Original Article

Safety and efficacy of probiotics in the prevention of necrotizing enterocolitis in premature and/or low-birthweight infants: a systematic review and meta-analysis

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Background: Neonatal necrotizing enterocolitis (NEC) is a serious pediatric gastrointestinal disease and a cause of death in neonates, especially in premature infants. The addition of probiotics to the diet can reduce the incidence and severity of neonatal NEC. This meta-analysis explored the preventive effect of probiotics on NEC.

Methods: Endnote X9 software was used to search for relevant studies in the Ovid, Embase, PubMed, and Web of Science databases. The search terms were "probiotics" and "necrotizing enterocolitis". After retrieval, screening, and quality evaluation of the studies, Stata 16.0 software was used to analyze the data.

Results: A total of 10 studies, which collectively included 3,227 patients, were selected for analysis. Of them, 5 used a multiple-strain probiotics, and 5 used single-strain probiotic. Meta-analysis showed that treatment with probiotics could reduce the incidence of severe NEC [risk ratio (RR) =0.66; 95% confidence interval (CI): (0.50, 0.87); Z=−2.978; P=0.003], reduce mortality in underweight premature children [RR =0.81; 95% CI: (0.70, 0.94); Z=−2.864; P=0.004], and reduce the incidence of feeding intolerance [RR =0.78; 95% CI: (0.67, 0.90); Z=−3.280; P=0.001].

Discussion: The addition of probiotics to the diet of low-birthweight and premature infants can reduce the incidence of severe NEC and reduce related mortality rates.

Keywords: Probiotics; preterm infants; low-birthweight infants; necrotizing enterocolitis; meta-analysis

Introduction

Background

Neonatal necrotizing enterocolitis (NEC) is a serious pediatric gastrointestinal disease and a cause of death in neonates, especially premature infants (1). In pediatric intensive care units, 90% of newborns with NEC are premature, and lower gestational ages and birthweights carry a higher probability of NEC. In very-low-birthweight (VLBW) premature infants, the incidence of NEC can reach 7% (2). The mortality rate of NEC is between 15% and 30%, while the mortality rate of severe NEC requiring surgical intervention is up to 40% or 50%. In the case of survivals of severe NEC, there are long-term adverse

effects, such as infection, malnutrition, dysplasia, and retinal disease which could bring low quality of life for the children (3). The occurrence of NEC may be associated with various factors such as immature intestinal development, ischemia and hypoxia, dysbacteriosis, and a disturbed immune response in premature infants, but there is no complete pathogenesis (4). NEC begins with intestinal epithelial injury and bacterial invasion, causing an inflammatory cascade that leads to intestinal perforation, necrosis, sepsis, and death. The symptoms of NEC in children are diverse, including bradycardia, unstable body temperature, abdominal distension, abdominal pain, vomiting, and hematochezia with intestinal obstruction and pneumatosis intestinalis observed with radiological examination (5).

Purpose

Previous studies have suggested that advocating breastfeeding, dietary additions of epidermal growth factor or erythropoietin, or oral arginine may prevent NEC (6,7). In recent years, many controlled clinical studies have investigated the role of probiotics in preventing NEC, and researchers have found that adding probiotics to the diet can reduce the incidence and severity of NEC (8). A meta-analysis conducted by Athalye-Jape *et al.* (9) included 29 studies and showed that the addition of probiotics reduced the incidence of NEC in premature VLBW newborns from 60% to 31.7%. However, but the outcome indicator of the meta-analysis was limited to NEC rates, not including different serious grade of NEC and the analysis did not distinguish between the species of probiotics. There is a need of meta-analysis to reassess the role of probiotics in NEC prevention with new indicators and subgroup analysis based on multiple- or single-strain probiotics. We carried out a new meta-analysis on this topic, adding several indicators and comparing the preventive effect of multiple- and single-strain probiotics on NEC to better understand the preventive effect of probiotics as a clinical reference.

We present the following article in accordance with the PRISMA reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-22-27/rc).

Methods

Inclusion criteria

We defined the inclusion of eligible studies according to the

PICOS criteria (Patients, Intervention, Control, Outcome, Study).

Study type

We searched for randomized controlled trials (RCTs). Quasi- and non-randomized controlled clinical trials (CCTs) were excluded.

Participants

All patients were premature infants. As the criteria for defining premature delivery varies, the studies selected for this analysis had to include a definition of premature delivery, clearly specifying the gestational age of the study participants, which was generally 34 weeks. Participants were all VLBW neonates, with a bodyweight lower than 1,500 g as the standard.

