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SYSTEMATIC REVIEW

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The effectiveness of treatment with probiotics in preventing necrotizing enterocolitis and related mortality: results from an umbrella meta-analysis on meta-analyses of randomized controlled trials

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Abstract

Introduction Probiotic supplementation has been proposed as a preventive measure for necrotizing enterocolitis (NEC) in preterm infants. This umbrella meta-analysis assesses the effects of probiotics, including single-strain and multi-strain formulations, on NEC and related mortality.

Methods A comprehensive search was conducted in PubMed, Scopus, ISI Web of Science, and Embase for studies up to August 2024. The AMSTAR2 tool assessed the quality of included studies. Meta-analysis studies were selected based on the PICOS framework, focusing on preterm neonates (< 37-week gestation), probiotic supplementation (single-strain or multi-strain), placebo or standard care comparison, and outcomes of NEC and mortality. Pooled relative risks (RR) and odds ratios (OR) with 95% confidence intervals (CI) were calculated using random-effects models.

Results Overall, 35 eligible studies were included into the study. Twenty-six and 32 probiotic intervention arms used single- and multi-strain probiotics, respectively. The findings revealed that probiotics decreased NEC significantly (ES_{RR}: 0.51; 95% CI: 0.46, 0.55, $p < 0.001$, and ES_{OR}: 0.59; 95%CI: 0.48, 0.72, $P < 0.001$), and mortality rate (ES_{RR}: 0.72; 95% CI: 0.68, 0.76, $P < 0.001$, and ES_{OR}: 0.77; 95%CI: 0.70, 0.84, $p < 0.001$).

Conclusion The present review suggests that supplementation with probiotics reduced NEC and related mortality. Probiotic supplementation can be recognized as a NEC-preventing approach in preterm and very preterm infants, particularly Multi-strain probiotics.

Keywords Probiotics, Necrotizing enterocolitis, Umbrella meta-analysis, Mortality

Introduction

Necrotizing enterocolitis (NEC) is a multifactorial disease and a leading cause of mortality and morbidity in premature infants. The incidence of NEC varies significantly across neonatal units, ranging from 2 to 13% in preterm and very low birth weight infants [1]. The incidence of NEC in very low birth weight infants (birth weight < 1500 g) is approximately 5–10% [2]. Several clinical risk factors for NEC have been identified, including immaturity, premature rupture of membranes, assisted ventilation, sepsis, and hypotension [3]. Additional contributors include formula feeding, the use of acid suppression, and prolonged antibiotic therapy [4]. The clinical presentation of NEC is often

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nonspecific, with symptoms ranging from apnea, abdominal distension, and bloody stools to severe complications such as intestinal perforation, peritonitis, sepsis, shock, and death. Standard treatment strategies encompass bowel rest, parenteral nutrition, broad-spectrum antibiotics, ventilatory support, blood pressure stabilization, and, in severe cases, peritoneal drainage or surgical resection of necrotic bowel tissue [5]. However, these treatments have significant limitations, including impaired bowel function, prolonged reliance on parenteral feeding with central catheter insertion, extended hospitalization, and substantial healthcare costs. As a result, there is a critical need for more effective treatments with fewer side effects. Probiotic supplementation has been proposed as a preventive strategy against NEC [6]. Although some meta-analysis studies have suggested that probiotic supplementation reduces the incidence of NEC and related mortality [7–9], these findings remain inconsistent [10, 11].

Probiotics are increasingly employed in neonatal intensive care units as a strategy to manage NEC. Commonly used probiotics include species from the *Bifidobacterium*, *Lactobacillus*, and *Saccharomyces* genera [12]. These microorganisms are stable and can survive the harsh gastrointestinal environment due to their resistance to low pH, acidic conditions, and proteolytic enzymes [13]. The human gastrointestinal tract harbors over 10,000 microbial species, each influencing the health and disease status of host. Research has demonstrated that probiotics can effectively prevent or manage a range of conditions, including intestinal inflammation and infections [14, 15], obesity, diabetes, liver disease [16], respiratory diseases [15], arthritis, pouchitis, ulcerative colitis, and Crohn disease [17]. Probiotics are also known to enhance both innate and adaptive immunity and are often used prophylactically to mitigate the adverse effects of cancer therapies [16]. Nevertheless, the exact mechanisms underlying the therapeutic benefits of probiotics remain incompletely understood [17].

