ORIGINAL ARTICLE

Probiotic strategies to prevent necrotizing enterocolitis in preterm infants: a meta-analysis

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Abstract

Purpose We aimed to compare probiotics with placebo for necrotizing enterocolitis in preterm infants and to evaluate the safety and effect and strict effect of specific probiotic genera.

Methods Data recorded until January 2019 were searched, and relevant academic articles from PubMed, MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials were selected by two independent reviewers. Two reviewers independently included randomized controlled trials that compared probiotics and placebo in preterm infants. The outcomes included more than one of the following outcomes: incidence of necrotizing enterocolitis, necrotizing enterocolitis-related mortality, incidence of sepsis, and all-cause mortality. Two reviewers independently extracted data and assessed the risk of bias and quality of evidence.

Results We identified 34 eligible studies of 9161 participants. This meta-analysis showed an overall advantage of probiotics to prevent the incidence of necrotizing enterocolitis (3.54%) and gut-associated sepsis (15.59%), and decrease mortality (5.23%) in preterm infants. A probiotic mixture showed a huge advantage and vitality in preventing necrotizing enterocolitis (2.48%) and gut-associated sepsis (18.39%), and in reducing mortality (5.57%) in preterm infants.

Conclusion The probiotic mixture showed advantages over the single strains to decrease the incidences of necrotizing enterocolitis and gut-associated sepsis, and mortality in preterm infants.

Keywords Probiotic genera · Necrotizing enterocolitis · Preterm infants · Meta-analysis

Introduction

Necrotizing enterocolitis (NEC) is one of the most serious complications in premature infants. NEC can cause perforation and irreversible bowel injury requiring resection, and the associated mortality rate is up to 30% [1, 2]. Especially among the very low birth weight infants, the survivors of NEC have an increased risk of severe long-term growth failure; therefore, optimal prevention strategies are essential [3]. Probiotics are a group of living microorganisms that are beneficial to one's health. They can reduce colonization of enteropathogenic bacteria and help modulate the intestinal microbiota in preterm infants [4, 5]. Probiotic supplementation might protect the intestine from NEC by reducing the inflammatory reaction, regulating the component of the mucus layer, and improving intestinal integrity. Several randomized clinical trials (RCTs) have reported that administration of probiotics is an effective treatment for NEC [6–8]. Recent meta-analyses and systematic reviews of such trials have yielded supportive conclusions. However, there are many types of products with probiotics. Previous studies have neglected to report the specific different action of each probiotic genus in preterm infants and failed to provide detailed treatment strategies to prevent NEC in preterm infants. Furthermore, as probiotics are a preparation of living bacteria, the safety of them is a concern as well. Therefore, it remains controversial to prevent NEC using probiotics in preterm infants.

To evaluate the safety and effect of probiotics and the strict effect of specific probiotic genera, we performed a meta-analysis to compare probiotics with placebo for NEC in preterm infants.

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Methods

We conducted a meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement standards [9].

Literature searches and search strategy

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 (randomized controlled trial OR controlled clinical trial OR randomly) AND (probiotics OR probiotic treatment). In addition, 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. In the case that more than one publication was available for a study, we used either the most recent publication or the one with the most relevant information.

Study selection

All enrolled RCTs met the following inclusion criteria: (1) it was published as a full report in a peer-reviewed journal; (2) population: probiotics were administered exclusively in preterm infants with a low birth weight (< 2500 g); (3) intervention: probiotics were used to prevent NEC; (4) comparison intervention: placebo or negative control; (5) outcomes, including more than one of the following outcomes: incidence of NEC, NEC-related mortality, incidence of sepsis, and all-cause mortality; and (6) 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.

Data extraction

We created an electronic data extraction spreadsheet to prefetch the relevant data. We adjusted the spreadsheet based on the results of the data extraction. Two independent reviewers included the following information according to the prior plan: general trial parameters, demographic characteristics, diagnostic measurements, treatment regimens, and outcomes. Disagreements were settled by discussion. All data are presented in Table 1.

Risk of bias assessment

Two independent reviewers used the Cochrane Collaboration's Risk of Bias Tool [10] to evaluate the methodological quality and risk of bias of the included RCTs. The following seven individual items were valued as low, high, or unclear by the two authors: (1) random sequence generation, (2) allocation sequence concealment, (3) blinding of participants and personnel, (4) blinding of outcome assessment, (5) completeness of outcome data, (6) selective reporting, and (7) other sources of bias.

Data synthesis and analysis

We summarized the numbers of dichotomous outcome variables, and a relative ratio (RR) with 95% confidence interval (CI) was calculated for the outcomes. A P value ≤ 0.05 was deemed as significant. We estimated the presence of statistical heterogeneity using the P value for heterogeneity and the degree of heterogeneity using the I^2 statistic. An I^2 statistic was considered to reflect low likelihood (0–25%), moderate likelihood (26–50%), and high likelihood (51–100%) of differences beyond chance, as was a P value ≤ 0.05 for heterogeneity. When there was evidence of significant heterogeneity ($I^2 > 50\%$), random-effects models were used; otherwise, fixed-effects models were used for analysis in accordance with the Cochrane Handbook for systematic reviews of Interventions (version 5.1.0) [11]. Publication bias was assessed with funnel plots. We also conducted a subgroup analysis to explore sources of heterogeneity. Statistical analyses were performed with Review Manager 5.3 (The Cochrane Collaboration, Copenhagen, Denmark).

Strength of evidence

Two senior reviewers independently evaluated the strength of evidence and arrived at the final grade based on five required domains: risk of bias, consistency, directness, precision, and publication bias. According to an established guideline, we finally assessed the strength of evidence as high, moderate, low, or insufficient [12].

Results

Description of studies

The result of the initial literature search strategy yielded 1630 potentially relevant articles. Overall, 1558 articles were

Table 1 Characteristics of the included studies

Study	Design	Setting	Simple size	Patients	Intervention	Outcomes
Braga et al. [6]	RCT	SC	Propbiotics: 119; placebo: 112	Preterm; BW 750–1499 g; Admission to Hospital	*L. casei* (3.5×10^9 CFU OD) and *B. breve* (3.5×10^7 CFU OD); from day 2 to day 30 or occurrence of primary outcomes	NEC, sepsis, death
Lin et al. [7]	RCT	NR	Propbiotics: 180; placebo: 187	Preterm; BW < 1500 g enteral nutrition; age > 7 days	*L. acidophilus* and *B. infantis* ($\geq 10^6$ CFU each probiotic, BD); from enteral feeding to discharge	NEC
Al-Hosni et al. [8]	RCT	MC	Propbiotics: 50; placebo: 51	Preterm; BW 501–1000 g, appropriate for GA, and ≤ 14 days of age at time of feeding initiation	*L. rhamnosus* GG and *B. infantis* (0.5×10^9 CFU, OD); from first enteral feeding to discharge or PMA > 34 weeks	Weight gain CLD, ROP, NEC, sepsis, mortality, mean volume of feeding
Chou et al. [13]	RCT	SC	Propbiotics: 153; placebo: 148	PVLBW infants; enteral feeding; age > 7 days	*L. acidophilus* and *B. infantis* ($\geq 10^6$ 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. [14]	RCT	MC	Propbiotics: 217; placebo: 217	Preterm; GA < 34 weeks; BW ≤ 1500 g; enteral feeding	*L. acidophilus* NCDO 1746 and *B. bifidum* NCDO 1453 (1×10^9 CFU; = 125 mg/kg BD); from day 2 of age; duration 6 weeks	NEC, sepsis, death, IVH, CLD, NICU stay, weight gain
Hays et al. [15]	RCT	MC	*B. lactis*: 50; *B. longum*: 48; *B. lactis* + *B. longum*: 47; placebo: 52	700–1600 g; GA 25–31 weeks	*B. lactis* (1×10^9 CFU OD), *B. longum* (1×10^9 CFU OD); from age < 7d to 4 weeks (GA 29 W), 6 weeks (GA 28 W)	Weight, length, HC, NEC, sepsis, body composition, bacterial count
Jacobs et al. [16]	RCT	MC	Propbiotics: 548; placebo: 551	Preterm; GA < 32 weeks BW < 1500 g	*B. infantis* BB-02 (300 CFU $\times 10^6$); *Streptococcus thermophilus* Th-4 (350×10^6 CFU); *B. lactis* BB-12 (350 CFU $\times 106$); 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 28d, ROP, CLD, IVH
Patole et al. [17]	RCT	SC	Propbiotics: 77; placebo: 76	Preterm; GA < 33 weeks; BW < 1500 g; enteral feeds for < 12 h	*B. breve* (3×10^9 CFU OD), from enteral feed to corrected age of 37 weeks	Discharge weight, death, hospitalization; NEC
Costalos et al. [18]	RCT	SC	Propbiotics: 51; placebo: 36	GA28–32 weeks; no major GI problem; not receiving antibiotics; not receiving breast milk	*Saccharomyces boulardii* (1×10^9 CFU BD); non-specified start time and end time	Weight gain and loss, daily milk in take, NEC, sepsis, bacterial counts

Table 1 (continued)

Study	Design	Setting	Simple size	Patients	Intervention	Outcomes
Roy et al. [19]	RCT	SC	Propbiotics: 56; placebo: 56	Preterm; GA <37weeks; BW <2500 g; enteral feeding; age <72 h	*L. acidophilus* (1.25×10^9 CFU/g); *B. longum* (0.125×10^9 CFU/g); *B. bifidum* (0.125×10^9 CFU×1 g) and *B. lactis* (1×10^9 CFU×1 g); from 72 h of life, duration 6 weeks or at discharge	Full feed establishment, candida, death, NEC, hospitalization
Sari et al. [20]	RCT	SC	Propbiotics: 86; placebo: 88	Preterm; GA <33 weeks; or BW <1500 g	*L. sporogenes* (0.35×10^9 CFU OD), from first feed to discharge.	Weight gain, length, HC, CLD, hospitalization, NEC, sepsis, feeding intolerance, oxygen days, fullfeeding days
Costeloe et al. [21]	RCT	MC	Propbiotics: 650; placebo: 660	GA 23–30 weeks	*B. breve* (1.6×10^9 CFU OD), until corrected age of 36w	ROP, death, NEC, sepsis
Fernández-Carrocera et al. [22]	RCT	MC	Propbiotics: 75; placebo: 75	Preterm; BW <1500 g Infants with NEC IA and IB were excluded	*Streptococcus thermophilus* (6.6×10^5 CFU/g); *B. infantis* (2.76×10^7 CFU/g); *L. plantarum* (1.76×10^8 CFU/g); *L. casei* (1×10^9 CFU/g); *L. rhamnosus* (4.4×10^8 CFU/g); *L. acidophilus* (1 CFU/g); from enteral feeding,non-specified end time	Weight, death, NEC
Manzoni et al. [23]	RCT	SC	Propbiotics: 39; placebo: 41	BW <1500 g, age >3 days, not receive any form of antifungal prophylaxis other than LGG	*L. rhamnosus* LGG (6×10^9 CFU/d); from the 3rd day of life to age of 6th week or discharge from the NICU	Hospitalization, time of achievement of full feedings, death, NEC
Mihatsch et al. [24]	RCT	SC	Propbiotics: 91; placebo: 89	Preterm; GA <30 weeks; BW ≤1500 g	*B. lactis* (2×10^9 CFU/kg 6 times/d), from enteral feeding, non-specified end time	NEC, death, nosocomial infections
Oncel et al. [25]	RCT	SC	Propbiotics: 200; placebo: 200	Preterm; GA ≤32 weeks; BW ≤1500 g, enteral feeding	*L. reuteri* (1×10^8 CFU OD); from first feed to death or discharge	NEC, sepsis, death, hospitalization, feeding intolerance
Rojas et al. [26]	RCT	MC	Propbiotics: 372; placebo: 378	Preterm; BW ≤2000 g, age ≤48 h; HS; enteral feeding	*L. reuteri* (1×10^8 CFU OD); from age ≤48 h to death or discharge	Death, duration of hospital, NEC, sepsis
Rougé et al. [27]	RCT	MC	Propbiotics: 45; placebo: 49	Preterm; GA <32 weeks; BW <1500 g, age ≤2 weeks; excluded non-preterm birth related diseases; enteral feeding	*B. longum* BB536 and *L. rhamnosus* GG (1×10^8 CFU/day); from enteral feeding to discharge	Nutrition–total calories delivered enterally, duration of hospital stays, death, oxygen therapy duration