Intervention

All selected studies randomly divided newborns into experimental and control groups. Newborns in the two groups were breastfed. Those in the experimental group had probiotics added to their diet, while those in the control group were given either a placebo or no addition to their diet. We did not limit the species of probiotics or whether the probiotics were multiple-strain. Studies that added non-probiotics, such as prebiotics and synbiotics, were excluded.

Control

All studies needed to be grouped according to random methods. We did not limit whether the allocation concealment was used in the study or whether the blind method was described, but we would conduct bias analysis and quality evaluation before analysis.

Outcome indicators

The studies did not need to have a set follow-up time to be considered, but they needed to describe the start and end points of the treatments. For our statistical analysis, the main outcome indicators were as follows: (I) incidence of patients with NEC stage ≥2; (II) mortality. Secondary outcomes indicators were the following: (I) total incidence of NEC; (II) incidence of feeding intolerance. NEC grading was performed according to Bell's criteria (10).

Search strategy

We used the literature search function in Endnote X9

software to search the Ovid, Embase, PubMed, and Web of Science databases using the search key words "probiotics" and "necrotizing enterocolitis", the search process was done from Oct to Nov of 2021.

Literature screening and data extraction

Two researchers independently reviewed the studies. After eliminating duplicate studies, the researchers screened the articles by title and abstract, following the inclusion criteria for article type, participant type, intervention description, and outcome indicators. Any conflicts of opinion between the two researchers were resolved with the intervention of a third researcher.

Once the initial screening process was complete, the two researchers independently read each selected study and extracted the data, recording it in a pre-prepared form. The extracted data included author name, publication time, number of participants, participants' gestational age, weight, gender, antibiotic use, number of cases in the two study groups, treatment method used in each group, and outcome indicator data for the two groups. All possible efforts were made to obtain data relevant to the selected studies. However, if the data from a study was not accessible, the study was excluded from the meta-analysis.

Bias assessment and quality evaluation

Risk of bias assessment was performed according to the Cochrane Collaboration Handbook for Systematic Reviews of Intervention from 6 aspects: (I) random allocation method; (II) blind method; (III) implementation of allocation concealment; (IV) data integrity; (V) selective reporting bias; (VI) other biases. We considered a study to be of high quality (grade A) if it met the following criteria: random method, allocation concealment, and blind method; and included the following information: withdrawal or loss to follow-up, intention analysis, and baseline conditions. Grade B was awarded to studies in which these quality evaluation criteria were only partially satisfied (or unclear), and studies in which the criteria were completely unsatisfied were given a grade C.

Statistical analysis

Stata 16.0 software (Stata Corp LLC, TX, USA) was used for statistical analysis in this meta-analysis. The Mantel-Haenszel model was used to analyze discrete data (NEC incidence and mortality). Effect sizes were expressed as risk ratios (RRs), and a forest plot was used to display results. We used the Cochran Q test and I^2 to assess the heterogeneity of the studies, with $I^2>50\%$ or $P<0.1$ indicating statistically significant heterogeneity. Subgroup heterogeneity was investigated and a L'abbé plot was introduced to show the overall heterogeneity. If the source of heterogeneity was not found, only general descriptive analysis was performed. Funnel plots were used to represent publication bias.

Results

Literature search results

Figure 1 shows the results of the literature search and screening process.

Basic characteristics and quality assessment of the included studies

A total of 10 articles, which collectively included 3,227 patients (11-20), were included in the meta-analysis. The studies were all published between 2005 and 2016. A total of 5 studies (11-14,17) used multi-strain probiotic mixtures in their treatments, while the other 5 (15,16,18-20) used single-strain probiotic. Newborns in all the studies had a gestational age below 34 weeks and birthweights under 1,500 g (*Table 1*).

Bias analysis of the selected studies

Although the randomization methods were not specified, all studies included in this meta-analysis claimed to be randomized. Two studies (11,17) did not mention allocation concealment or blinding. All studies included information about dropouts and withdrawals, with no selective reporting or other biases shown (*Table 2*).