Several mechanisms have been proposed to explain the effects of probiotics in the gastrointestinal tract. First, they restore endogenous microbiota by colonizing the gut, competing for adhesion sites and nutrients, and secreting beneficial metabolites [18]. Second, probiotics interact with the intestinal epithelial cells, promoting mucin production, modulating pro-inflammatory markers, and reinforcing tight junctions to prevent apoptosis [19]. Lastly, they modulate the innate immune system. Recent studies have highlighted the role of microbial overgrowth and a lack of microbial diversity in the gastrointestinal tracts of preterm infants as key contributors to NEC [20]. These findings suggest that disturbances in the microbiome, rather than a single pathogen, may drive NEC development. The lower prevalence of protective *Lactobacillus* and *Bifidobacterium* species in preterm infants compared to full-term infants underscores the potential of probiotics as a preventive intervention for NEC [21, 22].

Several meta-analysis studies have pointed to the effectiveness of probiotics in preventing the occurrence of NEC [7–9]. However, the results are not entirely conclusive [10, 11]. To address this uncertainty, we aim to conduct an umbrella meta-analysis to comprehensively evaluate the efficacy of probiotics in preventing and managing NEC.

Methods

This meta-analysis and systematic review followed the JBI Manual for Evidence Synthesis guidelines for comprehensive reporting and analysis [23]. The protocol of the current review was submitted to PROSPERO.

Search strategy

To identify the most relevant literature, a comprehensive search was conducted in PubMed, Scopus, ISI Web of Science, and Embase. The search was conducted up to August 15, 2024 using a detailed set of keywords and Boolean operators. The full search strategy, including specific Boolean terms and database query strings, is provided in the Supplementary Table 1 for transparency and reproducibility. No restrictions were imposed on the publication date or language. Additionally, the reference lists of relevant papers were thoroughly reviewed to identify additional publications.

Eligibility criteria

Relevant studies were selected based on the PICOS (population/intervention/comparison/outcome/study design) framework: population (preterm neonates < 37-week gestation), intervention (probiotic supplementation), comparison (placebo or standard care), outcome (standardized relative risk (RR) or odds ratio (OR) of necrotizing enterocolitis or NEC-related mortality), and study design (meta-analysis studies of randomized controlled trials). Animal studies, in-vitro studies, and non-meta-analytic designs were excluded.

Data extraction

Titles and abstracts were screened independently by JH, PZ, and CF, and the full texts of potentially eligible articles were reviewed based on the inclusion criteria. Data extraction was performed systematically and independently by two reviewers (JH and YR) using a predesigned extraction form to ensure consistency and accuracy. Information was extracted from each included meta-analysis to capture comprehensive details relevant to the study objectives. Extracted data included the citation (author and year of publication), number of studies included in the meta-analysis, geographical location of the studies, number of participants, age of the participants (e.g., gestational age or birth weight), the type of intervention (e.g., single-strain or multi-strain probiotics, specific probiotic strains), and the quality assessment methods used (e.g., Cochrane Risk of Bias, Jadad score, or NOS). Additionally, outcomes extracted included the relative risk (RR) or odds ratio (OR) for necrotizing enterocolitis (NEC) incidence and mortality.

Quality assessment

The methodological quality of the qualified meta-analysis was assessed independently by two reviewers using the assessment of multiple systematic reviews (AMSTAR2) questionnaire, which evaluates critical domains such as the comprehensiveness of literature search, adequacy of included study evaluation, and appropriateness of data synthesis [24]. According to AMSTAR2, there are four categories of quality, "high quality", "moderate quality", "low quality" and "critically low quality". While specific perspectives such as measurement, attrition, and reporting bias are not directly applicable at this level, AMSTAR2 indirectly addresses these concerns by requiring systematic reviews to evaluate these elements in their included studies. We also extracted and reported the results of the quality assessment of the clinical trials included in each meta-analysis, as well as the methods used for quality evaluation.