Table 1 (continued)

Study	Design	Setting	Simple size	Patients	Intervention	Outcomes
Samanta et al. [28]	RCT	MC	Propbiotics: 91; placebo: 95	Preterm; GA < 32 weeks; BW < 1500 g; enteral feeding; age > 48 h	*L. acidophilus*; *B. longum*; *B. bifidum* and *B. infantis* (2.5×10^9 CFU, BD); from enteral feeding to discharge.	NEC, death, hospitalization
Stratiki et al. [29]	RCT	SC	Propbiotics: 41; placebo: 34	Preterm; GA27–32 weeks, formula-fed, without major congenital anomalies	*B. lactis* (2×10^7 CFU/g of milk powder), from enteral feeding, non-specified end time	Weight, length, NEC, time to full enteral feed
Chowdhury et al. [30]	RCT	SC	Propbiotics: 52; placebo: 50	Preterm; GA < 33 weeks; BW < 1500 g; enteral feeding; age > 48 h	Capsule TS6 probiotic + containing Bifidobacterium; species, and Lactobacillus (6×10^9 CFU/d); from first feeding to discharged	NEC, hospital stay
Demirel et al. [31]	RCT	SC	Propbiotics: 91; placebo: 90	GA < 32 weeks (lowest GA 24 weeks); BW < 1500 g (lowest BW 500 g)	*Saccharomyces boulardii* (5×10^9 CFU OD), from first feed to discharge	Feeding amount, full feeding day, weight, NEC, sepsis
Dani et al. [32]	RCT	MC	Propbiotics: 295; placebo: 290	GA < 33 weeks; or BW < 1500 g	*L. rhamnosus* GG (6×10^9 CFU OD); from first feed to discharge	antibiotic treatment; UTI; sepsis; NEC
Sari et al. [33]	RCT	SC	Propbiotics: 110; placebo: 111	GA < 32 weeks or BW < 1500 g; enteral feeding	*L. sporogenes* (0.35×10^9 CFU OD), from first feed to discharge	NICU stay; NEC; Death; Weight gain; Feeding intolerance
Demirel et al. [34]	RCT	SC	Propbiotics: 135; placebo: 136	GA ≤ 32 weeks; BW ≤ 1500 g, enteral feeding	*Saccharomyces boulardii* (5×10^9 CFU OD), from first feed to discharge	Feeding amount, full feeding day, weight, NEC, sepsis
Dilli et al. [35]	RCT	MC	Propbiotics: 100; placebo: 100	GA < 32 weeks 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 d7 after birth to death or discharge (max 8w)	Height, weight, HC, NEC, sepsis, feeding intolerance, RDS, stay at NICU, mortality
Fujii et al. [36]	RCT	SC	Propbiotics: 11; placebo: 8	GA < 34 weeks	*B. breve* M-16 V (1×10^9 CFU BD), non-specified start, until discharge	Duration of hospital stay, NEC, Chronic lung disease, Retinopathy of prematurity, Infection
Kanic et al. [37]	RCT	SC	Propbiotics: 40; placebo: 40	GA < 33 weeks; 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, urinary tract infection, and omphalitis
Saengtawesin et al. [38]	RCT	SC	Propbiotics: 31; placebo: 29	GA ≤ 34 weeks BW ≤ 1500 g	*L. acidophilus* (1×10^9 CFU) and *B. bifidum* (1×10^9 CFU); from feeding to 6 weeks of age or discharge	Feeding amount; Weight gain; Length of stay; NEC; sepsis; BPD; ROP; PVL; IVH

Table 1 (continued)

Study	Design	Setting	Sample size	Patients	Intervention	Outcomes
Serce et al. [39]	RCT	MC	Probiotics: 104; placebo: 104	GA ≤ 32 weeks; BW ≤ 1500 g, enteral feeding	*Saccharomyces 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. [40]	RCT	MC	Probiotics: 123; placebo: 121	GA <34 weeks; admitted to the NICU	*B. clausii* (8×10^8 CFU, TD), from age 24 h to postnatal age of 6 weeks, discharge, or death	Sepsis; NEC; Feed intolerance; Death
Totsu et al. [41]	RCT	MC	Probiotics: 153; placebo: 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 establishment of enteral feeding, death, NEC, CLD
Kitajima et al. [42]	RCT	SC	Probiotics: 45; placebo: 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 apnea; Duration of antibiotics; NEC
Bin-Nun et al. [43]	RCT	SC	Probiotics: 72; placebo: 73	Preterm; BW <1500 g; enteral feeding	*Streptococcus thermophilus*; *B. bifidus* and *B. infantis* (0.35×10^9 CFU, OD); from enteral feeding to 36 weeks postconceptual age	Weight gain NEC, TPN

RCT randomized controlled trial, *SC* single center, *BW* birth weight, *NEC* necrotizing enterocolitis, *NR* no reported, *TPN* total parenteral nutrition, *MC* multi-center, *CLD* chronic lung disease, *ROP* retinopathy of prematurity, *PVLBW* preterm very low birth weight, *HC* head circumference, *GA* gestational age, *IVH* intraventricular hemorrhage, *NICU* neonatal intensive care unit, *HS* hemodynamically stable, *UTI* urinary tract infection, *OD* once daily, *BD* twice daily, *CFU* colony-forming unit, *PMA* postmenstrual age, *RDS* respiratory distress syndrome, *PVL* periventricular Leukō malacia, *NCDO* National Collection of Dairy Organisms, *GI* gastrointestinal

identified by checking the titles and abstracts, and then of these, 112 trials were considered after rigorously reviewing the full text. We excluded 78 trials because of several reasons (data unavailable, article from the same trial, and

patients had other relevant disease). After finishing the literature search and assessing the entire text of articles, 34 studies were finally included in the meta-analysis (Fig. 1) [6–8, 13–43]. These studies included 9161 premature infants, of
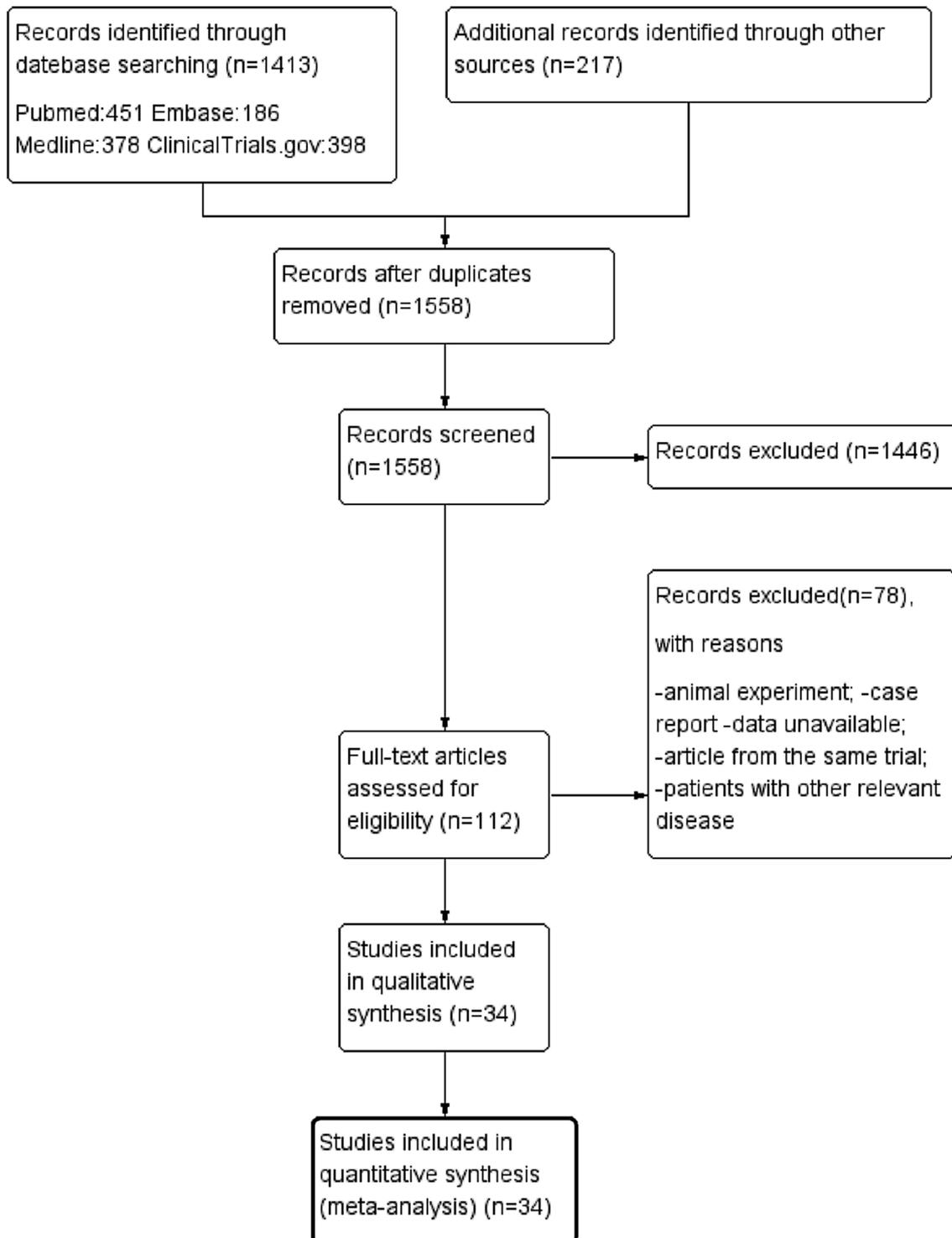
Fig. 1 Flowchart showing the search strategy and search results. The relevant number of papers at each point is given

whom 4648 were treated with probiotics. All trials in our analysis were RCTs and they met the inclusion criteria. More details of included RCTs are summarized in Table 1.

Ten probiotic strategies were used in all the eligible studies: (1) *Bacillus clausii* alone in one study [40], (2) *Bifidobacterium bifidum* in one study [41], (3) *B. breve* in four studies [17, 21, 36, 42], (4) *B. lactis* in four studies [15, 24, 29, 35], (5) *B. longum* in one study [15], (6) *Lactobacillus reuteri* in two studies [25, 26], (7) *L. GG* in two studies [23, 32], (8) *L. sporogenes* in two studies [20, 33], (9) *Saccharomyces boulardii* in four studies [18, 31, 34], and (10) probiotic mixture in 15 studies [6–8, 13–16, 19, 22, 27, 28, 30, 37, 38, 43]. We integrated the probiotic genus depending on strain characteristics as follows: *Lactobacilli*, *Bifidobacteria*, *Bacillus*, *Saccharomyces*, and probiotic mixture.

Quality of the included studies

We used the Cochrane Risk of Bias Tool to assess the quality of the included RCTs (Fig. 2). The results showed that most of the included trials followed the strict blind method for the researchers, outcome assessors, and participants in the intervention, and one trial had a high risk in both randomization and blindness. We evaluated the level of evidence of outcomes based on the GRADE approach (Table 2).

Effect of probiotics on NEC

The incidence of NEC was assessed in all 34 trials. The 9171 premature infants were divided into two arms: probiotic arm (4648 cases) and control arm (4523 cases). The result showed that the risk of incidence of NEC was significantly reduced by 42% after the administration of probiotics (3.54%) compared with placebo (6.23%) (RR = 0.58, 95% CI 0.48–0.69, $P < 0.05$; Fig. 3). There was a low level of heterogeneity ($I^2 = 9\%$, $P = 0.32$), and no significant risk of publication bias was shown in the funnel plots (Fig. 4a).