Meta-analysis results

Incidences of stage ≥2 NEC
A total of 7 studies (12-18) reported incidences of stage ≥2 NEC, including 4 studies using multi-strain probiotic treatment and 3 studies using single-strain probiotic treatment. The collective experimental group included 947 patients, and the control group included 946 patients. There was no statistical heterogeneity among the 7 studies ($I^2=0\%$; $P=0.581$). Fixed effects model analysis yielded a pooled RR
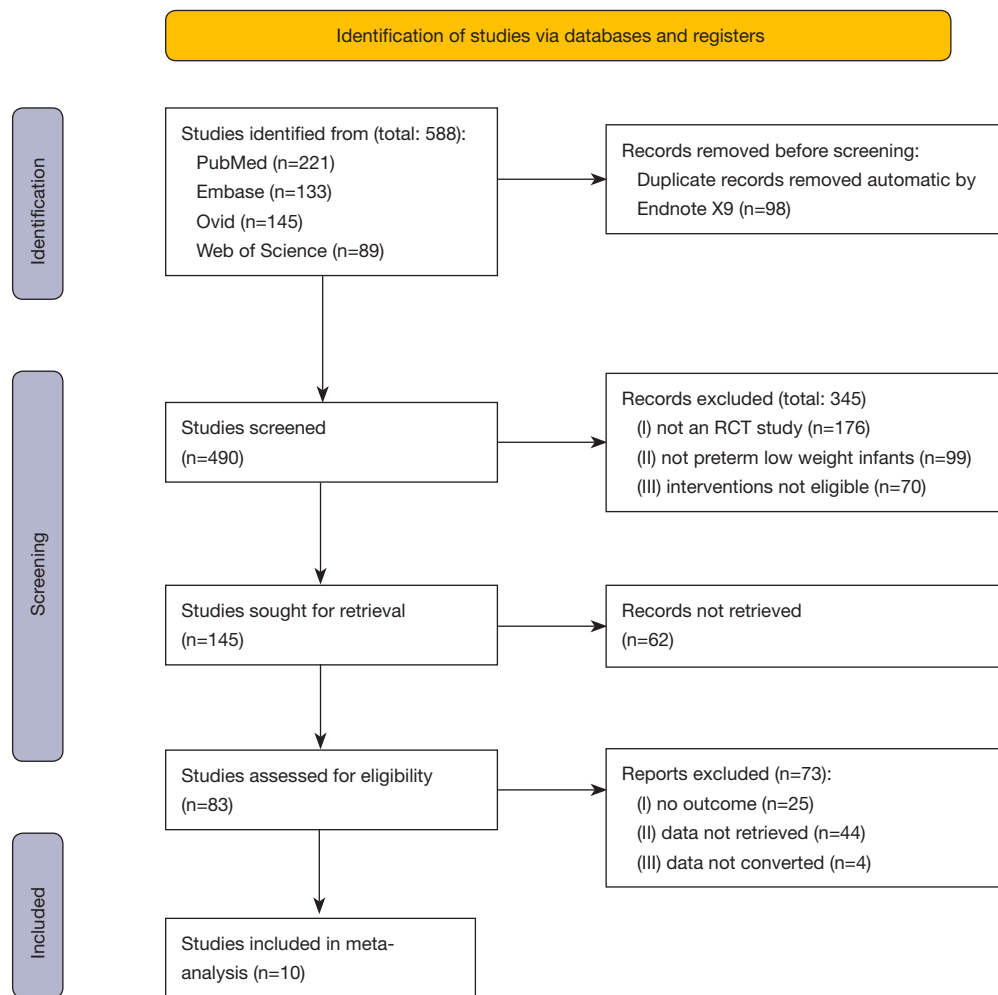
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Identification

Studies identified from (total: 588):
  PubMed (n=221)
  Embase (n=133)
  Ovid (n=145)
  Web of Science (n=89)

Records removed before screening:
  Duplicate records removed automatic by
  Endnote X9 (n=98)

Screening

Studies screened
(n=490)

Records excluded (total: 345)
  (I) not an RCT study (n=176)
  (II) not preterm low weight infants (n=99)
  (III) interventions not eligible (n=70)

Studies sought for retrieval
(n=145)

Records not retrieved
(n=62)

Studies assessed for eligibility
(n=83)

Reports excluded (n=73):
  (I) no outcome (n=25)
  (II) data not retrieved (n=44)
  (III) data not converted (n=4)

Included

Studies included in meta-
analysis (n=10)

Figure 1 Literature search and screening process flow chart. RCT, randomized controlled trial.

statistic of 0.66, 95% confidence interval (CI): (0.50, 0.87), suggesting that intervention with probiotics could reduce the incidence rate of severe NEC ($Z=-2.978$; $P=0.003$). Our meta-analysis divided studies into two subgroups (multi- and single-strain), according to whether multiple- or single-strain probiotic were used in the treatment. There was no heterogeneity in either subgroup. The pooled RR for the multi-strain probiotics group was 0.50, 95% CI: (0.32, 0.78), with a statistically significant difference ($P=0.002$). The pooled RR for the single-strain probiotic group was 0.85, 95% CI: (0.61, 1.20). The difference was not statistically significant ($P=0.361$) (*Figure 2*).