Statistical analysis

The ESs and their CIs for NEC in the probiotics and control groups/ periods were utilized to calculate the overall ESs. A random-effects model was applied to generate overall effect sizes, accounting for variations between studies. Heterogeneity was assessed using the I^2 statistic and Cochrane's Q test. We considered the I^2 value > 50% or $P < 0.05$ for the Q-test as significant heterogeneity between studies. To explore the potential sources of heterogeneity, subgroup analyses were conducted based on the predefined variables such as age, weight, and type of strain probiotics. Sensitivity analysis was performed to assess the influence of a specific study on the overall ES was done using the leave-one-out method [25]. The potential for publication bias was assessed using funnel plots and statistical asymmetry tests (Begg's adjusted rank correlation test and Egger's test). All analyses were carried out using STATA software (version 16 software). We considered P-value < 0.05 as a significant level.

Results

Study selection

A total of 278 articles were detected through electronic search of databases. After removing 27 duplicates, 251 remained studies were screened based on title and abstract. After removing irrelevant studies, 51 studies were selected to be considered by full-text evaluation and 16 studies excluded. Finally, 35 studies met our specified inclusion criteria that 33 studies were in relation to NEC and 28 studies have reported the mortality. The study search process is illustrated as PRISMA flow diagram in Fig. 1.

Study characteristics

In the umbrella meta-analysis of interventional studies, there were 36 meta-analysis studies that 30 studies have reported RR for NEC (52 effect sizes), 3 studies OR for NEC (6 effect sizes), 25 studies RR for mortality (45 effect sizes) and 4 OR for mortality (10 effect sizes). The included studies were conducted between 2008 up to 2024. Twelve studies were conducted in China [10, 11, 26–34], 6 in Australia [35–40], 4 in UK [41–43], 3 in Saudi Arabia [7, 44], 2 in Germany [45], 2 in the USA [9, 46], one in India [47], Taiwan [48], Canada [49], Italy [8], Denmark [50], Poland [51]. The mean age of participants was < 37 weeks and most of the included studies have evaluated the effect of probiotic mixture [7–11, 26, 30, 33, 38, 40, 41, 43–48] in relation to NEC or mortality. In addition, *Bifidobacterium* [8, 11, 26, 29, 30, 36, 42, 48, 51, 52], *Lactobacillus* [8, 11, 26, 35, 42, 48], *Sacchromyces* [11, 26, 42, 48], *Bacillus* [26, 42] and *Enterococcus* [32] were used in the included studies. The Cochrane Risk of Bias Tool [7, 26, 28, 29, 31, 39, 42, 44, 45, 49, 52] and Jadad scores [10, 11, 30, 32, 34, 43, 48] and Grading of Recommendations Assessment, Development and Evaluation (GRADE) [8, 27, 38, 47] were used for the quality assessment of included RCTs in the current meta-analysis. Detailed characteristics of the included studies are outlined in Table 1.

Risk of bias assessment

Overall, all of the included studies in the current umbrella review were evaluated as high quality. The results of the quality assessment of included studies using AMSTAR2 questionnaire are summarized in Supplementary Table 2.

The effect of probiotics supplementation on NEC

Thirty studies with 52 effect sizes enrolling 312,438 patients evaluated the effect of probiotics using RR for the NEC value. Combining the results of included studies due to the random-effects model illustrated that probiotics decreased RR of NEC significantly (ES_{RR}: 0.51; 95% CI: 0.46, 0.55, $p < 0.001$). Also, a significant

