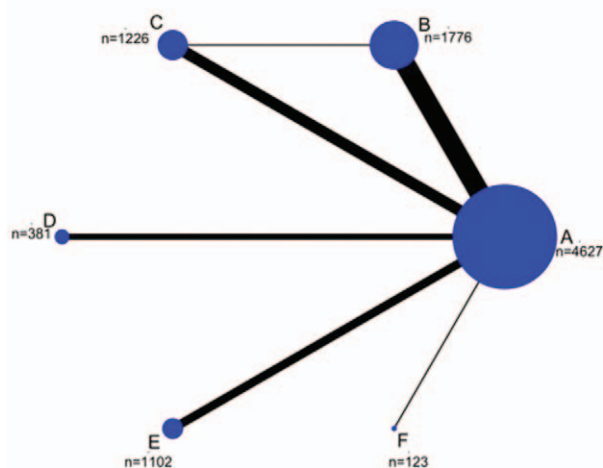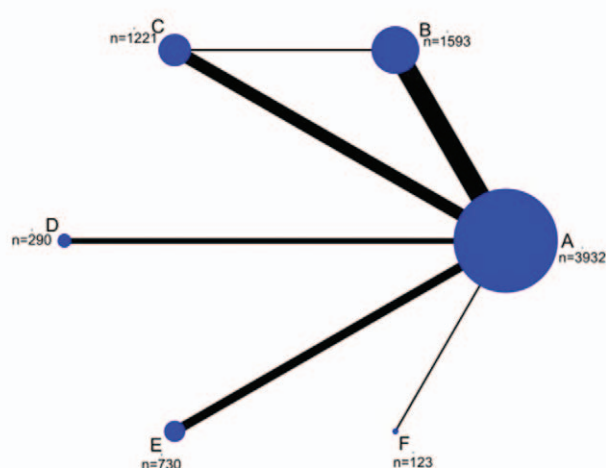
Figure 2. Network plot of RCTs comparing different probiotic treatment strategies for preterm-related complications. The width of the lines is proportional to the number of trials comparing each pair of treatments with numbers on the lines illustrating the exact number. The size of the circles represents the cumulative number of patients for each intervention. A: Placebo; B: Probiotic mixture; C: *Bifidobacterium*; D: *Saccharomyces*; E: *Lactobacillus*; F: *Bacillus*.

However, other results of our analysis showed no significant statistical difference (Table 3).

3.5. NMA results for NEC incidence, gut associated sepsis and mortality

Preterm infants fed with *Bifidobacterium* or probiotic mixture showed a significantly lower risk of the incidence of NEC when compared with those with placebo (*Bifidobacterium*: OR = 0.33, 95%CI: 0.13–0.67; probiotic mixture: OR = 0.38, 95%CI: 0.22–0.61, Table 4). However, we found no significant difference between probiotic supplement and placebo in the incidence of gut associated sepsis (Table 4). Furthermore, there is a significant decrease in preterm infants' mortality with the probiotic mixture when compared placebo (probiotic mixture: OR = 0.49, 95%CI: 0.32–0.69, Table 4).

3.6. Ranking of 5 probiotic strategies and cluster analysis

We used the SUCRA value for each probiotic supplement to show their potential ranks for each outcome (Table 5). *Bifidobacterium* exhibited the highest SUCRA values with respect to NEC incidence (SUCRA = 0.50). *Bacillus* showed a potential advantage in reducing the risk of gut associated sepsis incidence with highest SUCRA values (SUCRA = 0.38). Additionally, the performance of the probiotic mixture appeared to have the highest SUCRA value under the outcome mortality (SUCRA = 0.66). The funnel plots showed a clear visual asymmetry (Fig. 4). We identified no strong evidence of publication bias in our study.

