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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{9} The function of probiotics is species specific, depending on morphological, physiological, and biochemical characteristics of the different probiotic genera.\textsuperscript{7} Recent review studies showed that Bifidobacterium significantly decreases the incidence of NEC and...
mortality rates.\textsuperscript{[10]} Bifidobacterium breve may improve intestinal tolerance, which was reported by Kitajima et al.\textsuperscript{[11]} Additionally, in other literature, the effect of a single strain might also differ from combined strains in NEC.\textsuperscript{[12]} Reports showed that supplements of a combination of strains of Bifidobacterium species\textsuperscript{[13]} or a combination of Bifidobacterium and Lactobacillus acidophilus\textsuperscript{[14]} might achieve an earlier total gastrointestinal nutrition. Moreover, other clinical trials considered different strains of probiotics including Lactobacillus\textsuperscript{[15,16]} and Saccharomyces boulardii.\textsuperscript{[18]} 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 sporo-
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.\textsuperscript{[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\textsuperscript{2}. When I\textsuperscript{2} was <50%, there was no obvious heterogeneity in the analysis, and the fixed effect model was used. When the I\textsuperscript{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.
| Study | Design | Simple size (P/C) | Patients | Probiotic strain(s) | Probiotic duration | Outcomes |
|-------|--------|------------------|----------|--------------------|--------------------|----------|
| Braga et al[12] | RCT | 119/12 | Preterm; BW 750–1499 g; admission to hospital | L. casei (0.5 × 10^9 CFU OD) and B. breve (3.5 × 10^6 CFU OD) | From day 2 to day 30 or the occurrence of NEC, sepsis | NEC, sepsis, death |
| Lin et al[13] | RCT | 180/187 | Preterm; BW < 1500 g; enteral nutrition; age > 7 d | L. acidophilus and B. infantis (≥10^9 CFU each) | From enteral feeding to discharge | NEC |
| Al-Hosni et al[23] | RCT | 50/51 | Preterm; BW 501–1000 g; appropriate for GA, and <14 d of age at the time of feeding initiation | L. casei and L. acidophilus (≥10^7 CFU OD) | From first enteral feeding to discharge or PMA > 34 wk | Weight gain, NEC, sepsis, mortality, mean volume of feeding growth, height, weight, HC, neurodevelopmental and sensory outcomes, NEC, death, CLD, sepsis, hospitalization, visual impairment, deafness, cerebral palsy |
| Chou et al[24] | RCT | 153/148 | Preterm very low birth weight infants; enteral feeding; age > 7 d | L. acidophilus and B. infantis (≥10^9 CFU each) | From enteral feeding to discharge | Growth, weight, NEC, sepsis, hospitalization, duration of parenteral nutrition, days to full enteral feeds, weight at 28 d, ROP, NEC, discharge weight, NEC |
| Lin et al[25] | RCT | 217/217 | Preterm; GA < 32 wk; BW < 1500 g; age ≥10 d of age at the time of feeding initiation | L. acidophilus NCDO 1748 and B. infantis (≥10^9 CFU OD) | From first enteral feeding to discharge | NEC, sepsis, death, IVH, CLD, NCU stay, weight gain |
| Hays et al[14] | RCT | 54/551 | Preterm; GA < 32 wk; BW < 1500 g; enteral nutrition | B. infantis BB-02 (0.5 × 10^9 CFU OD) and S. thermophilus Th-4 (350 CFU × 10^6) | From enteral feed to discharge or term corrected age | NEC, sepsis, death, hospitalization, duration of parenteral nutrition, days to full enteral feeds, weight at 28 d, ROP, QD, MH |
| Jacobs et al[26] | RCT | 77/76 | Preterm; GA < 33 wk; BW < 1500 g; enteral feeds for <12 h | B breve (3 × 10^8 CFU OD) | From enteral feed to corrected age of 37 wk | NEC, sepsis, death, hospitalization, NEC |
| Patole et al[27] | RCT | 51/56 | GA 28–32 wk; no major PDI problems; not receiving antibiotics; not receiving breast milk | S. thermophilus (1 × 10^8 CFU OD) | 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 |
| Costiloe et al[28] | RCT | 56/56 | Preterm; GA < 37 wk; BW < 2500 g; enteral feeding; age ≥72 h | L. acidophilus (1.25 × 10^9 CFU OD) and B. bifidum (1.25 × 10^8 CFU OD) | 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. acidophilus (3.0 × 10^9 CFU OD) | From first feed to discharge | Weight gain, length, HC, CLD, hospitalization, NEC, sepsis, feeding intolerance, oxygen days, full-feeding days |
| Costiloe et al[30] | RCT | 604/660 | GA 23–30 wk | B breve (1.6 × 10^9 CFU OD) | Until corrected age of 36 wk | NEC, sepsis, death, NEC |
| Fernández-Carrozana et al[31] | RCT | 75/75 | Preterm; BW < 1500 g infants with NEC IA and IB were excluded | S. thermophilus (6.6 × 10^8 CFU/g) and L. rhamnosus (1 CFU/g) | From enteral feeding, non-specified end time | Weight gain, length, HC, CLD, hospitalization, NEC, sepsis, feeding intolerance, oxygen days, full-feeding days |
| Minazzi et al[32] | RCT | 39/41 | BW < 1500 g, age ≥ 3 d, not receive any form of antimicrobial prophylaxis other than LGG | B breve (2 × 10^8 CFU/g) and L. rhamnosus (4.4 × 10^9 CFU/g) | From the third day of life to the age of sixth week or discharge from the NICU | Hospitalization, time of achievement of full feedings, NEC |
| Mihaطب et al[33] | RCT | 91/93 | Preterm; GA < 30 wk; BW < 1500 g | B lactis (2 × 10^9 CFU/g) | From enteral feeding, non-specified end time | NEC, death, necrotizing enterocolitis |
| Onnel et al[34] | RCT | 200/200 | Preterm; GA < 32 wk; BW < 1500 g; enteral feeding | L. reuteri (1 × 10^9 CFU/g) | From first feed to death or discharge | NEC, sepsis, death, hospitalization, feeding intolerance |
| Rajo et al[35] | RCT | 372/378 | Preterm; BW ≤ 2000 g, age ≥ 48 h; HS; enteral feeding | L. reuteri (1 × 10^9 CFU/g) | From age ≥ 48 h to death or discharge | Death, duration of a hospital, NEC, sepsis |