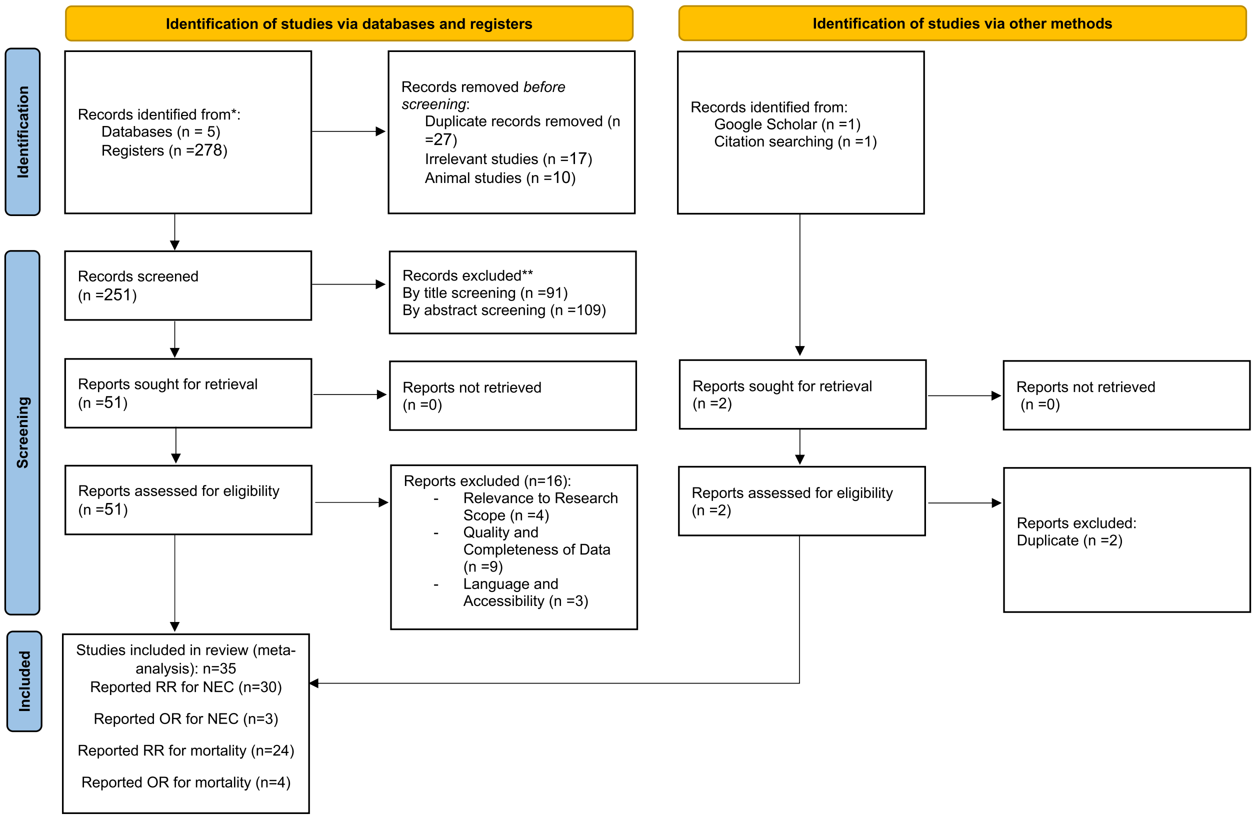

Fig. 1 PRISMA flow chart indicating the study selection process

heterogeneity was detected among studies ($I^2 = 66.2\%$, $p < 0.001$) (Fig. 2A). According to subgroup analysis, mean age, mean weight and type of strain probiotics were identified as potential source of heterogeneity. Likewise, subgroup analysis revealed that preterm infants age (32–37 weeks), weighted lower than 2500 g and which were treated with *Bifidobacterium* and Multi-strain probiotics were possible source of heterogeneity (Table 2). Moreover, we found no significant effect of any individual study on the overall effect size of study outcomes using sensitivity analysis. Also, three studies with six effects sizes reported the effect of probiotics supplementation based on OR of NEC. Moreover, random-effects analysis showed a significant reduction in OR of NEC following probiotics supplementation (ES_{OR}: 0.59; 95%CI: 0.48, 0.72, $P < 0.001$) with almost low between study heterogeneity ($I^2 = 57.5\%$, $p = 0.038$) (Fig. 2B). Egger's ($p = 0.002$) and Begg's ($p = 0.032$) tests indicated a small study effect. In addition, visual inspection of the funnel plot showed an asymmetry distribution. Therefore, the trim-and-fill method was carried out with eight imputed studies (RR: 0.45; 95% CI: 0.42, 0.49, $p < 0.05$), and the result is still significant (Supplementary Figure 1).