3.7. Quality of evidence evaluation

Among all 45 direct and indirect comparisons for outcomes, the quality of evidence was down because of serious publication bias
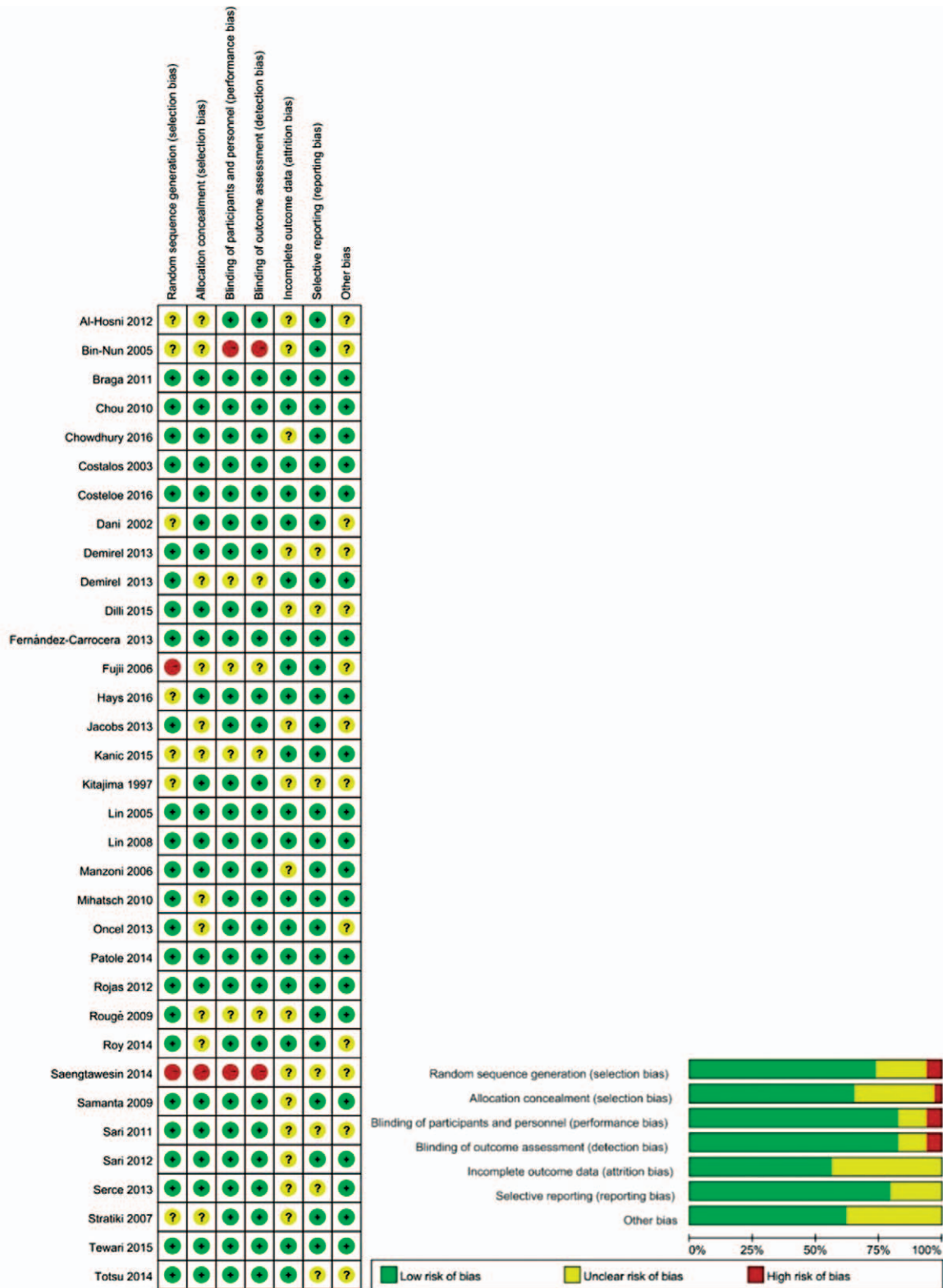
Figure 3. Risk of bias summary and graph showing authors judgement about each risk of bias item for the randomized trial.

Table 3

Meta-analysis results for incidence of NEC, sepsis and all-cause mortality.

compare with placebo	NEC	Sepsis	Mortality
Probiotic mixture	**0.38 [0.27, 0.54]**	**0.82 [0.69, 0.98]**	**0.54 [0.42, 0.71]**
Lactobacillus	**0.58 [0.37, 0.91]**	0.96 [0.70, 1.31]	0.76 [0.51, 1.13]
Bifidobacterium	**0.68 [0.50, 0.94]**	0.82 [0.65, 1.04]	0.85 [0.60, 1.21]
Bacillus	0.98 [0.14, 7.10]	0.75 [0.39, 1.43]	0.83 [0.37, 1.87]
Saccharomyces	0.82 [0.44, 1.54]	0.81 [0.51, 1.28]	1.35 [0.56, 3.24]

in all comparisons, for serious imprecision in 41 comparisons, and serious inconsistency in 35 comparisons. Node splitting found an obvious incoherence in comparisons for the NEC incidence (probiotic mixture vs *Bifidobacterium*), whereas no significant incoherence for other outcomes (sepsis and mortality). Ultimately, we determined the level of evidence for NMA in the 4 comparisons to be moderate, 3 to be low, and 38 to be very low (Table 6).