| Raoufi et al[36] | RCT | 45/49 | Preterm; GA < 32 wk; BW < 1500 g, age ≤ 2 wk, excluded non-preterm birth related diseases; enteral feeding | B longum 88838 and L. rhamnosus GG (1 × 10^9 CFU/g) | From enteral feeding to discharge | Nutrition–total calories delivered enterally, duration of hospital stays, death, oxygen therapy duration |
| Samanta et al[37] | RCT | 91/95 | Preterm; GA < 32 wk; BW < 1500 g; enteral feeding; age ≥ 48 h | L. acidophilus; B. bifidum and B. infantis (2.5 × 10^9 CFU OD, BD) | From enteral feeding to discharge, | NEC, death, hospitalization |
| Strakl et al[38] | RCT | 41/34 | Preterm; GA 27–32 wk, formula-fed, without major congenital anomalies | B lactis (2 × 10^6 CFU/g of milk powder) | From enteral feeding, non-specified end time | Weight, length, NEC, time to full enteral feed |
| Choudhury et al[39] | RCT | 52/50 | Preterm; GA < 32 wk; BW < 1500 g; enteral feeding; age ≥ 48 h | Capsule TS6 probiotic containing Bifidobacterium spp, and Lactobacillus (6 × 10^9 CFU) | From first feeding to discharged | NEC, hospital stay |
| Denisel et al[40] | RCT | 91/90 | GA < 32 wk; lowest GA; BW 24 wk; BW < 1500 g (lowest BW 500 g) | B. longum 88838 and L. rhamnosus GG (1 × 10^9 CFU/g) | From first feed to discharge | Feeding amount, full feeding day, weight, NEC, sepsis |
| Dari et al[41] | RCT | 295/290 | GA < 32 wk; or BW < 1500 g | L. reuteri (1 × 10^9 CFU OD) | From first feed to discharge | Antibiotic treatment, UTI, sepsis, NEC |
| Sari et al[42] | RCT | 110/111 | GA < 32 wk or BW < 1500 g; enteral feeding | L. casei (0.35 × 10^9 CFU OD) | From first feed to discharge | (continued)
| Study          | Design | Simple size (P/C) | Patients                                                                 | Probiotic strain(s)                        | Probiotic duration                                                                 | Outcomes                                                                 |
|---------------|--------|-------------------|--------------------------------------------------------------------------|--------------------------------------------|------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Demirel et al[41] | RCT    | 135/136           | GA ≤ 32 w; BW ≤ 1500 g, enteral feeding                                  | *S. boulardii* (5 × 10⁹ CFU OD)            | From first feed to discharge                                                       | NICU stay; NEC; death; weight gain; feeding intolerance; Feeding amount; full feeding day; weight; NEC; sepsis                  |
| DHL 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 × 10⁹ CFU)                 | From beyond d 7 after birth to death or discharge (max 6 w)                        | Height, weight, HC, NEC, sepsis, feeding intolerance, PVL, stay at NICU, mortality                                    |
| Fuji et al[43] | RCT    | 11/8              | GA < 34 wk                                                              | *B. breve* MI-186V (1 × 10¹⁰ CFU BD)       | Non-specified start, until discharge                                               | Duration of hospital stay, NEC, CLD, ROP, infection                      |
| Karic et al[44] | RCT    | 40/40             | GA < 33 wk; BW < 1500 g                                               | *L. acidophilus*, *Enterococcus faecium* and *B. infantis* (0.6 × 10⁹ CFU BD) | From enteral feeding to discharge                                                 | Hospitalization, late-onset sepsis, pneumonia, NEC, death, meningitis, URT, and emphysema                          |
| Sarto et al[45] | RCT    | 31/29             | GA < 34 wk; BW ≤ 1500 g                                               | *L. acidophilus* (1 × 10⁹ CFU) *B. bifidum* (1 × 10⁹ CFU) | From feeding to 6 w of age or discharge                                            | Feeding amount; weight gain; length of stay; NEC; sepsis; ROP; PVL; NH, NEC, weight gain; death                       |
| Terai et al[46] | RCT    | 104/104           | GA ≤ 32 wk; BW ≤ 1500 g, enteral feeding                              | *S. boulardii* (0.5 × 10⁹ CFU/kg BD)       | Non-specified start time and end time                                               | NIC; weight gain; death; hospitalization; apneas, time to reach 100 ml/kg/d of oral feeding                          |
| Totsu et al[47] | RCT    | 153/130           | BW < 1500 g                                                            | *B. breve* (2.5 × 10⁹ CFU, divided in two doses) | From age 24 h to postnatal age of 6 wk; discharge or death                         | Sepsis; NEC; feed intolerance; death                                      |
| Kitajima et al[48] | RCT    | 45/46             | BW < 1500 g                                                            | *B. breve* M4010 (0.5 × 10⁹ CFU OD)        | Start within 24 h after birth, until body weight 2000 g                           | Body weight; hospitalization; sepsis, HC; accelerated the establishment of enteral feeding; death; NEC, CLD            |
| Bin-Nun et al[22] | RCT    | 72/73             | Preterm; BW < 1500 g, enteral feeding                                  | *S. thermophilus*, *B. bifidus* and *B. infantis* (0.35 × 10⁹ CFU OD) | From enteral feeding to 36 wk of age; postconceptional age.                        | Weekly change of aspirated air volume from stomach, Frequency of vomiting and apneas; Duration of antibiotics; NEC, Weight gain | NEC, TPN                                                                                                                                 |