Mortality

Mortality was reported in all articles (11-20), including 5 studies using multi-strain probiotics and 5 studies using

single-strain probiotic. The collective experimental group included 1,723 patients, and there were 1,735 in the control group. There was no statistical heterogeneity among the 10 articles ($I^2=36.9\%$; $P=0.114$). A fixed effects model analysis yielded a pooled RR of 0.81, 95% CI: (0.70, 0.94), suggesting that intervention with probiotics could reduce mortality in underweight preterm infants ($Z=-2.864$; $P=0.004$). The pooled RR statistic for the multi-strain probiotics group was 0.63, 95% CI: (0.48, 0.82), with a statistically significant difference ($P=0.001$). The pooled RR for the single-strain probiotic group was 0.94, 95% CI: (0.80, 1.11), and the difference was not statistically significant ($P=0.470$) (*Figure 3*).

Incidence of NEC

Four studies (11,17,19,20) reported the total incidence rate

Table 1 Basic characteristics of included studies and participants and study quality evaluation scores

Author, year	Gestational age (weeks)	Birthweight (g)	E:C samples	Experimental group method	Control group method	Feeding method	Outcome indicators	Grade
Bin-Nun et al., 2005 (11)	29.8±2.6	1,152±262	72:73	Probiotics mixture 1	Placebo	HM	(a)(b)(d)	A
Lin et al., 2008 (12)	N/A	1,028.9±246.0	217:217	Probiotics mixture 2	Placebo	HM	(c)(d)	B
Samanta et al., 2009 (13)	30.12±1.63	1,172±143	91:95	Probiotics mixture 1	Placebo	HM	(c)(d)(e)	B
Braga et al., 2011 (14)	29.5±2.5	1,194.7±206.3	119:112	Probiotics mixture 2	Placebo	HM	(c)(d)	B
Sari et al., 2011 (15)	29.5±2.4	1,231±262	110:111	Lactobacillus sporogenes	Placebo	HM	(c)(d)(f)	A
Demirel et al., 2013 (16)	28.0 (range, 17–45)	1,164±261	135:136	Saccharomyces boulardii	Placebo	HM	(c)(d)(e)(f)	B
Fernández-Carrocera et al., 2013 (17)	31.2 (range, 26–35.4)	1,090 (range, 580–1,495)	75:75	Probiotics mixture 3	Placebo	HM	(a)(c)(d)(e)	A
Oncel et al., 2014 (18)	28.2±2.4	1,071±274	200:200	Lactobacillus reuteri	Placebo	HM	(a)(c)(d)(e)(f)	A
Van Niekerk et al., 2015 (19)	24–34	500–1,250	54:56	Lactobacillus rhamnosus GG	Placebo	HM	(a)(d)	A
Costeloe et al., 2016 (20)	28 (26.1–29.1)	1,039±312	650:660	Bifidobacterium breve	Placebo	HM	(a)(d)	A

multiple-strain probiotics: 1= *Bifidobacteria infantis, Streptococcus thermophilus*, and *Bifidobacteria bifidus*; 2= *Bifidobacterium* and *Lactobacillus*; 3= *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Bifidobacteruim infantis*, and *Streptococcus thermophilus*. Outcome indicators: (a) overall NEC incidence, (b) NEC severity score, (c) NEC stage ≥2, (d) mortality, (e) length of stay, (f) feeding intolerance. E:C, experimental: control; N/A, not available; HM, human milk; NEC, neonatal necrotizing enterocolitis.