4. Discussion

The intestine of the preterm infants easily trended to colonized by pathogenic bacteria in the neonatal intensive care units (NICUs), which may be because of the delayed breastfeeding, early antibiotic intervention, and/or total parenteral nutrition.[20] This contributed to the higher incidence of NEC and gut associated sepsis. The recent literature reported the incidence of NEC up to 10% and the NEC-related mortality rate of up to 20%, which greatly affects the health and survival of preterm infants especially in NICUs.[20,49] There has been evidence that probiotics have the effect to prevent the severe complications of the preterm infants such as NEC, sepsis and mortality.[36,37,43] Probiotics act through many different mechanisms which are genera-specific. Unfortunately, there is no trial to explore the comparison of the effect of different strains to prevent preterm related complications. Network meta-analysis allowed comparisons of relative effectiveness between interventions that have never been compared head to head. Therefore, we performed an NMA to address the relative efficacy of different probiotic genera strategy of preventing severe complications in preterm infants.

Neither the traditional meta results or the NMA results both indicated that probiotic mixture and *Bifidobacterium* might show a greater availability to prevent the NEC incidence in preterm infants. Similarly, the performance of the probiotic mixture was still superior to other probiotic supplements under the outcome of all-cause mortality. The possible explanation is because normal flora is diverse in the gut, it might mean that the combination of probiotic strains is more rational, and our conclusions appear to support this hypothesis. The recent systematic review draws a same conclusion that the multistrain products performed more significant decline of NEC incidence when compared with a single organism.[16] Similarly, *Bifidobacterium* has its unique advantages to prevent the NEC incidence and inflammatory reactions in preterm infants in recent literature. Because *Bifidobacterium* might have more affinity with immature intestine, and reduce the butyric acid and up-regulate transforming growing factor A1 (that included potent anti-inflammatory effects and promoted epithelial cell proliferation and differentiation) to provide protection from preterm related complications.[11,22,27,36] In our analysis, traditional results revealed that *Lactobacillus* had the ability to reduce the incidence of NEC, while the NMA showed that it had no effect. We failed to confirm the accurate results from these paradoxical statistic results. These might provide a possibility that *Lactobacillus* was worth of deep investigation. Therefore, it might imply that probiotic mixture and *Bifidobacterium* could be the better option for preterm infants.

Furthermore, selecting an appropriate probiotic strategy merely according to the efficacy for preventing NEC and mortality might result in biased results. This is of specific importance, considering that safety remains a concern in the use of probiotics because probiotics are live bacteria supplement. It is

Table 4

Network meta-analysis results for the incidence of NEC (a), sepsis (b) and all-cause mortality (c).

Bacillus					
3.18 (0.22, 48.47)	Bifidobacterium				
1.91 (0.13, 25.61)	0.60 (0.18, 1.56)	Lactobacillus			
1.25 (0.08, 19.89)	0.40 (0.10, 1.24)	0.67 (0.21, 2.05)	Saccharomyces		
2.69 (0.19, 36.25)	0.86 (0.31, 1.95)	1.42 (0.60, 3.42)	2.15 (0.76, 6.22)	Probiotic mixture	
1.02 (0.08, 12.87)	**0.33 (0.13, 0.67)**	0.55 (0.26, 1.08)	0.82 (0.32, 2.01)	**0.38 (0.22, 0.62)**	Placebo
(a)					
Bacillus					
1.00 (0.33, 3.19)	Bifidobacterium				
0.78 (0.24, 2.48)	0.77 (0.40, 1.46)	Lactobacillus			
0.92 (0.26, 3.31)	0.91 (0.40, 2.04)	1.18 (0.51, 2.79)	Saccharomyces		
0.92 (0.31, 2.75)	0.92 (0.54, 1.50)	1.19 (0.66, 2.12)	1.01 (0.47, 2.15)	Probiotic mixture	
0.74 (0.25, 2.13)	0.74 (0.48, 1.09)	0.95 (0.58, 1.55)	0.80 (0.40, 1.61)	0.80 (0.58, 1.09)	Placebo
(b)					
Bacillus					
1.06 (0.27, 4.18)	Bifidobacterium				
1.10 (0.30, 4.41)	1.07 (0.43, 2.53)	Lactobacillus			
0.59 (0.12, 3.08)	0.56 (0.17, 1.91)	0.54 (0.16, 1.80)	Saccharomyces		
1.68 (0.50, 5.92)	1.57 (0.78, 3.36)	1.50 (0.75, 3.19)	2.81 (0.94, 8.69)	Probiotic mixture	
0.82 (0.26, 2.63)	0.78 (0.40, 1.43)	0.74 (0.39, 1.35)	1.37 (0.49, 3.86)	**0.49 (0.32, 0.69)**	Placebo
(c)					