We performed a subgroup analysis of the incidence of NEC based on different probiotic genera. We observed that the risk of the incidence of NEC in the probiotic mixture group (2.48%) was about 40% of that in the placebo group (6.33%) (RR = 0.40, 95% CI 0.29–0.56, $P < 0.05$; heterogeneity, $I^2 = 0.0\%$, $P = 0.47$; Fig. 3). Additionally, *Lactobacilli* and *Bifidobacteria* both reduced the risk of incidence of NEC compared with the placebo (RR = 0.59, 95% CI 0.38–0.91, $P < 0.05$; heterogeneity, $I^2 = 0.0\%$, $P = 0.97$ and RR = 0.70, 95% CI 0.52–0.94, $P < 0.05$; heterogeneity, $I^2 = 56\%$, $P = 0.05$, respectively; Fig. 3). However, the results of all studies using *Bacillus* and *Saccharomyces* showed no significant statistical difference between the two groups (RR = 0.98, 95% CI 0.14–6.87, $P = 0.99$ and RR = 0.83, 95% CI 0.47–1.49, $P = 0.53$, respectively; heterogeneity, $I^2 = 0.0\%$, $P = 0.91$; Fig. 3).

Effect of probiotics on sepsis

Twenty-eight of the total included publications reported outcomes related to the incidence of sepsis. Overall, 7785 subjects were recruited in our analysis (3957 in the probiotic group and 3828 in the control group). Our analysis found a significantly lower risk of sepsis in the probiotic group (15.59%) than in the placebo (17.95%), and the statistical result had significant heterogeneity (RR = 0.87, 95% CI 0.79–0.96, $P < 0.05$; heterogeneity, $I^2 = 35\%$, $P = 0.04$; Fig. 5). Funnel plots identified no strong evidence of publication bias (Fig. 4b).

The subgroup analysis of the incidence of sepsis based on different probiotic genera showed the following. Compared to the placebo (21.32%), the probiotic mixture (18.39%) decreased the risk of the incidence of sepsis to 0.86 (RR = 0.86, 95% CI 0.75–0.99, $P < 0.05$; heterogeneity, $I^2 = 53\%$, $P = 0.01$; Fig. 5); all other subgroups failed to find a significant statistical difference in the risk of the incidence of sepsis (Fig. 5).

Effect of probiotics on mortality

We analyzed 28 trials that reported 228 cases of mortality in the probiotic group (4362 participants) and 316 cases of mortality in the control group (4261 participants). Significantly decreased mortality was observed in the probiotic group (5.23%) compared with the control group (7.41%) (RR = 0.72, 95% CI 0.61–0.85, $P < 0.05$). No significant heterogeneity was found in our analysis ($I^2 = 15\%$, $P = 0.24$; Fig. 6). The funnel plots showed no apparent publication bias (Fig. 4c). We performed a subgroup analysis based on NEC-related mortality. Fifty-nine cases of mortality related to NEC were reported in nine studies. Unfortunately, we observed that the effect of probiotics on NEC-related mortality was not significantly altered by probiotics (1.15%) compared with placebo (1.79%) (RR = 0.66, 95% CI 0.40–1.09, $P = 0.11$; heterogeneity, $I^2 = 0.0\%$, $P = 0.85$; Fig. 7). However, the pooled analysis failed to include 10 studies so that the funnel plot could not be used to assess publication bias.

The subgroup analysis of the incidence of sepsis based on different probiotic genera showed the following. Compared to placebo (9.51%), the probiotic mixture (5.57%) significantly decreased the risk of mortality (RR = 0.58, 95% CI 0.46–0.74], $P < 0.05$; heterogeneity, $I^2 = 34\%$, $P = 0.11$; Fig. 6); all other subgroups showed no significant statistical difference in the risk of mortality (Fig. 6).

Discussion

Gastrointestinal complications such as NEC and gut-associated sepsis are linked to poor neurodevelopmental and growth outcomes in preterm infants, and they are one of
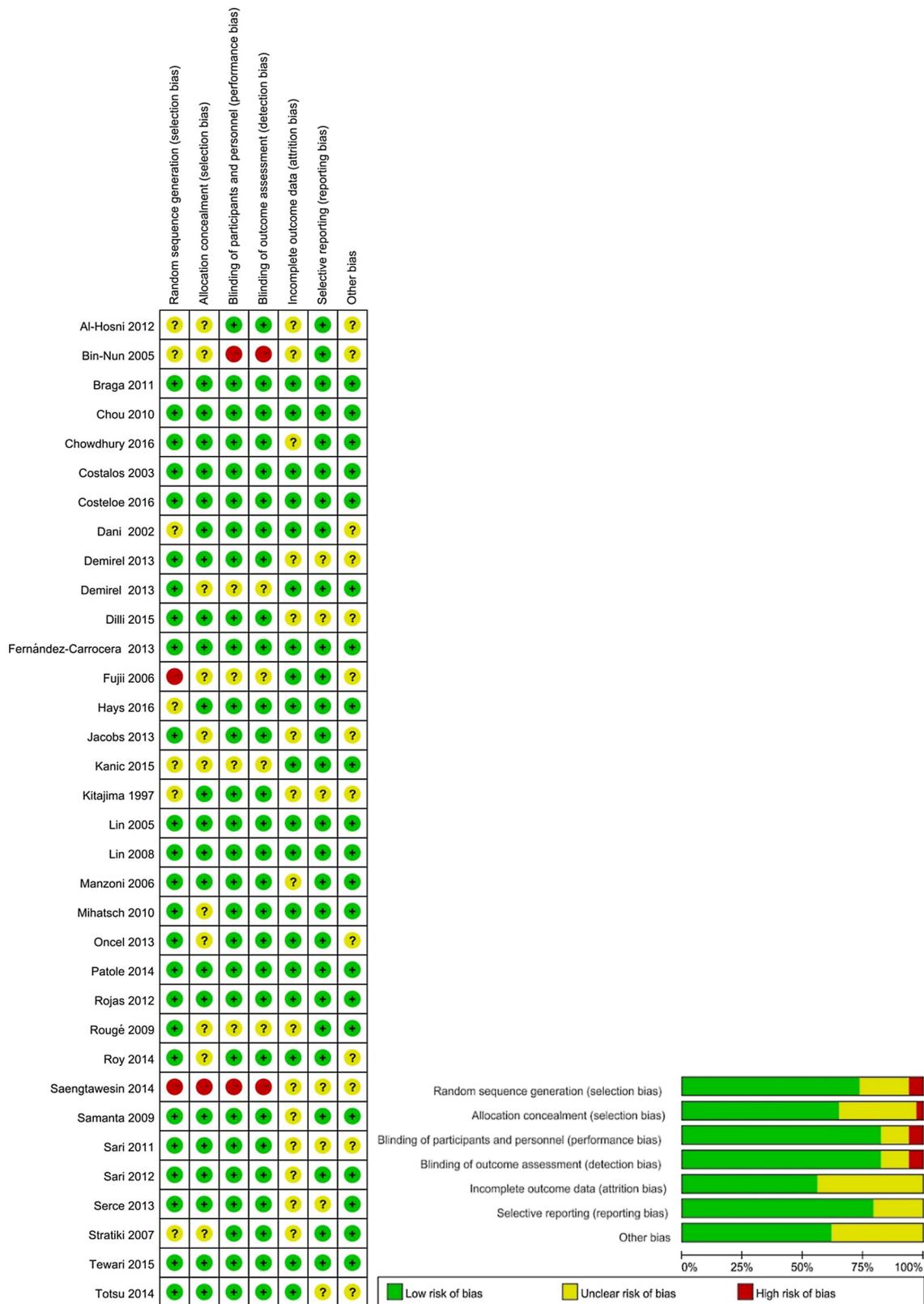
Fig. 2 Risk of bias summary and graph showing authors judgement about each risk of bias item for the randomized trial

Table 2 The level of the evidence of each result based on GRADE approach

Probiotics compared to placebo for severe clinical complications in preterm infants

Patient or population: Preterm infants with severe clinical complications
Settings:
Intervention: Probiotics
Comparison: Placebo

Outcomes	Illustrative comparative risks[a] (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	Probiotics				
Incidence of NEC	Study population		RR 0.58 (0.48–0.69)	9275 (34 studies)	⊕⊕⊕⊕ high	
	62 per 1000	36 per 1000 (30–43)				
	Moderate					
	52 per 1000	30 per 1000 (25–36)				
Incidence of NEC—probiotic mixture	Study population		RR 0.4 (0.29–0.56)	3561 (15 studies)	⊕⊕⊕⊖ moderate[b]	
	63 per 1000	25 per 1000 (18–35)				
	Moderate					
	54 per 1000	22 per 1000 (16–30)				
Incidence of NEC—*Lactobacillus*	Study population		RR 0.59 (0.38–0.91)	2210 (6 studies)	⊕⊕⊖⊖ low[c,d]	
	48 per 1000	28 per 1000 (18–44)				
	Moderate					
	62 per 1000	37 per 1000 (24–56)				
Incidence of NEC—*Bifidobacterium*	Study population		RR 0.7 (0.52–0.94)	2513 (9 studies)	⊕⊖⊖⊖ very low[b,c,d,e]	
	79 per 1000	55 per 1000 (41–74)				
	Moderate					
	45 per 1000	31 per 1000 (23–42)				
Incidence of NEC—*Bacillus*	Study population		RR 0.98 (0.14–6.87)	244 (1)	See comment	
	17 per 1000	16 per 1000 (2–114)				
	Moderate					
	17 per 1000	17 per 1000 (2–117)				
Incidence of NEC—*Saccharomyces*	Study population		RR 0.83 (0.47–1.49)	747 (4 studies)	⊕⊖⊖⊖ very low[c,d]	
	60 per 1000	50 per 1000 (28–90)				
	Moderate					
	59 per 1000	49 per 1000 (28–88)				
Incidence of sepsis	Study population		RR 0.87 (0.79–0.96)	7889 (28 studies)	⊕⊕⊕⊕ high	
	180 per 1000	156 per 1000 (142–173)				
	Moderate					
	193 per 1000	168 per 1000 (152–185)				
Incidence of sepsis—probiotic mixture	Study population		RR 0.86 (0.75–0.99)	3197 (12 studies)	⊕⊕⊕⊖ moderate[e]	
	213 per 1000	183 per 1000 (160–211)				
	Moderate					
	234 per 1000	201 per 1000 (175–232)				
Incidence of sepsis—*Lactobacillus*	Study population		RR 0.97 (0.76–1.23)	1460 (5 studies)	⊕⊕⊖⊖ low[b,c,d]	
	142 per 1000	138 per 1000 (108–175)				
	Moderate					
	216 per 1000	210 per 1000 (164–266)				
Incidence of sepsis—*Bifidobacterium*	Study population		RR 0.85 (0.71–1.03)	2422 (8 studies)	⊕⊕⊖⊖ low[c,d]	
	156 per 1000	132 per 1000 (111–160)				
	Moderate					
	144 per 1000	122 per 1000 (102–148)				
Incidence of sepsis—*Bacillus*	Study population		RR 0.79 (0.46–1.34)	244 (1)	See comment	
	207 per 1000	163 per 1000 (95–277)				
	Moderate					
	207 per 1000	164 per 1000 (95–277)				

Table 2 (continued)