**Notes:**
- BW = birth weight, CLD = chronic lung disease, GA = gestational age, GI = gastrointestinal, HS = hemodynamically stable, IH = 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, RES = respiratory distress syndrome, ROP = retinopathy of prematurity, TPN = total parenteral nutrition, URT = 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). 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. 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 approach. The lower level of evidence is used as the level of evidence for indirect comparison represented 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 level of 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 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). The characteristics of included studies 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.

3.2. 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: Lactobacillus 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. The risk assessment of all included RCTs 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, Lactobacillus (OR = 0.58, 95% CI: 0.37–0.91) and Bifidobacterium (OR = 0.68, 95% CI: 0.30–0.94) 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)
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 judgment about each risk of bias item for the randomized trial.
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] |

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. 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. There has been evidence that probiotics have the effect to prevent the severe complications of the preterm infants such as NEC, sepsis and mortality. Probiotics act through many different mechanisms which are genus-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. 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. 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
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

### Table 5

| 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      |

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.

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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 probiotic mixture (n/N) | Direct odds ratios (95%CI) | QOE | Indirect odds ratios (95%CI) | QOE | Node splitting P value$^*$ | Network odds ratio (95%CI) | QOE |
|-------------------------|----------------------------------|---------------------------|-----|-----------------------------|-----|---------------------------|---------------------------|-----|
| Bacillus                | NA NA NA NA 1.10 (0.30, 4.41) VL | NA NA NA NA 0.38 (0.27, 0.54) Mod | NA NA NA NA 0.82 (0.44, 1.54) VL | NA NA NA NA 0.82 (0.08, 7.10) VL |

#### (B) incidence of gut associated sepsis

| Events in control (n/N) | Events in probiotic mixture (n/N) | Direct odds ratios (95%CI) | QOE | Indirect odds ratios (95%CI) | QOE | Node splitting P value$^*$ | Network odds ratio (95%CI) | QOE |
|-------------------------|----------------------------------|---------------------------|-----|-----------------------------|-----|---------------------------|---------------------------|-----|
| Bacillus                | NA NA NA NA 1.10 (0.30, 4.41) VL | NA NA NA NA 1.10 (0.30, 4.41) VL | NA NA NA NA 1.10 (0.30, 4.41) VL | NA NA NA NA 1.10 (0.30, 4.41) VL |

#### (C) Incidence of all-cause mortality

| Events in control (n/N) | Events in probiotic mixture (n/N) | Direct odds ratios (95%CI) | QOE | Indirect odds ratios (95%CI) | QOE | Node splitting P value$^*$ | Network odds ratio (95%CI) | QOE |
|-------------------------|----------------------------------|---------------------------|-----|-----------------------------|-----|---------------------------|---------------------------|-----|
| Bacillus                | NA NA NA NA 1.10 (0.30, 4.41) VL | NA NA NA NA 1.10 (0.30, 4.41) VL | NA NA NA NA 1.10 (0.30, 4.41) VL | NA NA NA NA 1.10 (0.30, 4.41) VL |

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

$^*$ 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.

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Author contributions
IW Bi contributed the most to the study and should be considered first author. BL Yan contributed the same as the first authors. Le-wei 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 and agree to be accountable for all aspects of the work.

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