Table 5

SUCRA values for the treatments under 3 endpoints.

Drug	NEC	Sepsis	Mortality
Bacillus	0.16	**0.38**	0.17
Bifidobacterium	**0.50**	0.24	0.06
Lactobacillus	0.06	0.05	0.08
Saccharomyces	0.02	0.23	0.02
probiotic mixture	0.25	0.10	**0.66**
placebo	0.00	0.00	0.00

reported that probiotics had the potential to cause probiotics related sepsis. We aimed at the end point of gut related sepsis to perform an analysis. The performance of all elected probiotic genus was superior to placebo under the outcome of sepsis. However, it did not mean that probiotics are safe absolutely. As with our concern, a few reported *Lactobacillus* bacteremia cases occurred in extremely sick infants who accessed to high doses of *Lactobacillus*.[50] *Lactobacillus* should be used with caution because excessive ingestion may cause a high risk of sepsis, and this may cause adverse effects on preterm infants.

In spite of the value of this question (namely, which is the best probiotic strategy to prevent preterm related complications), the evaluated data was not comprehensive. The statistical power of our network meta-analyses is relatively low because of a few direct comparisons as well as a few studies and patients in each indirect comparison.

Our study only aimed at the genera of probiotics to analysis, which may merely provide a research direction, not a specific probiotic treatment strategy. None of the studies reported the results according to dose categories albeit excess of probiotics might be connected with safety. Similarly, it is impossible to perform a subanalysis with small patient cohorts according to birthweight categories in every probiotic group. Considering the known increased morbidity of the preterm related complications for the very low birth weight infants, probiotic intervention in this subgroup might be even more hazardous and less efficacious. In addition, we found only one study reported long-term outcomes of oral probiotics and the study found no effect on neurodevelopment and growth.[29] Their long-term outcomes remain to be evaluated.