Outcomes	Illustrative comparative risks[a] (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	Probiotics				
Incidence of sepsis—*Saccharomyces*	Study population		RR 0.84 (0.58–1.22)	566 (3 studies)	⊕⊖⊖⊖ very low[b,c,d]	
	178 per 1000	149 per 1000 (103–217)				
	Moderate					
	154 per 1000	129 per 1000 (89–188)				
NEC-related mortality	Study population		RR 0.66 (0.4–1.09)	4006 (9 studies)	⊕⊕⊖⊖ low[c,d]	
	18 per 1000	12 per 1000 (7–20)				
	Moderate					
	14 per 1000	9 per 1000 (6–15)				
All-cause mortality	Study population		RR 0.71 (0.61–0.84)	8825 (28 studies)	⊕⊕⊕⊕ high	
	74 per 1000	52 per 1000 (45–62)				
	Moderate					
	74 per 1000	53 per 1000 (45–62)				
All-cause mortality—probiotic mixture	Study population		RR 0.58 (0.46–0.74)	3557 (14 studies)	⊕⊕⊕⊖ moderate[b]	
	95 per 1000	55 per 1000 (44–70)				
	Moderate					
	88 per 1000	51 per 1000 (40–65)				
All-cause mortality—*Lactobacillus*	Study population		RR 0.77 (0.53–1.12)	2036 (5 studies)	⊕⊕⊖⊖ low[b,c,d]	
	58 per 1000	45 per 1000 (31–65)				
	Moderate					
	74 per 1000	57 per 1000 (39–83)				
All-cause mortality—*Bifidobacterium*	Study population		RR 0.86 (0.63–1.19)	2328 (6 studies)	⊕⊖⊖⊖ very low[c,d]	
	65 per 1000	56 per 1000 (41–77)				
	Moderate					
	35 per 1000	30 per 1000 (22–42)				
All-cause mortality—*Bacillus*	Study population		RR 0.84 (0.41–1.75)	244 (1)	See comment	
	116 per 1000	97 per 1000 (47–202)				
	Moderate					
	116 per 1000	97 per 1000 (48–203)				
All-cause mortality—*Saccharomyces*	Study population		RR 1.33 (0.57–3.12)	660 (3 studies)	⊕⊕⊕⊖ moderate[b,d]	
	27 per 1000	36 per 1000 (16–85)				
	Moderate					
	22 per 1000	29 per 1000 (13–69)				

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate

Very low quality: We are very uncertain about the estimate

CI confidence interval, *RR* risk ratio

[a]The basis for the assumed risk (e.g., the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI)

[a]Funnel plot shows the bias of publication

[b]Fewer patients were included in the analysis, and the confidence interval was wide

[c]The number of the literatures is less

[d]Higher heterogeneity

Study or Subgroup	Probiotics		Placebo			Risk Ratio	Risk Ratio
	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
1.2.2 probiotic mixture							
Al-Hosni 2012	2	50	2	51	0.7%	1.02 [0.15, 6.96]	
Bin-Nun 2005	3	72	12	73	4.1%	0.25 [0.07, 0.86]	
Braga 2011	0	119	4	112	1.6%	0.10 [0.01, 1.92]	
Chou 2010	0	153	2	148	0.9%	0.19 [0.01, 4.00]	
Chowdhury 2016	1	52	6	50	2.1%	0.16 [0.02, 1.28]	
Fernández-Carrocera 2013	6	75	12	75	4.1%	0.50 [0.20, 1.26]	
Hays 2016	5	47	3	52	1.0%	1.84 [0.47, 7.30]	
Jacobs 2013	11	548	24	551	8.2%	0.46 [0.23, 0.93]	
Kanic 2015	0	40	5	40	1.9%	0.09 [0.01, 1.59]	
Lin 2005	2	180	10	187	3.4%	0.21 [0.05, 0.94]	
Lin 2008	4	217	14	217	4.8%	0.29 [0.10, 0.85]	
Rougé 2009	2	45	1	49	0.3%	2.18 [0.20, 23.21]	
Roy 2014	2	56	2	56	0.7%	1.00 [0.15, 6.85]	
Saengtawesin 2014	1	31	1	29	0.4%	0.94 [0.06, 14.27]	
Samanta 2009	5	91	15	95	5.1%	0.35 [0.13, 0.92]	
Subtotal (95% CI)		1776		1785	39.2%	0.40 [0.29, 0.56]	
Total events	44		113				
Heterogeneity: Chi² = 13.72, df = 14 (P = 0.47); I² = 0%							
Test for overall effect: Z = 5.32 (P < 0.00001)							
1.2.3 Lactobacillus							
Dani 2002	4	295	8	290	2.8%	0.49 [0.15, 1.61]	
Manzoni 2006	1	39	3	41	1.0%	0.35 [0.04, 3.23]	
Oncel 2013	8	200	10	200	3.4%	0.80 [0.32, 1.99]	
Rojas 2012	9	372	15	378	5.1%	0.61 [0.27, 1.38]	
Sari 2011	6	110	10	111	3.4%	0.61 [0.23, 1.61]	
Sari 2012	3	86	7	88	2.4%	0.44 [0.12, 1.64]	
Subtotal (95% CI)		1102		1108	18.2%	0.59 [0.38, 0.91]	
Total events	31		53				
Heterogeneity: Chi² = 0.94, df = 5 (P = 0.97); I² = 0%							
Test for overall effect: Z = 2.38 (P = 0.02)							
1.2.4 Bifidobacterium							
Costeloe 2016	61	650	66	660	22.6%	0.94 [0.67, 1.31]	
Dilli 2015	2	100	18	100	6.2%	0.11 [0.03, 0.47]	
Fujii 2006	0	11	0	8		Not estimable	
Hays 2016	3	98	6	104	2.0%	0.53 [0.14, 2.06]	
Kitajima 1997	0	45	0	46		Not estimable	
Mihatsch 2010	2	91	4	89	1.4%	0.49 [0.09, 2.60]	
Patole 2014	0	77	1	76	0.5%	0.33 [0.01, 7.95]	
Stratiki 2007	0	41	3	34	1.3%	0.12 [0.01, 2.23]	
Totsu 2014	0	153	0	130		Not estimable	
Subtotal (95% CI)		1266		1247	34.0%	0.70 [0.52, 0.94]	
Total events	68		98				
Heterogeneity: Chi² = 11.25, df = 5 (P = 0.05); I² = 56%							
Test for overall effect: Z = 2.34 (P = 0.02)							
1.2.5 Bacillus							
Tewari 2015	2	123	2	121	0.7%	0.98 [0.14, 6.87]	
Subtotal (95% CI)		123		121	0.7%	0.98 [0.14, 6.87]	
Total events	2		2				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.02 (P = 0.99)							
1.2.6 Saccharomyces							
Costalos 2003	5	51	6	36	2.4%	0.59 [0.19, 1.78]	
Demirel 2013	2	91	2	90	0.7%	0.99 [0.14, 6.87]	
Demirel 2013	6	135	7	136	2.4%	0.86 [0.30, 2.50]	
Serce 2013	7	104	7	104	2.4%	1.00 [0.36, 2.75]	
Subtotal (95% CI)		381		366	7.9%	0.83 [0.47, 1.49]	
Total events	20		22				
Heterogeneity: Chi² = 0.54, df = 3 (P = 0.91); I² = 0%							
Test for overall effect: Z = 0.62 (P = 0.53)							
Total (95% CI)		4648		4627	100.0%	0.58 [0.48, 0.69]	
Total events	165		288				
Heterogeneity: Chi² = 34.09, df = 31 (P = 0.32); I² = 9%							
Test for overall effect: Z = 5.85 (P < 0.00001)							
Test for subgroup differences: Chi² = 7.96, df = 4 (P = 0.09), I² = 49.8%							

Scale: 0.01 0.1 1 10 100
probiotics   Placebo

the most severe illnesses in neonatal intensive care units [44]. In preterm infants, establishment of the microbiome is delayed and less diverse, potentially increasing the chance of pathogenic proliferation and leading to the occurrence of NEC [45, 46]. Probiotics have been one of the hot topics in neonatal medicine in recent years. An increasing number of pediatricians suggested that probiotics prevented NEC and gut-associated sepsis, and reduced mortality in preterm infants [47–50]. Although there have been good quality RCTs and systematic literature reviews confirming that the correct use of probiotics can reduce the incidence of gastrointestinal complications, probiotics have not truly been implemented worldwide. Their effectiveness and safety still need to be validated.

The results of this meta-analysis indicated that compared with placebo, probiotics showed an overall advantage in preventing the incidence of NEC and gut-associated sepsis and decreasing mortality in preterm infants. Our results are consistent with those of a previous systematic review and meta-analysis by Dermyshi et al. that included both RCTs and observational studies and showed that probiotics decrease the incidence of NEC and sepsis and the risk of all-cause mortality [51]. In studies published recently [50], researchers outlined several protective intestinal mechanisms of probiotics: (1) probiotics can produce short-chain fatty acids and low oxygen tension, which results in a low pH in the distal small bowel and colon; (2) short-chain fatty acids produced by probiotics also serve as a nutrient source for host colonocytes without the release of oxygen into the lumen; (3) probiotics can produce bacteriocins with direct antimicrobial activity; and (4) probiotics can decrease intestinal permeability in the immature gut. Unfortunately, because the number of trials that referred to NEC-related mortality is small, we only analyzed nine studies and failed to find a significant decrease in NEC-related mortality in the probiotic group. However, the low level of statistical heterogeneity among trials and low risk of publication bias strengthens the reliability of our research results. Additionally, the levels of evidence for the results we explored were high.

Despite the advantages of the overall probiotic effect, it is remarkable that different treatment strategies of probiotics have been used in different trials. It is important to emphasize that the effect of a live microorganism used as a probiotic is strictly genera specific [52]. Recent meta-analyses and systemic reviews also only reported the positive role of probiotic supplementation and failed to distinguish different genera of probiotics [53–55]. We tried to investigate the effect of different probiotic genera among key clinical results such as the incidence of NEC, gut-associated sepsis, and mortality in preterm infants.

We divided all included studies into five different subgroups to analyze the characteristics of probiotic genera. Our study showed that compared with placebo, the probiotic mixture showed a huge advantage and vitality in preventing the occurrence of NEC and gut-associated sepsis, reducing mortality in preterm infants. A possible explanation for this result is that normal flora is diverse in the gut, so use of a combination of probiotic strains might be more logical. Aceti et al. performed a subgroup analysis of the incidence of NEC according to the probiotic genera and reported a similar result to our study [56]. Similarly, two meta-analyses reported that a combination of *Lactobacillus* and *Bifidobacterium* should be strongly considered to reduce the risk of incidence of NEC and gut-associated sepsis and overall-cause mortality [51, 57, 58]. A recent network meta-analysis was performed for each strain, and each result included in the literature was inadequate, the quality of evidence was lower, and the non-probiotic treatment was included; however, it concluded that the mixture of *Bifidobacterium* and *Lactobacillus* can prevent severe complications in premature infants [59]. In summary, the results of the analysis indicated that the use of probiotic mixtures should be recommended to prevent severe clinical complications in preterm infants.