Table 2 Risk of bias assessment based on Cochrane Collaboration criteria

| Study	Random sequence generation	Allocation concealment	Blind method	Data integrity	Optional reporting	Other bias
Bin-Nun et al., 2005 (11)	Low	Unclear	Unclear	Low	Low	Low
Lin et al., 2008 (12)	Low	Low	Low	Low	Low	Low
Samanta et al., 2009 (13)	Low	Low	Low	Low	Low	Low
Braga et al., 2011 (14)	Low	Low	Low	Low	Low	Low
Sari et al., 2011 (15)	Low	Low	Low	Low	Low	Low
Demirel et al., 2013 (16)	Low	Low	Low	Low	Low	Low
Fernández-Carrocera et al., 2013 (17)	Low	Unclear	Unclear	Low	Low	Low
Oncel et al., 2014 (18)	Low	Low	Low	Low	Low	Low
Van Niekerk et al., 2015 (19)	Low	Low	Low	Low	Low	Low
Costeloe et al., 2016 (20)	Low	Low	Low	Low	Low	Low
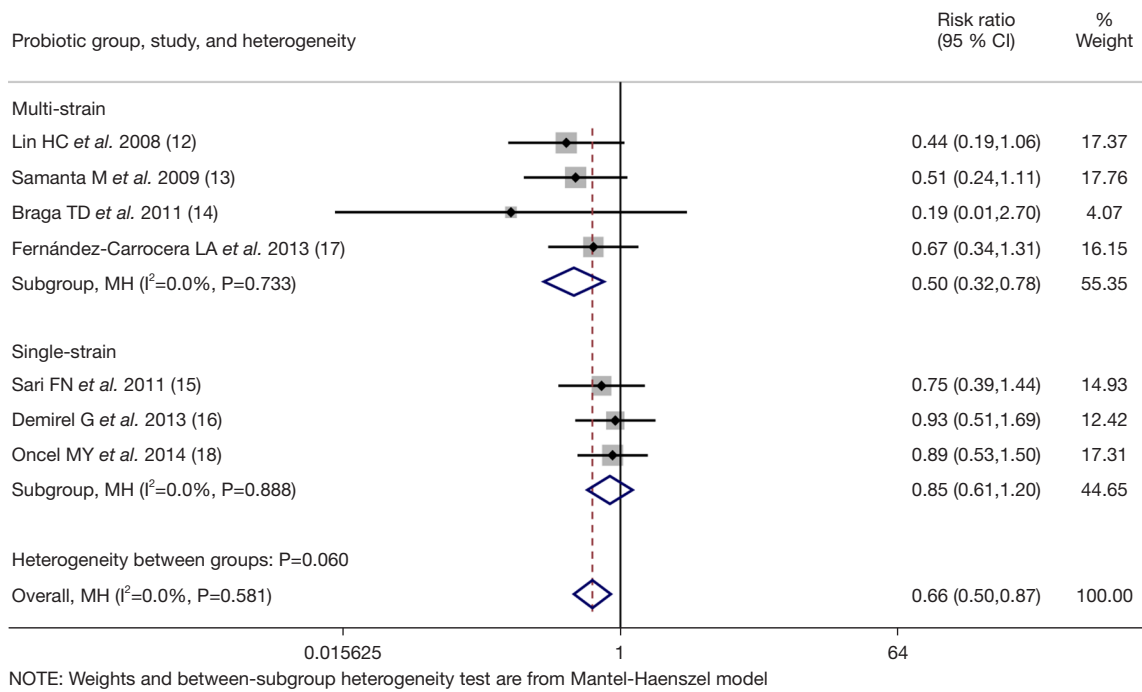
Figure 2 Preventive effect of probiotics on severe NEC (12-18). NEC, neonatal necrotizing enterocolitis; CI, confidence interval.
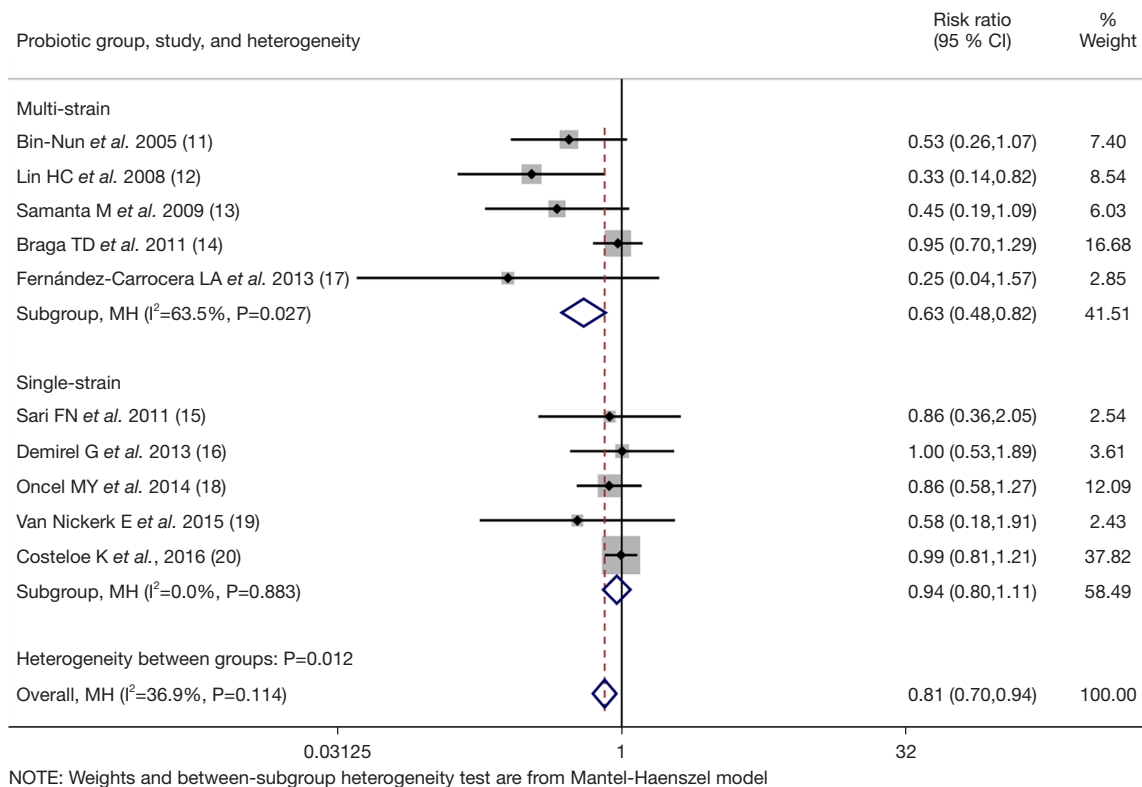