The outcomes of our network meta-analysis suggested that further investigations are necessary to explore the suitable
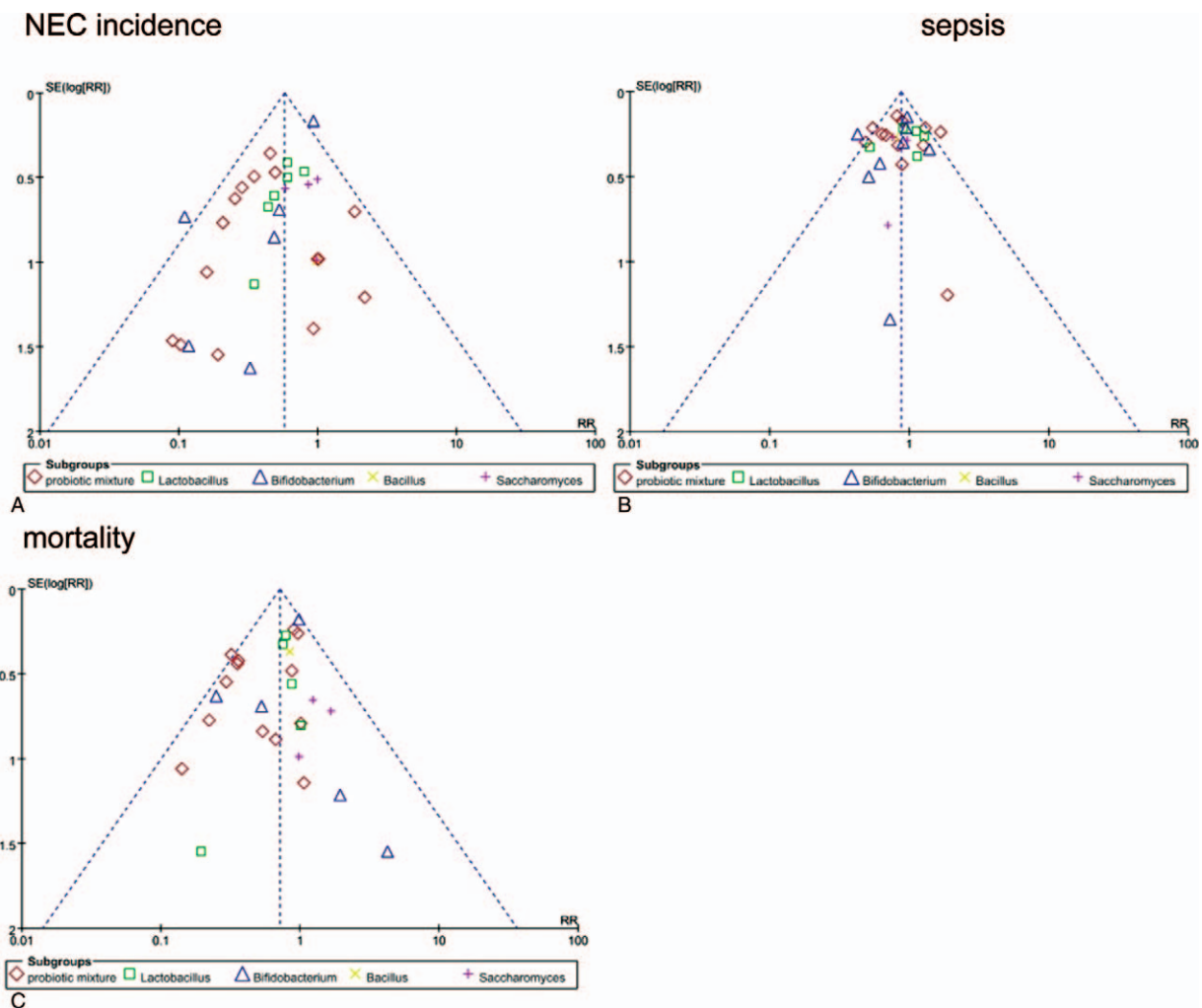

Figure 4. Comparison funnel plots for publication bias analysis.

Table 6

Network meta-analysis for all outcomes and quality-of-evidence assessment.

(A) incidence of NEC (the primary outcome)

	Events in control (n/N)[*]	Events in control (n/N)[*]	Direct odds ratios (95%CI)	QOE	Indirect odds ratios (95%CI)	QOE	Node splitting P value[†]	Network odds ratio (95%CI)	QOE
VS. placebo		Placebo							
Probiotic mixture	44/1776	113/1785	0.38 (0.27, 0.54)	Mod	NA	NA	NA	0.38 (0.27, 0.54)	Mod
Bifidobacterium	68/1266	98/1247	0.33 (0.13, 0.67)	VL	NA	NA	NA	0.33 (0.13, 0.67)	VL
Lactobacillus	31/1102	53/1108	0.58 (0.37, 0.91)	L	NA	NA	NA	0.58 (0.37, 0.91)	L
Saccharomyces	20/381	22/366	0.82 (0.44, 1.54)	VL	NA	NA	NA	0.82 (0.44, 1.54)	VL
Bacillus	2/123	2/121	0.82 (0.08, 7.10)	VL	NA	NA	NA	0.82 (0.08, 7.10)	VL
VS. probiotic mixture		Probiotic mixture							
Bifidobacterium	3/98	10/94	1.39 (0.01, 2.89)	VL	−0.10 (−1.13, 0.53)	VL	0.03	0.86 (0.31, 1.95)	VL
Lactobacillus	NA	NA	NA	NA	1.42 (0.60, 3.42)	VL	NA	1.42 (0.60, 3.42)	VL
Saccharomyces	NA	NA	NA	NA	2.15 (0.76, 6.22)	VL	NA	2.15 (0.76, 6.22)	VL
Bacillus	NA	NA	NA	NA	2.69 (0.19, 36.25)	VL	NA	2.69 (0.19, 36.25)	VL
VS. Saccharomyces		Saccharomyces							
Lactobacillus	NA	NA	NA	NA	0.67 (0.21, 2.05)	VL	NA	0.67 (0.21, 2.05)	VL
Bifidobacterium	NA	NA	NA	NA	0.40 (0.10, 1.24)	VL	NA	0.40 (0.10, 1.24)	VL
Bacillus	NA	NA	NA	NA	1.91 (0.13, 25.61)	VL	NA	1.25 (0.08, 19.89)	VL
VS. Lactobacillus		Lactobacillus							
Bifidobacterium	NA	NA	NA	NA	0.60 (0.18, 1.56)	VL	NA	0.60 (0.18, 1.56)	VL
Bacillus	NA	NA	NA	NA	1.91 (0.13, 25.61)	VL	NA	1.91 (0.13, 25.61)	VL
VS. Bifidobacterium		Bifidobacterium							
Bacillus	NA	NA	NA	NA	3.18 (0.22, 48.47)	VL	NA	3.18 (0.22, 48.47)	VL