Lactobacillus and *Bifidobacterium* are normally colonizing microorganisms of the human gastrointestinal tract, and they are the most frequently used bacteria in trials [60, 61]. In our analysis, unlike the probiotic mixture, *Lactobacillus* or *Bifidobacterium* was marginally superior in only reducing the risk of occurrence of NEC, whereas there was no definite decrease in the risk of gut-associated sepsis and mortality. As for *Lactobacillus*, our results are close to those of the recent meta-analysis by Dermyshi et al. that showed no change of the incidence of NEC and mortality using *L. reuteri* in preterm infants [51]. Regarding *Bifidobacterium*, the results contrasted the findings of Sun et al. [62]. They demonstrated that *B. infantis* can play an important role in decreasing the risk of NEC, gut-associated sepsis, and mortality. Similarly, in a meta-analysis, Zhu et al. drew the conclusion that *Bifidobacterium* may have a beneficial effect and be safe in preventing NEC in preterm infants [63]. A possible explanation for this difference in results is that *Bifidobacterium* enters the gastrointestinal tract and its activity settles within 1 or 2 days after birth; it is the main dominant bacteria in the neonatal period. It has the function of immune activation, can enhance and mobilize the immune function of the body, and can activate the mononuclear macrophage system and induce secretory immunoglobulin A (sIgA) [64, 65]. sIgA is an important local mucosal immunoglobulin, which has an obvious bactericidal effect and a role in neutralizing toxins, and it effectively makes up for the deficiency of
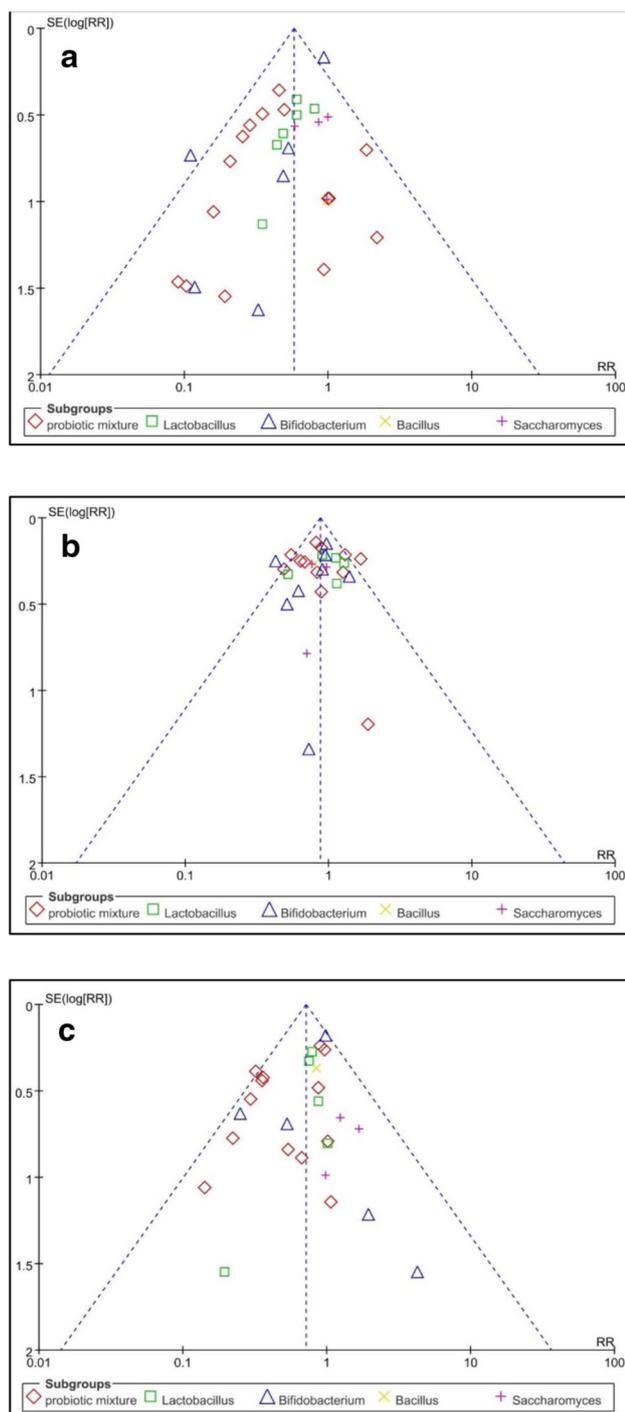
Fig. 4 Funnel plot of the included studies: **a** necrotizing enterocolitis; **b** gut-associated sepsis; **c** all-cause mortality. The funnel plot does not show any clear visual asymmetry

low sIgA in the intestinal tract of premature infants; thus, it effectively prevents the occurrence of intestinal complications such as NEC. However, we reviewed merely one trial that used *Bacillus* so a sub-meta-analysis was not performed in this study.

In addition, the results of our analysis demonstrated that Saccharomyces alone failed to prevent all severe clinical outcomes in preterm infants. Thus far, no study has proved that the use of *Saccharomyces* alone can reduce risk of NEC incidence [39]. Because of the difference between bacterial and yeast strains, their underlying mechanism of action is poorly understood. Reports suggested that yeast and bacterial probiotics have a synergistic effect and promote mutually to their survival and growth [66]. Even so, the use of Saccharomyces alone cannot be recommended to prevent the risk of NEC or gut-associated sepsis in preterm infants until a higher level of evidence is provided.

An important fact is that probiotic supplements are living microorganisms that may cause infection. Therefore, their safety and long-term effect must receive attention as well. Some recent studies have already reported cases of *Lactobacillus* sepsis in infants who received treatment with probiotics [67–70]. Among the cases, most affected infants had severe diseases, such as immunocompromised [67] and short-gut syndrome [70]. Similarly, some cases of *Bifidobacterium* sepsis have been reported in the literature [71–73]. The other concern is that probiotics might spread to someone who did not need to receive probiotics, and their safety may be at risk. Probiotic-associated sepsis, although rare and/or mild in general, should not be ignored. More investigations are needed to solve this problem. Additionally, a previous study was conducted particularly on the long-term outcomes of oral probiotics [20]. The authors concluded that oral probiotics had no effect on neurodevelopment and growth in infants with a corrected age of 18–22 months [20]. Nevertheless, their long-term safety remains to be evaluated.

In addition, a recently published Cochrane review reported about the role of maternal probiotic supplementation in preventing NEC in preterm infants [74]. The authors provided a new idea that probiotics may alter the microbiome of the preterm infant if given to mothers during pregnancy/lactation. They aimed to enhance the safety of probiotic supplementation in this regard. Unfortunately, they failed to find an efficient way to prove its effectiveness. Thus, more research is needed for gestational/postnatal administration of probiotics.

Despite the fact that the application of probiotics has been increasingly popular, their effect and safety need to be further investigated before a consensus is made. The published data still have limitations: (1) the recent studies made it difficult for us to perform a meta-analysis according to each strain of probiotics so we could only reveal a tendency of the optimal choice; (2) as for optimal dose, there is no clear standard to distinguish the effective and safe dose; (3) as aforementioned, investigation of the long-term outcome of probiotics is limited, but the possibility that one will carry probiotics for life is of concern; (4) furthermore, none of the literature addressed
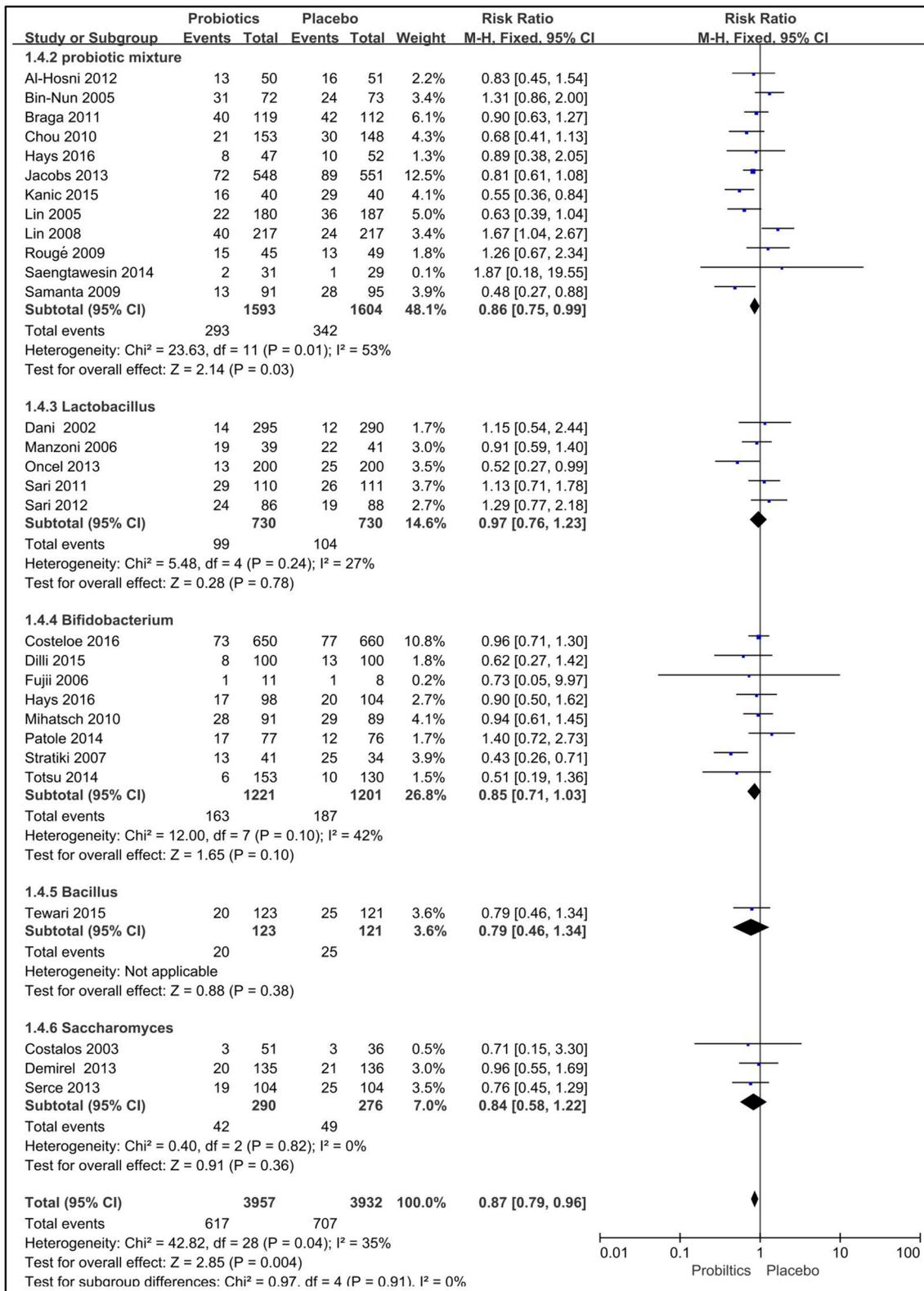
Study or Subgroup	Probiotics Events	Total	Placebo Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI
1.4.2 probiotic mixture							
Al-Hosni 2012	13	50	16	51	2.2%	0.83 [0.45, 1.54]	
Bin-Nun 2005	31	72	24	73	3.4%	1.31 [0.86, 2.00]	
Braga 2011	40	119	42	112	6.1%	0.90 [0.63, 1.27]	
Chou 2010	21	153	30	148	4.3%	0.68 [0.41, 1.13]	
Hays 2016	8	47	10	52	1.3%	0.89 [0.38, 2.05]	
Jacobs 2013	72	548	89	551	12.5%	0.81 [0.61, 1.08]	
Kanic 2015	16	40	29	40	4.1%	0.55 [0.36, 0.84]	
Lin 2005	22	180	36	187	5.0%	0.63 [0.39, 1.04]	
Lin 2008	40	217	24	217	3.4%	1.67 [1.04, 2.67]	
Rougé 2009	15	45	13	49	1.8%	1.26 [0.67, 2.34]	
Saengtawesin 2014	2	31	1	29	0.1%	1.87 [0.18, 19.55]	
Samanta 2009	13	91	28	95	3.9%	0.48 [0.27, 0.88]	
Subtotal (95% CI)		1593		1604	48.1%	**0.86 [0.75, 0.99]**	
Total events	293		342				
Heterogeneity: Chi² = 23.63, df = 11 (P = 0.01); I² = 53%							
Test for overall effect: Z = 2.14 (P = 0.03)							
1.4.3 Lactobacillus							
Dani 2002	14	295	12	290	1.7%	1.15 [0.54, 2.44]	
Manzoni 2006	19	39	22	41	3.0%	0.91 [0.59, 1.40]	
Oncel 2013	13	200	25	200	3.5%	0.52 [0.27, 0.99]	
Sari 2011	29	110	26	111	3.7%	1.13 [0.71, 1.78]	
Sari 2012	24	86	19	88	2.7%	1.29 [0.77, 2.18]	
Subtotal (95% CI)		730		730	14.6%	**0.97 [0.76, 1.23]**	
Total events	99		104				
Heterogeneity: Chi² = 5.48, df = 4 (P = 0.24); I² = 27%							
Test for overall effect: Z = 0.28 (P = 0.78)							
1.4.4 Bifidobacterium							
Costeloe 2016	73	650	77	660	10.8%	0.96 [0.71, 1.30]	
Dilli 2015	8	100	13	100	1.8%	0.62 [0.27, 1.42]	
Fujii 2006	1	11	1	8	0.2%	0.73 [0.05, 9.97]	
Hays 2016	17	98	20	104	2.7%	0.90 [0.50, 1.62]	
Mihatsch 2010	28	91	29	89	4.1%	0.94 [0.61, 1.45]	
Patole 2014	17	77	12	76	1.7%	1.40 [0.72, 2.73]	
Stratiki 2007	13	41	25	34	3.9%	0.43 [0.26, 0.71]	
Totsu 2014	6	153	10	130	1.5%	0.51 [0.19, 1.36]	
Subtotal (95% CI)		1221		1201	26.8%	**0.85 [0.71, 1.03]**	
Total events	163		187				
Heterogeneity: Chi² = 12.00, df = 7 (P = 0.10); I² = 42%							
Test for overall effect: Z = 1.65 (P = 0.10)							
1.4.5 Bacillus							
Tewari 2015	20	123	25	121	3.6%	0.79 [0.46, 1.34]	
Subtotal (95% CI)		123		121	3.6%	**0.79 [0.46, 1.34]**	
Total events	20		25				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.88 (P = 0.38)							
1.4.6 Saccharomyces							
Costalos 2003	3	51	3	36	0.5%	0.71 [0.15, 3.30]	
Demirel 2013	20	135	21	136	3.0%	0.96 [0.55, 1.69]	
Serce 2013	19	104	25	104	3.5%	0.76 [0.45, 1.29]	
Subtotal (95% CI)		290		276	7.0%	**0.84 [0.58, 1.22]**	
Total events	42		49				
Heterogeneity: Chi² = 0.40, df = 2 (P = 0.82); I² = 0%							
Test for overall effect: Z = 0.91 (P = 0.36)							
Total (95% CI)		3957		3932	100.0%	**0.87 [0.79, 0.96]**	
Total events	617		707				
Heterogeneity: Chi² = 42.82, df = 28 (P = 0.04); I² = 35%							
Test for overall effect: Z = 2.85 (P = 0.004)							
Test for subgroup differences: Chi² = 0.97, df = 4 (P = 0.91), I² = 0%							