Figure 3 Effect of probiotics on reducing mortality in underweight neonates (11-20). CI, confidence interval.
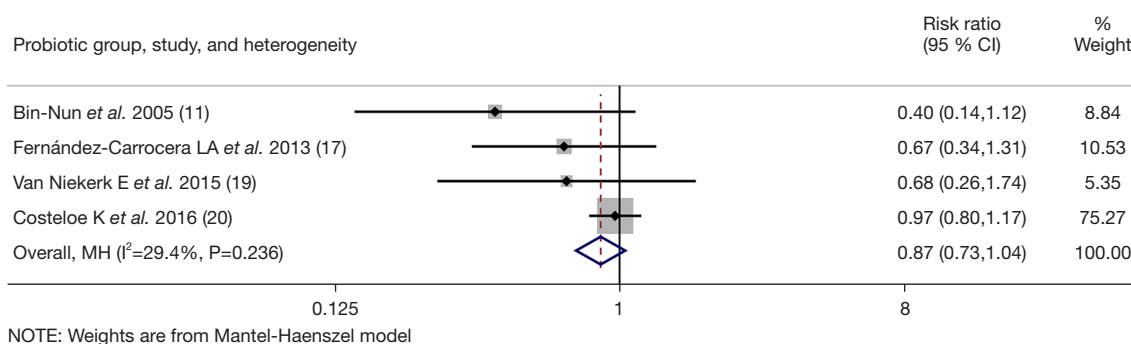
Figure 4 Effect of probiotics on reducing the overall incidence of NEC (11,17,19,20). NEC, neonatal necrotizing enterocolitis; CI, confidence interval.
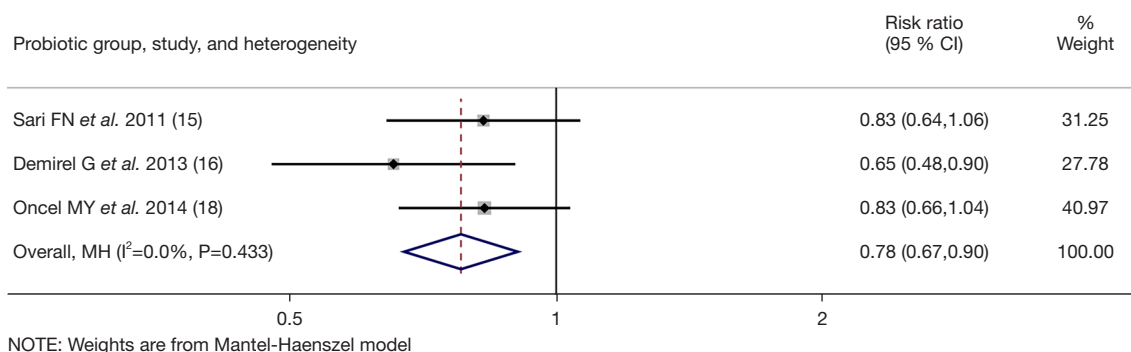

Figure 5 Effect of probiotics on feeding intolerance (15,16,18). CI, confidence interval.

of NEC. There was no statistical heterogeneity among the 4 studies (I^2=29.4%; P=0.236). A fixed effects model analysis yielded a pooled RR of 0.87, 95% CI: (0.73, 1.04), Z=−1.513, and the difference was not statistically significant (P=0.130), suggesting that probiotics did not reduce the total incidence rate of NEC (*Figure 4*).