See the legend at the end of this table series.

B) incidence of gut associated sepsis

	Events in control (n/N)[*]	Events in control (n/N)[*]	Direct odds ratios (95%CI)	QOE	Indirect odds ratios (95%CI)	QOE	Node splitting P value[†]	Network odds ratio (95%CI)	QOE
VS. Placebo		Placebo							
Probiotic mixture	293/1593	342/1604	0.82 (0.69, 0.98)	Mod	NA	NA	NA	0.82 (0.69, 0.98)	Mod
Bifidobacterium	163/1221	187/1201	0.82 (0.65, 1.04)	VL	NA	NA	NA	0.82 (0.65, 1.04)	VL
Lactobacillus	99/730	104/730	0.96 (0.70, 1.31)	L	NA	NA	NA	0.96 (0.70, 1.31)	L
Saccharomyces	42/290	49/276	0.81 (0.51, 1.28)	VL	NA	NA	NA	0.81 (0.51, 1.28)	VL
Bacillus	20/123	25/121	0.75 (0.39, 1.43)	VL	NA	NA	NA	0.75 (0.39, 1.43)	VL
VS. probiotic mixture		Probiotic mixture							
Bifidobacterium	17/98	16/94	0.00 (−1.17, 1.14)	VL	0.10 (−0.43, 0.67)	VL	0.87	0.92 (0.54, 1.50)	VL
Lactobacillus	NA	NA	NA	NA	1.19 (0.66, 2.12)	VL	NA	1.19 (0.66, 2.12)	VL
Saccharomyces	NA	NA	NA	NA	1.01 (0.47, 2.15)	VL	NA	1.01 (0.47, 2.15)	VL
Bacillus	NA	NA	NA	NA	0.92 (0.31, 2.75)	VL	NA	0.92 (0.31, 2.75)	VL
VS. Saccharomyces		Saccharomyces							
Lactobacillus	NA	NA	NA	NA	1.18 (0.51, 2.79)	VL	NA	1.18 (0.51, 2.79)	VL
Bifidobacterium	NA	NA	NA	NA	0.91 (0.40, 2.04)	VL	NA	0.91 (0.40, 2.04)	VL
Bacillus	NA	NA	NA	NA	0.92 (0.26, 3.31)	VL	NA	0.92 (0.26, 3.31)	VL
VS. Lactobacillus		Lactobacillus							
Bifidobacterium	NA	NA	NA	NA	0.77 (0.40, 1.46)	VL	NA	0.77 (0.40, 1.46)	VL
Bacillus	NA	NA	NA	NA	0.78 (0.24, 2.48)	VL	NA	0.78 (0.24, 2.48)	VL
VS. Bifidobacterium		Bifidobacterium							
Bacillus	NA	NA	NA	NA	1.00 (0.33, 3.19)	VL	NA	1.00 (0.33, 3.19)	VL