0.01 0.1 1 10 100
Probiltics   Placebo

Fig. 5 Forest plot of the included studies. The forest plot shows the association between the use of probiotics and gut-associated sepsis in the overall population of preterm infants. *M–H* Mantel–Haenszel method
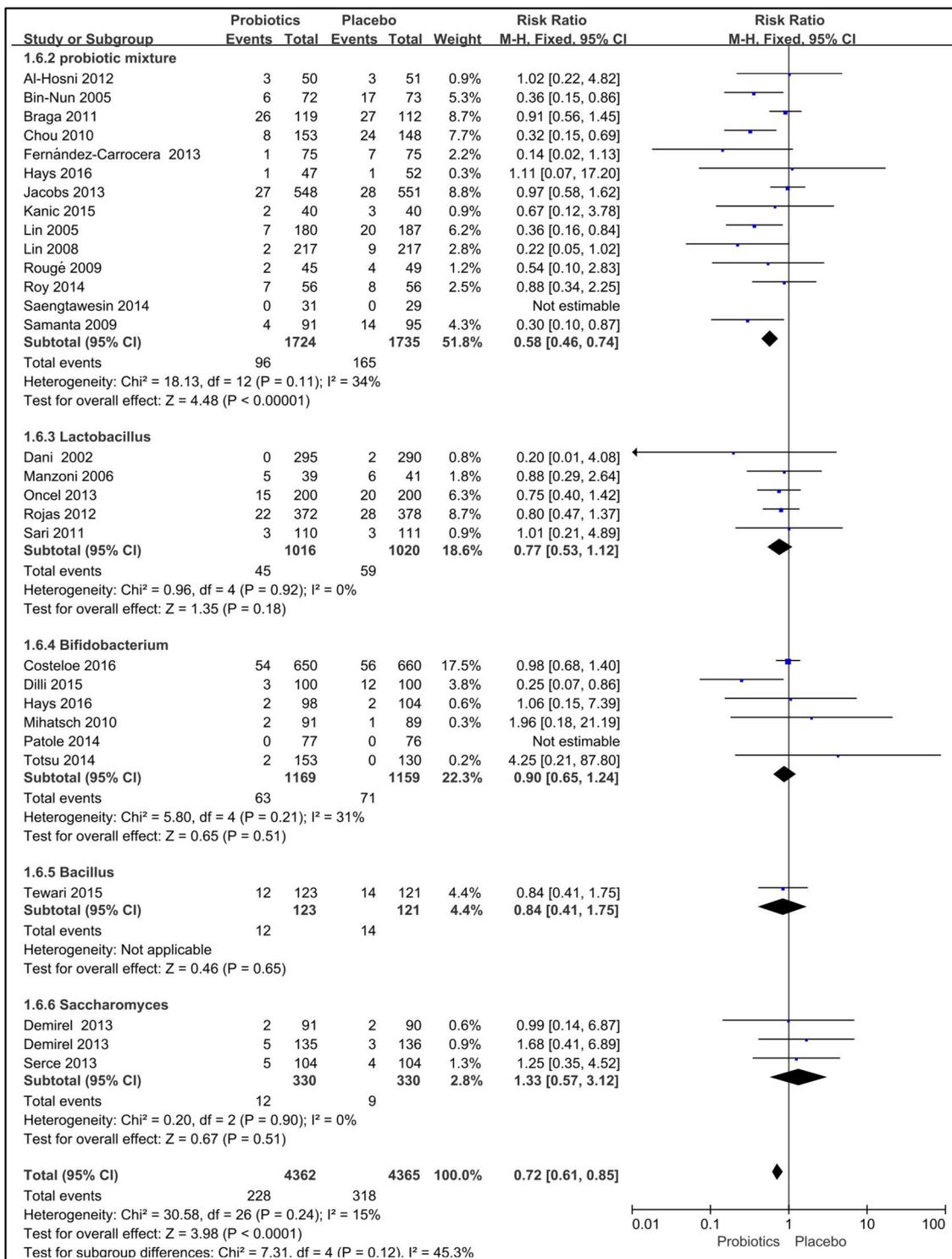
Study or Subgroup	Probiotics Events	Total	Placebo Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.6.2 probiotic mixture						
Al-Hosni 2012	3	50	3	51	0.9%	1.02 [0.22, 4.82]
Bin-Nun 2005	6	72	17	73	5.3%	0.36 [0.15, 0.86]
Braga 2011	26	119	27	112	8.7%	0.91 [0.56, 1.45]
Chou 2010	8	153	24	148	7.7%	0.32 [0.15, 0.69]
Fernández-Carrocera 2013	1	75	7	75	2.2%	0.14 [0.02, 1.13]
Hays 2016	1	47	1	52	0.3%	1.11 [0.07, 17.20]
Jacobs 2013	27	548	28	551	8.8%	0.97 [0.58, 1.62]
Kanic 2015	2	40	3	40	0.9%	0.67 [0.12, 3.78]
Lin 2005	7	180	20	187	6.2%	0.36 [0.16, 0.84]
Lin 2008	2	217	9	217	2.8%	0.22 [0.05, 1.02]
Rougé 2009	2	45	4	49	1.2%	0.54 [0.10, 2.83]
Roy 2014	7	56	8	56	2.5%	0.88 [0.34, 2.25]
Saengtawesin 2014	0	31	0	29		Not estimable
Samanta 2009	4	91	14	95	4.3%	0.30 [0.10, 0.87]
Subtotal (95% CI)		1724		1735	51.8%	0.58 [0.46, 0.74]
Total events	96		165			

Heterogeneity: Chi² = 18.13, df = 12 (P = 0.11); I² = 34%
Test for overall effect: Z = 4.48 (P < 0.00001)

Study or Subgroup	Events	Total	Events	Total	Weight	Risk Ratio
1.6.3 Lactobacillus						
Dani 2002	0	295	2	290	0.8%	0.20 [0.01, 4.08]
Manzoni 2006	5	39	6	41	1.8%	0.88 [0.29, 2.64]
Oncel 2013	15	200	20	200	6.3%	0.75 [0.40, 1.42]
Rojas 2012	22	372	28	378	8.7%	0.80 [0.47, 1.37]
Sari 2011	3	110	3	111	0.9%	1.01 [0.21, 4.89]
Subtotal (95% CI)		1016		1020	18.6%	0.77 [0.53, 1.12]
Total events	45		59			

Heterogeneity: Chi² = 0.96, df = 4 (P = 0.92); I² = 0%
Test for overall effect: Z = 1.35 (P = 0.18)

Study or Subgroup	Events	Total	Events	Total	Weight	Risk Ratio
1.6.4 Bifidobacterium						
Costeloe 2016	54	650	56	660	17.5%	0.98 [0.68, 1.40]
Dilli 2015	3	100	12	100	3.8%	0.25 [0.07, 0.86]
Hays 2016	2	98	2	104	0.6%	1.06 [0.15, 7.39]
Mihatsch 2010	2	91	1	89	0.3%	1.96 [0.18, 21.19]
Patole 2014	0	77	0	76		Not estimable
Totsu 2014	2	153	0	130	0.2%	4.25 [0.21, 87.80]
Subtotal (95% CI)		1169		1159	22.3%	0.90 [0.65, 1.24]
Total events	63		71			

Heterogeneity: Chi² = 5.80, df = 4 (P = 0.21); I² = 31%
Test for overall effect: Z = 0.65 (P = 0.51)

Study or Subgroup	Events	Total	Events	Total	Weight	Risk Ratio
1.6.5 Bacillus						
Tewari 2015	12	123	14	121	4.4%	0.84 [0.41, 1.75]
Subtotal (95% CI)		123		121	4.4%	0.84 [0.41, 1.75]
Total events	12		14			

Heterogeneity: Not applicable
Test for overall effect: Z = 0.46 (P = 0.65)

Study or Subgroup	Events	Total	Events	Total	Weight	Risk Ratio
1.6.6 Saccharomyces						
Demirel 2013	2	91	2	90	0.6%	0.99 [0.14, 6.87]
Demirel 2013	5	135	3	136	0.9%	1.68 [0.41, 6.89]
Serce 2013	5	104	4	104	1.3%	1.25 [0.35, 4.52]
Subtotal (95% CI)		330		330	2.8%	1.33 [0.57, 3.12]
Total events	12		9			

Heterogeneity: Chi² = 0.20, df = 2 (P = 0.90); I² = 0%
Test for overall effect: Z = 0.67 (P = 0.51)

Study or Subgroup	Events	Total	Events	Total	Weight	Risk Ratio
Total (95% CI)		4362		4365	100.0%	0.72 [0.61, 0.85]
Total events	228		318			

Heterogeneity: Chi² = 30.58, df = 26 (P = 0.24); I² = 15%
Test for overall effect: Z = 3.98 (P < 0.0001)
Test for subgroup differences: Chi² = 7.31, df = 4 (P = 0.12); I² = 45.3%