Feeding intolerance

Three studies (15,16,18) reported the incidence of neonatal feeding intolerance. There was no statistical heterogeneity among the studies (I^2=0%; P=0.433). A fixed effects model analysis yielded a pooled RR statistic of 0.78, 95% CI: (0.67, 0.90), Z=−3.280, and the difference was statistically significant (P=0.001), suggesting that fewer cases of feeding intolerance occurred with probiotic intervention (*Figure 5*).

Heterogeneity and subgroup analysis

A L'abbé plot was used to assess heterogeneity in the

subgroup analysis of mortality rates. It was found that the studies clustered into two groups, corresponding to the two probiotic-based subgroups (multiple- and single-strain). The main source of heterogeneity between the studies was whether the probiotics used were single- or multiple-strain (*Figure 6*).

Sensitivity analysis

In the sensitivity analysis for mortality, impact factor analysis showed that all 10 studies had a sensitivity of between 0.70 and 0.94, indicating that the stability of the results was good (*Figure 7*).

Analysis of publication bias

A funnel plot was drawn up to assess bias analysis. The distribution of all 10 studies included in this meta-analysis remained uneven within the funnel, suggesting a small

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possibility of publication bias (*Figure 8*).

Discussion

This meta-analysis evaluated 10 high-quality controlled clinical studies that collectively included a total of 3,227 premature infants with low bodyweight. Our results showed that the proportion of newborns with severe NEC (stage ≥2) was significantly lower in the experimental groups who took preventive probiotics than that in the control groups. The mortality rate was also significantly lower

in the experimental groups than in the control groups, consistent with the results of previous studies (21,22). It should be noted that concerning the overall incidence rate of NEC, we found differences between the experimental and control groups to be not statistically significant, which is inconsistent with the results of a previous study (9). This discrepancy may have arisen because only 4 studies in the present meta-analysis reported the overall incidence rate of NEC, resulting in insufficient data. Despite this, the results of this meta-analysis confirmed the role of probiotics in preventing severe NEC and reducing mortality in preterm, underweight neonates.

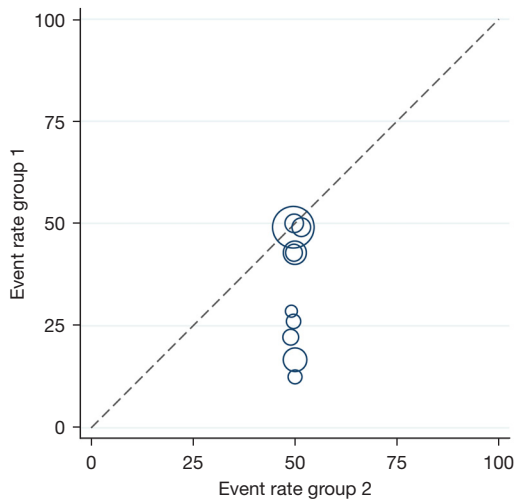Dysregulation of intestinal bacteria is an important cause of neonatal NEC, which is characterized by reduced

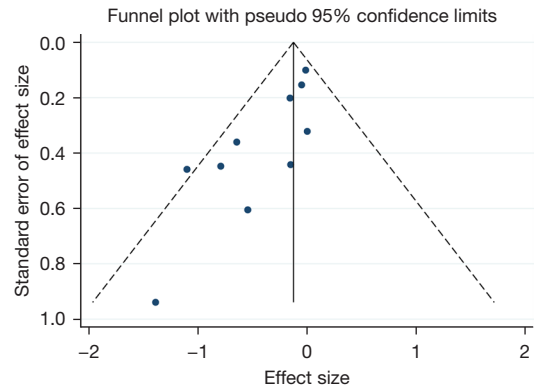
Figure 6 Heterogeneity analysis for mortality.

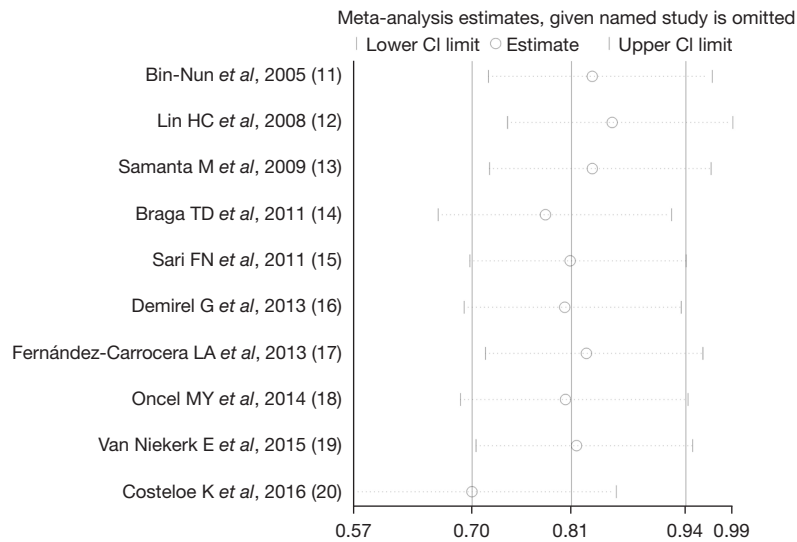**Figure 8** Funnel plot analysis of bias for mortality.