See the legend at the end of this table series

C) incidence of all-cause mortality

	Events in control (n/N)[*]	Events in control (n/N)[*]	Direct odds ratios (95%CI)	QOE	Indirect odds ratios (95%CI)	QOE	Node splitting P value[†]	Network odds ratio (95%CrI)	QOE
VS. Placebo		Placebo							
probiotic mixture	98/1822	165/1735	0.54 (0.42, 0.71)	Mod	NA	NA	NA	0.54 (0.42, 0.71)	Mod
Bifidobacterium	64/1169	75/1159	0.85 (0.60, 1.21)	VL	NA	NA	NA	0.85 (0.60, 1.21)	VL
Lactobacillus	45/1016	59/1020	0.76 (0.51, 1.13)	L	NA	NA	NA	0.76 (0.51, 1.13)	L
Saccharomyces	12/330	9/330	1.35 (0.37, 1.87)	Mod	NA	NA	NA	1.35 (0.37, 1.87)	Mod
Bacillus	12/123	14/121	0.83 (0.37, 1.87)	VL	NA	NA	NA	0.83 (0.37, 1.87)	VL
VS. probiotic mixture		Probiotic mixture							
Bifidobacterium	3/98	6/94	0.72 (−1.13., 2.82)	VL	0.45 (−0.03, 1.27)	VL	0.78	1.57 (0.78, 3.36)	VL
Lactobacillus	NA	NA	NA	NA	1.50 (0.75, 3.19)	VL	NA	1.50 (0.75, 3.19)	VL
Saccharomyces	NA	NA	NA	NA	2.81 (0.94, 8.69)	VL	NA	2.81 (0.94, 8.69)	VL
Bacillus	NA	NA	NA	NA	1.68 (0.50, 5.92)	VL	NA	1.68 (0.50, 5.92)	VL
VS. Saccharomyces		Saccharomyces							
Lactobacillus	NA	NA	NA	NA	0.54 (0.16, 1.80)	VL	NA	0.54 (0.16, 1.80)	VL
Bifidobacterium	NA	NA	NA	NA	0.56 (0.17, 1.91)	VL	NA	0.56 (0.17, 1.91)	VL
Bacillus	NA	NA	NA	NA	0.59 (0.12, 3.08)	VL	NA	0.59 (0.12, 3.08)	VL
VS. Lactobacillus		Lactobacillus							
Bifidobacterium	NA	NA	NA	NA	1.07 (0.43, 2.53)	VL	NA	1.07 (0.43, 2.53)	VL
Bacillus	NA	NA	NA	NA	1.10 (0.30, 4.41)	VL	NA	1.10 (0.30, 4.41)	VL
VS. Bifidobacterium		Bifidobacterium							
Bacillus	NA	NA	NA	NA	1.06 (0.27, 4.18)	VL	NA	1.06 (0.27, 4.18)	VL

NA = Not available due to no direct comparison or no indirect comparison; VL = very low.

[*] The numbers (n/N) showed the sums of the numbers of infants who had the outcome in each strategy group (n) /the sums of the numbers of infants included in each strategy group (N) in trials directly comparing the two strategies.

[†] The direct and indirect odds ratios were estimated by node splitting when the comparisons had both direct and indirect comparisons. A smaller P value indicated a higher probability of incoherence between direct and indirect effect estimates. A P value of <.05 indicated significant incoherence.

probiotic treatment strategy, the optimal dose, the long-term safety and the preterm infants who benefit the most.

5. Conclusion

The recent literature has reported a total of 5 probiotic strategies, including *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Saccharomyces*, and probiotic mixture. Our thorough review and NMA provided a piece of available evidence to choose optimal probiotics prophylactic strategy for premature infants. The results indicated that probiotic mixture and *Bifidobacterium* showed a stronger advantage to use in preterm infants; the other probiotic genera failed to show an obvious effect to reduce the incidence of NEC, sepsis and all-cause death. More trials need to be performed to determine the optimal probiotic treatment strategy to prevent preterm related complications.

Acknowledgments

Author contributions

LW Bi contributed the most to the study and should be considered first author. BL Yan contributed the same as the first authors. Le-wee Bi conceptualized and designed the study, drafted the initial manuscript, and interpreted the data; Bei-lei Yan conducted the initial analyses and drafted the initial manuscript; Qian-yu Yang conceptualized and designed the study and supervised the analysis; Miao-miao Li conducted the meta-analyses, interpreted the data, and reviewed the manuscript; and Hua-lei Cui conceptualized and designed the study, supervised the analysis, interpreted the data, and reviewed the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.
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