Fig. 6 Forest plot of the included studies. The forest plot shows the association between the use of probiotics and all-cause mortality in the overall population of preterm infants. *M–H* Mantel–Haenszel method

contraindications for probiotics; and (5) the problems associated with the use of different milks and antibiotics need to be solved, although these problems did not seem to affect the results of any trials. In the future, rigorous studies need to be conducted to further investigate and improve the treatment strategy of probiotics, and to determine their value and critical role in the prevention of severe disease in preterm infants.
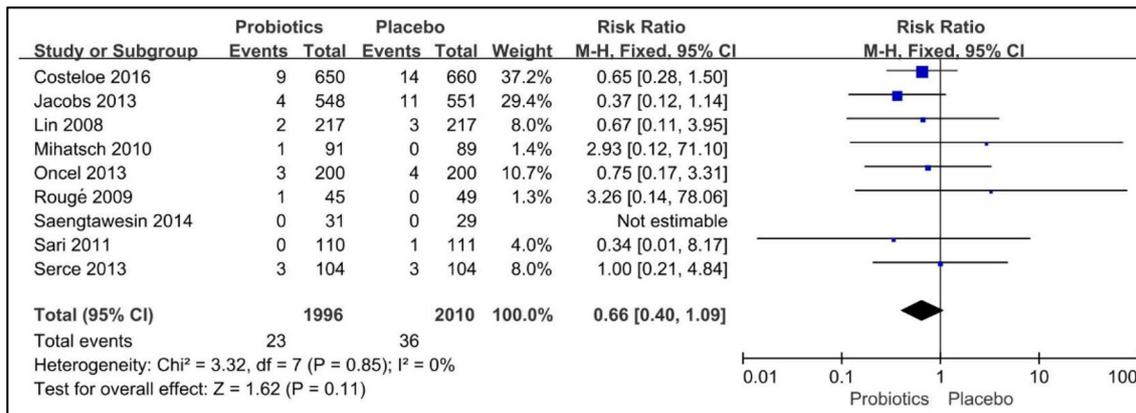
Fig. 7 Forest plot of the included studies. The forest plot shows the association between the use of probiotics and NEC-related mortality in the overall population of preterm infants. *M–H* Mantel–Haenszel method

Conclusions

Probiotic supplementation may offer an avenue to decrease the incidence of NEC and gut-associated sepsis and mortality in preterm infants. A definite conclusion can be drawn from available data: the probiotic mixture might show advantages over single probiotic strains. However, larger studies are needed to better understand the effect and safety of probiotic and to further solve the lack of clarity regarding the optimal dose of probiotics, which type of probiotic strain should be chosen, and feeding time of probiotics.

Author contributors LB conceptualized and designed the study, drafted the initial manuscript, and interpreted the data; BY conducted the initial analyses and drafted the initial manuscript; QY conceptualized and designed the study and supervised the analysis; ML conducted the meta-analyses, interpreted the data, and reviewed the manuscript; and HC 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.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

References

1. Arbra CA, Oprisan A, Wilson DA, Ryan RM, Lesher AP (2018) Time to reintroduction of feeding in infants with nonsurgical necrotizing enterocolitis. J Pediatr Surg 53(6):1187–1191. https ://doi.org/10.1016/j.jpedsurg.2018.02.082

2. Barron LK, Warner BB, Tarr PI, Shannon WD, Deych E, Warner BW (2017) Independence of gut bacterial content and neonatal necrotizing enterocolitis severity. J Pediatr Surg 52(6):993–998. https://doi.org/10.1016/j.jpedsurg.2017.03.029

3. Hong CR, Fullerton BS, Mercier CE, Morrow KA, Edwards EM, Ferrelli KR, Soll RF, Modi BP, Horbar JD, Jaksic T (2018) Growth morbidity in extremely low birth weight survivors of necrotizing enterocolitis at discharge and two-year follow-up. J Pediatr Surg 53(6):1197–1202. https://doi.org/10.1016/j.jpeds urg.2018.02.085

4. Hammerman C, Bin-Nun A, Kaplan M (2004) Germ warfare: probiotics in defense of the premature gut. Clin Perinatol 31(3):489–500. https://doi.org/10.1016/j.clp.2004.04.015

5. Martin CR, Walker WA (2008) Probiotics: role in pathophysiology and prevention in necrotizing enterocolitis. Semin Perinatol 32(2):127–137. https://doi.org/10.1053/j.semperi.2008.01.006

6. Braga TD, da Silva GA, de Lira PI, de Carvalho Lima M (2011) Efficacy of *Bifidobacterium breve* and *Lactobacillus casei* oral supplementation on necrotizing enterocolitis in very-low-birth-weight preterm infants: a double-blind, randomized, controlled trial. Am J Clin Nutr 93(1):81–86. https://doi.org/10.3945/ajcn.2010.29799

7. Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, Oh W (2005) Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Pediatrics 115(1):1–4. https://doi.org/10.1542/peds.2004-1463

8. Al-Hosni M, Duenas M, Hawk M, Stewart LA, Borghese RA, Cahoon M, Atwood L, Howard D, Ferrelli K, Soll R (2012) Probiotics-supplemented feeding in extremely low-birth-weight infants. J Perinatol 32(4):253–259. https://doi.org/10.1038/jp.2011.51

9. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA (2015) Preferred reporting items for

systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ 350:g7647. https://doi.org/10.1136/bmj.g7647

10. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA (2011) The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ 343:d5928. https://doi.org/10.1136/bmj.d5928

11. Higgins JPT, Green S (2011) Cochrane handbook for systematic reviews of interventions, version 5.1.0. The cochrane collaboration. https://training.cochrane.org/handbook. Accessed 20 July 2019

12. Pottie K, Welch V, Morton R, Akl EA, Eslava-Schmalbach JH, Katikireddi V, Singh J, Moja L, Lang E, Magrini N, Thabane L, Stanev R, Matovinovic E, Snellman A, Briel M, Shea B, Tugwell P, Schunemann H, Guyatt G, Alonso-Coello P (2017) GRADE equity guidelines 4: considering health equity in GRADE guideline development: evidence to decision process. J Clin Epidemiol 90:84–91. https://doi.org/10.1016/j.jclinepi.2017.08.001))

13. Chou IC, Kuo HT, Chang JS, Wu SF, Chiu HY, Su BH, Lin HC (2010) Lack of effects of oral probiotics on growth and neurodevelopmental outcomes in preterm very low birth weight infants. J Pediatr 156(3):393–396. https://doi.org/10.1016/j.jpeds.2009.09.051

14. Lin HC, Hsu CH, Chen HL, Chung MY, Hsu JF, Lien RI, Tsao LY, Chen CH, Su BH (2008) Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. Pediatrics 122(4):693–700. https://doi.org/10.1542/peds.2007-3007

15. Hays S, Jacquot A, Gauthier H, Kempf C, Beissel A, Pidoux O, Jumas-Bilak E, Decullier E, Lachambre E, Beck L, Cambonie G, Putet G, Claris O, Picaud JC (2016) Probiotics and growth in preterm infants: a randomized controlled trial, PREMAPRO study. Clin Nutr 35(4):802–811. https://doi.org/10.1016/j.clnu.2015.06.006

16. Jacobs SE, Tobin JM, Opie GF, Donath S, Tabrizi SN, Pirotta M, Morley CJ, Garland SM, ProPrems Study G (2013) Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. Pediatrics 132(6):1055–1062. https://doi.org/10.1542/peds.2013-1339

17. Patole S, Keil AD, Chang A, Nathan E, Doherty D, Simmer K, Esvaran M, Conway P (2014) Effect of *Bifidobacterium breve* M-16 V supplementation on fecal bifidobacteria in preterm neonates—a randomised double blind placebo controlled trial. PLoS One 9(3):e89511. https://doi.org/10.1371/journal.pone.0089511

18. Costalos C, Skouteri V, Gounaris A, Sevastiadou S, Triandafilidou A, Ekonomidou C, Kontaxaki F, Petrochilou V (2003) Enteral feeding of premature infants with *Saccharomyces boulardii*. Early Hum Dev 74(2):89–96. https://doi.org/10.1016/s0378-3782(03)00090-2

19. Roy A, Chaudhuri J, Sarkar D, Ghosh P, Chakraborty S (2014) Role of enteric supplementation of probiotics on late-onset sepsis by candida species in preterm low birth weight neonates: a randomized, double blind, placebo-controlled trial. N Am J Med Sci 6(1):50–57. https://doi.org/10.4103/1947-2714.125870

20. Sari FN, Eras Z, Dizdar EA, Erdeve O, Oguz SS, Uras N, Dilmen U (2012) Do oral probiotics affect growth and neurodevelopmental outcomes in very-low-birth-weight preterm infants? Am J Perinatol 29(8):579–586. https://doi.org/10.1055/s-0032-1311981

21. Costeloe K, Hardy P, Juszczak E, Wilks M, Millar MR (2016) *Bifidobacterium breve* BBG-001 in very preterm infants: a randomised controlled phase 3 trial. Lancet 387(10019):649–660. https://doi.org/10.1016/s0140-6736(15)01027-2

22. Fernández-Carrocera LA, Solis-Herrera A, Cabanillas-Ayón M, Gallardo-Sarmiento RB, García-Pérez CS, Montaño-Rodríguez R, Echániz-Aviles MOL (2013) Double-blind, randomised clinical assay to evaluate the efficacy of probiotics in preterm newborns weighing less than 1500 g in the prevention of necrotising enterocolitis. Arch Dis Child Fetal Neonatal Ed 98(1):F5–F9. https://doi.org/10.1136/archdischild-2011-300435

23. Manzoni P, Mostert M, Leonessa ML, Priolo C, Farina D, Monetti C, Latino MA, Gomirato G (2006) Oral supplementation with *Lactobacillus casei* subspecies rhamnosus prevents enteric colonization by Candida species in preterm neonates: a randomized study. Clin Infect Dis 42(12):1735–1742. https://doi.org/10.1086/504324

24. Mihatsch WA, Vossbeck S, Eikmanns B, Hoegel J, Pohlandt F (2010) Effect of Bifidobacterium lactis on the incidence of nosocomial infections in very-low-birth-weight infants: a randomized controlled trial. Neonatology 98(2):156–163. https://doi.org/10.1159/000280291

25. Oncel MY, Sari FN, Arayici S, Guzoglu N, Erdeve O, Uras N, Oguz SS, Dilmen U (2014) *Lactobacillus reuteri* for the prevention of necrotising enterocolitis in very low birthweight infants: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed 99(2):F110–F115. https://doi.org/10.1136/archdischild-2013-304745

26. Rojas MA, Lozano JM, Rojas MX, Rodriguez VA, Rondon MA, Bastidas JA, Perez LA, Rojas C, Ovalle O, Garcia-Harker JE, Tamayo ME, Ruiz GC, Ballesteros A, Archila MM, Arevalo M (2012) Prophylactic probiotics to prevent death and nosocomial infection in preterm infants. Pediatrics 130(5):e1113–e1120. https://doi.org/10.1542/peds.2011-3584

27. Rouge C, Piloquet H, Butel MJ, Berger B, Rochat F, Ferraris L, Des Robert C, Legrand A, de la Cochetiere MF, N'Guyen JM, Vodovar M, Voyer M, Darmaun D, Roze JC (2009) Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 89(6):1828–1835. https://doi.org/10.3945/ajcn.2008.26919

28. Samanta M, Sarkar M, Ghosh P, Jk Ghosh, Mk Sinha, Chatterjee S (2008) Prophylactic probiotics for prevention of necrotizing enterocolitis in very low birth weight newborns. J Trop Pediatr 55(2):128–131. https://doi.org/10.1093/tropej/fmn091

29. Stratiki Z, Costalos C, Sevastiadou S, Kastanidou O, Skouroliakou M, Giakoumatou A, Petrohilou V (2007) The effect of a bifidobacter supplemented bovine milk on intestinal permeability of preterm infants. Early Hum Dev 83(9):575–579. https://doi.org/10.1016/j.earlhumdev.2006.12.002

30. Chowdhury T, Ali MM, Hossain MM, Singh J, Yousuf AN, Yasmin F, Chowdhury FR (2016) Efficacy of probiotics versus placebo in the prevention of necrotizing enterocolitis in preterm very low birth weight infants: a double-blind randomized controlled trial. J Coll Phys Surg Pak JCPSP 26(9):770–774