Figure 7 Sensitivity analysis for mortality (11-20). CI, confidence interval.

intestinal microbial diversity, reduced colonization of beneficial microorganisms such as *Bifidobacteria*, *Pirobacterium* and *Lactobacilli*, and an increase in the number of harmful bacteria (23). Probiotics can play a 3-fold role, regulating the structure of intestinal bacteria and inhibiting the growth of harmful germs, promoting the improvement of intestinal barrier function structure and accelerating the maturation of intestinal function, and regulating intestinal immunity and inhibiting the inflammatory response (24). *Bifidobacteria* can reduce plasma endotoxin levels by inhibiting bacterial translocation in the large intestine (25). *Lactobacillus rhamnosus* can then increase the intestinal lactic acid bacteria content and inhibit the transfer of pathogens through the intestinal barrier to extraintestinal organs (26).

However, some studies (20) have also revealed that although the application of *Bifidobacterium breve* in premature infants is safe, it has no significant effect on the incidence of NEC. In this meta-analysis, studies were divided into multi-strain and single-strain groups according to the characteristics of the probiotic treatment. Results showed that multi-strain mixtures of probiotics had a significant effect on reducing severe NEC and mortality, while single-strain probiotic had no significant effect. Jiang *et al.* (27) conducted a meta-analysis of studies that included 1,869 Asian neonates treated with multi- and single-strain probiotic. Their results showed that multiple-strain probiotics had a better preventive effect for severe NEC than single-strain probiotic, consistent with the present meta-analysis. It has been speculated that the combination of multiple probiotics can significantly increase the number of beneficial intestinal flora in underweight, premature infants while reducing *Clostridium perfringens* and *Enterobacteriaceae*. Hence, the multiple-strain-strain probiotics are better for the prevention of NEC (28).

In the studies included in our meta-analysis, the most frequently used probiotics were *Lactobacillus rhamnosus GG*, *Bifidobacterium*, and *Lactobacillus*. Although the effectiveness of the different probiotics was not compared in this meta-analysis, a reticular meta-analysis by Beghetti *et al.* (29), included 29 different probiotic treatments and showed that *Lactobacillus rhamnosus GG* was the most effective in reducing the incidence of NEC, while *Lactobacillus acidophilus LB* was the most effective in preventing severe NEC.

Although some studies (30) claim that the use of probiotics may increase the probability of neonatal infection, no serious adverse reactions were reported in the 10 studies included in our meta-analysis. In addition, the incidence of feeding intolerance in the experimental groups was lower than in the control groups, which demonstrated that the use of probiotics was safe. The children included in these 10 studies were breastfed. Study (31) has shown that combining *Bifidobacteria* with breastfeeding promoted the bacteria's colonization of the intestinal tract and reduced the number of *Enterobacteriaceae*, thereby reducing the incidence of NEC.

To investigate the source of heterogeneity in the studies, we analyzed them according to the type of probiotics used (multi- or single-strain), and our results showed that the type of probiotics used was the greatest source of heterogeneity. Sensitivity analysis showed that the results of our meta-analysis were stable.

We also assessed the quality and bias of the 10 selected controlled clinical studies. Our evaluation showed that the quality of the studies was high, however, neither literature (11) nor literature (17) described the allocation concealment method and blind method, which might resulting in some implementation bias for the analysis. Publication bias analysis showed that the literature on both sides of the funnel was unevenly distributed, which might indicate a small publication bias. And there were some limitations to our meta-analysis, including the lack of subgroup analysis for the different feeding methods (breastfeeding or formula feeding) or different probiotic species, which are worthy of further research.

Conclusions

In summary, the addition of probiotics to the diet of low-birthweight premature infants can reduce the incidence of severe NEC and associated mortality. However, based on the small implementation bias and publication bias in the literatures, the preventive effect of probiotics on NEC is still worthy of further discussion.

Footnote

Reporting Checklist: The authors have completed the PRISMA reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-22-27/rc

Conflicts of Interest: All authors have completed the ICMJE

uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-22-27/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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