31. Demirel G, Celik IH, Erdeve O, Saygan S, Dilmen U, Canpolat FE (2013) Prophylactic *Saccharomyces boulardii* versus nystatin for the prevention of fungal colonization and invasive fungal infection in premature infants. Eur J Pediatr 172(10):1321–1326. https://doi.org/10.1007/s00431-013-2041-4

32. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF (2002) Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biol Neonate 82(2):103–108. https://doi.org/10.1159/000063096

33. Sari FN, Dizdar EA, Oguz S, Erdeve O, Uras N, Dilmen U (2011) Oral probiotics: lactobacillus sporogenes for prevention of necrotizing enterocolitis in very low-birth weight infants: a randomized, controlled trial. Eur J Clin Nutr 65(4):434–439. https://doi.org/10.1038/ejcn.2010.278

34. Demirel G, Erdeve O, Celik IH, Dilmen U (2013) *Saccharomyces boulardii* for prevention of necrotizing enterocolitis in

preterm infants: a randomized, controlled study. Acta Paediatr 102(12):e560–e565. https://doi.org/10.1111/apa.12416

35. Dilli D, Aydin B, Fettah ND, Ozyazici E, Beken S, Zenciroglu A, Okumus N, Ozyurt BM, Ipek MS, Akdag A, Turan O, Bozdag S (2015) The propre-save study: effects of probiotics and prebiotics alone or combined on necrotizing enterocolitis in very low birth weight infants. J Pediatr 166(3):545–551. https://doi.org/10.1016/j.jpeds.2014.12.004 **(e541)**

36. Fujii T, Ohtsuka Y, Lee T, Kudo T, Shoji H, Sato H, Nagata S, Shimizu T, Yamashiro Y (2006) *Bifidobacterium breve* enhances transforming growth factor beta1 signaling by regulating Smad7 expression in preterm infants. J Pediatr Gastroenterol Nutr 43(1):83–88. https://doi.org/10.1097/01.mpg.0000228100.04702.f8

37. Kanic Z, Micetic Turk D, Burja S, Kanic V, Dinevski D (2015) Influence of a combination of probiotics on bacterial infections in very low birthweight newborns. Wien Klin Wochenschr 127(Suppl 5):S210–S215. https://doi.org/10.1007/s00508-015-0845-0

38. Saengtawesin V, Tangpolkaiwalsak R, Kanjanapattankul W (2014) Effect of oral probiotics supplementation in the prevention of necrotizing enterocolitis among very low birth weight preterm infants. J Med Assoc Thail 97(Suppl 6):S20–S25

39. Serce O, Benzer D, Gursoy T, Karatekin G, Ovali F (2013) Efficacy of *Saccharomyces boulardii* on necrotizing enterocolitis or sepsis in very low birth weight infants: a randomised controlled trial. Early Hum Dev 89(12):1033–1036. https://doi.org/10.1016/j.earlhumdev.2013.08.013

40. Tewari VV, Dubey SK, Gupta G (2015) *Bacillus clausii* for prevention of late-onset sepsis in preterm infants: a randomized controlled trial. J Trop Pediatr 61(5):377–385. https://doi.org/10.1093/tropej/fmv050

41. Totsu S, Yamasaki C, Terahara M, Uchiyama A, Kusuda S, Probiotics Study Group in J (2014) Bifidobacterium and enteral feeding in preterm infants: cluster-randomized trial. Pediatr Int 56(5):714–719. https://doi.org/10.1111/ped.12330

42. Kitajima H, Sumida Y, Tanaka R, Yuki N, Takayama H, Fujimura M (1997) Early administration of *Bifidobacterium breve* to preterm infants: randomised controlled trial. Arch Dis Child Fetal Neonatal Ed 76(2):F101–F107. https://doi.org/10.1136/fn.76.2.f101

43. Bin-Nun A, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, Hammerman C (2005) Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. J Pediatr 147(2):192–196. https://doi.org/10.1016/j.jpeds.2005.03.054

44. Eyal F, Sagi E, Arad I, Avital A (1982) Necrotising enterocolitis in the very low birthweight infant: expressed breast milk feeding compared with parenteral feeding. Arch Dis Child 57(4):274–276. https://doi.org/10.1136/adc.57.4.274

45. van Elburg RM, Fetter WP, Bunkers CM, Heymans HS (2003) Intestinal permeability in relation to birth weight and gestational and postnatal age. Arch Dis Child Fetal Neonatal Ed 88(1):F52–F55. https://doi.org/10.1136/fn.88.1.f52

46. Frost BL, Modi BP, Jaksic T, Caplan MS (2017) New medical and surgical insights into neonatal necrotizing enterocolitis: a review. JAMA Pediatr 171(1):83–88. https://doi.org/10.1001/jamapediatrics.2016.2708

47. Hagen PC, Skelley JW (2019) Efficacy of bifidobacterium species in prevention of necrotizing enterocolitis in very-low birth weight infants. A systematic review. J Pediatri Pharmacol Ther JPPT 24(1):10–15. https://doi.org/10.5863/1551-6776-24.1.10

48. Baranowski JR, Claud EC (2019) Necrotizing enterocolitis and the preterm infant microbiome. Adv Exp Med Biol. https://doi.org/10.1007/5584_2018_313

49. Underwood MA (2019) Arguments for routine administration of probiotics for NEC prevention. Curr Opin Pediatr 31(2):188–194. https://doi.org/10.1097/mop.0000000000000732

50. Underwood MA (2019) Probiotics and the prevention of necrotizing enterocolitis. J Pediatr Surg 54(3):405–412. https://doi.org/10.1016/j.jpedsurg.2018.08.055

51. Dermyshi E, Wang Y, Yan C, Hong W, Qiu G, Gong X, Zhang T (2017) The "golden age" of probiotics: a systematic review and meta-analysis of randomized and observational studies in preterm infants. Neonatology 112(1):9–23. https://doi.org/10.1159/000454668

52. Guarner F, Malagelada JR (2003) Gut flora in health and disease. Lancet 361(9356):512–519. https://doi.org/10.1016/s0140-6736(03)12489-0

53. Deshpande G, Rao S, Patole S (2007) Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. Lancet 369(9573):1614–1620. https://doi.org/10.1016/s0140-6736(07)60748-x

54. Barclay AR, Stenson B, Simpson JH, Weaver LT, Wilson DC (2007) Probiotics for necrotizing enterocolitis: a systematic review. J Pediatr Gastroenterol Nutr 45(5):569–576. https://doi.org/10.1097/MPG.0b013e3181344694

55. Alfaleh K, Anabrees J, Bassler D (2010) Probiotics reduce the risk of necrotizing enterocolitis in preterm infants: a meta-analysis. Neonatology 97(2):93–99. https://doi.org/10.1159/000235684

56. Aceti A, Gori D, Barone G, Callegari ML, Di Mauro A, Fantini MP, Indrio F, Maggio L, Meneghin F, Morelli L, Zuccotti G, Corvaglia L (2015) Probiotics for prevention of necrotizing enterocolitis in preterm infants: systematic review and meta-analysis. Ital J Pediatr. https://doi.org/10.1186/s13052-015-0199-2

57. Baucells BJ, Hally MM, Sánchez ATÁ, Aloy JF (2016) Probiotic associations in the prevention of necrotising enterocolitis and the reduction of late-onset sepsis and neonatal mortality in preterm infants under 1500g. A systematic review. Anales de Pediatría (English Edition) 85(5):247–255. https://doi.org/10.1016/j.anpede.2015.07.021

58. Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T (2011) Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. https://doi.org/10.1002/14651858.cd005496.pub3

59. van den Akker CHP, van Goudoever JB, Szajewska H, Embleton ND, Hojsak I, Reid D, Shamir R (2018) Probiotics for preterm infants. J Pediatr Gastroenterol Nutr 67(1):103–122. https://doi.org/10.1097/mpg.0000000000001897

60. Di Cerbo A, Palmieri B, Aponte M, Morales-Medina JC, Iannitti T (2016) Mechanisms and therapeutic effectiveness of lactobacilli. J Clin Pathol 69(3):187–203. https://doi.org/10.1136/jclinpath-2015-202976

61. Hidalgo-Cantabrana C, Delgado S, Ruiz L, Ruas-Madiedo P, Sanchez B, Margolles A (2017) Bifidobacteria and their health-promoting effects. Microbiol Spectr. https://doi.org/10.1128/microbiolspec.bad-0010-2016

62. Sun J, Marwah G, Westgarth M, Buys N, Ellwood D, Gray PH (2017) Effects of probiotics on necrotizing enterocolitis, sepsis, intraventricular hemorrhage, mortality, length of hospital stay, and weight gain in very preterm infants: a meta-analysis. Adv Nutr 8(5):749–763. https://doi.org/10.3945/an.116.014605

63. Zhu XL, Tang XG, Qu F, Zheng Y, Zhang WH, Diao YQ (2019) Bifidobacterium may benefit the prevention of necrotizing enterocolitis in preterm infants: a systematic review and meta-analysis. Int J Surg 61:17–25. https://doi.org/10.1016/j.ijsu.2018.11.026

64. Isolauri E, Sutas Y, Kankaanpaa P, Arvilommi H, Salminen S (2001) Probiotics: effects on immunity. Am J Clin Nutr 73(2 Suppl):444s–450s. https://doi.org/10.1093/ajcn/73.2.444s

65. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, Belzer C, Delgado Palacio S, Arboleya Montes S, Mancabelli L, Lugli GA, Rodriguez JM, Bode L, de Vos W, Gueimonde M, Margolles A, van Sinderen D, Ventura M (2017) The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. Microbiol Mol Biol Rev MMBR. https://doi.org/10.1128/mmbr.00036-17

66. Katakura Y, Sano R, Hashimoto T, Ninomiya K, Shioya S (2010) Lactic acid bacteria display on the cell surface cytosolic proteins that recognize yeast mannan. Appl Microbiol Biotechnol 86(1):319–326. https://doi.org/10.1007/s00253-009-2295-y

67. Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK (2005) Lactobacillus sepsis associated with probiotic therapy. Pediatrics 115(1):178–181. https://doi.org/10.1542/peds.2004-2137

68. Broughton RA, Gruber WC, Haffar AA, Baker CJ (1983) Neonatal meningitis due to Lactobacillus. Pediatr Infect Dis 2(5):382–384

69. Hammerman C, Bin-Nun A, Kaplan M (2006) Safety of probiotics: comparison of two popular strains. BMJ 333(7576):1006–1008. https://doi.org/10.1136/bmj.39010.630799.BE

70. Kunz AN, Noel JM, Fairchok MP (2004) Two cases of Lactobacillus bacteremia during probiotic treatment of short gut syndrome. J Pediatr Gastroenterol Nutr 38(4):457–458

71. Ohishi A, Takahashi S, Ito Y, Ohishi Y, Tsukamoto K, Nanba Y, Ito N, Kakiuchi S, Saitoh A, Morotomi M, Nakamura T (2010) Bifidobacterium septicemia associated with postoperative probiotic therapy in a neonate with omphalocele. J Pediatr 156(4):679–681. https://doi.org/10.1016/j.jpeds.2009.11.041

72. Zbinden A, Zbinden R, Berger C, Arlettaz R (2015) Case series of *Bifidobacterium longum* bacteremia in three preterm infants on probiotic therapy. Neonatology 107(1):56–59. https://doi.org/10.1159/000367985

73. Bertelli C, Pillonel T, Torregrossa A, Prod'hom G, Fischer CJ, Greub G, Giannoni E (2015) *Bifidobacterium longum* bacteremia in preterm infants receiving probiotics. Clin Infect Dis 60(6):924–927. https://doi.org/10.1093/cid/ciu946

74. Grev J, Berg M, Soll R (2018) Maternal probiotic supplementation for prevention of morbidity and mortality in preterm infants. Cochrane Database Syst Rev 12:cd012519. https://doi.org/10.1002/14651858.cd012519.pub2

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