The effect of probiotics supplementation on mortality

Twenty-five eligible studies with 44 effect sizes enrolling 229,699 patients evaluated the effect of probiotics using RR for the mortality rate. In the pooled-effect analysis, probiotics supplementation significantly reduced mortality rate (ES_{RR}: 0.72; 95% CI: 0.68, 0.76, $P < 0.001$). The amount of heterogeneity was low between the studies ($I^2 = 30.2\%$; $P = 0.033$) (Fig. 3A). However, type of probiotic's strain may be introduced as the possible source of heterogeneity based on subgroup analysis. Specifically, *Lactobacillus* strain could affect the mortality rate (Table 2). Also, four studies with ten effects sizes reported the effect of probiotics supplementation based on OR of mortality. Combined results from the random-effects model presented a significant reduction in mortality rate after probiotics supplementation (ES_{OR}: 0.77; 95%CI: 0.70, 0.84, $p < 0.001$) (Fig. 3B). Moreover, sensitivity analysis demonstrated that no study is likely to affect the pooled ES of mortality rate. No significant small-study effect was found using Egger's and Begg's tests ($p = 0.144$ and $p = 0.440$, respectively). Additionally, visual inspection of the funnel plot revealed publication bias. Accordingly, the trim-and-fill method was carried out with two

Table 1 Characteristics of the included meta-analyses studies

Citation (First author et al., year)	No. of Studies in Meta-analysis/ Location	No. of Participants in Meta-analysis	Age (year)	Intervention	Health conditions	Weight	Quality Assessment Scale and Outcome	
Batta et al. (a) 2023 [36]	15 Australia	4962	< 34 week	*Bifidobacterium longum (B. infantis)*	LBW Infants	< 2500	Yes (ROB)	RR for NEC
	14 Australia	4288						RR for mortality
Batta et al. (b) 2023 [36]	48 Australia	9080	< 34 week	Probiotic Mix without *Bifidobacterium longum* (*B. infantis*)	LBW Infants	< 2500	Yes (ROB)	RR for NEC
	40 Australia	6424						RR for mortality
Guo et al. (2023) [29]	6 China	3152	< 37 weeks	*Bifidobacterium longum* or *Bifidobacterium subsp. longum* or Bifidobacterium subsp. *infantis*	Preterm infants + young children aged 0–3 years	NR	Yes (Cochrane ROB 2.0) 2/6 high	RR for NEC
Men et al. 2023 [32]	6 China	1237	< 37 weeks	*Enterococcus faecium*	Preterm infants	NR	Yes (Jadad score) 2/6 high	RR for NEC
Wang et al. (a) 2023 [33]	28 China	17,602	< 35 weeks	Single-strain	Preterm and term neonates	NR	Yes (NOS) 70/73	RR for NEC
								RR for mortality
Wang et al. (b) 2023 [33]	21 China	17,602	< 35 weeks	Two-strain	Preterm and term neonates	NR	Yes (NOS) 70/73	RR for NEC
								RR for mortality
Wang et al. (c) 2023 [33]	20 China	17,602	< 35 weeks	Multiple-strain	Preterm and term neonates	NR	Yes (NOS) 70/73	RR for NEC
								RR for mortality
Wang et al. 2023 [33]	73 China	17,602	< 35 weeks	Probiotic Mix	Preterm and term neonates	NR	Yes (NOS) 70/73	RR for NEC
								RR for mortality
Ang et al. 2023 [53]	8 Australia	3900	< 37 weeks	*Lactobacillus reuteri*	LBW & preterm infants	< 2500	Yes (NOS) 8/8 high	RR for NEC
								RR for mortality
Liu et al. 2022 [31]	7 China	947	< 34 week	Probiotic Mix	VLBW neonates	< 1500	Yes (Cochrane)	RR for NEC
	10 China	1723						RR for mortality
Deshmukh and Patole 2021 [37]	30 WesternAustralia	77,018	< 37 weeks	Probiotic Mix	ELBW neonates	< 1000	Yes (NOS)	RR for NEC
	30 WesternAustralia	77,018						OR for NEC
	27 WesternAustralia	70,977						OR for mortality
Balasubramanian et al. 2020 [47]	9 India	1514	< 37 week	Probiotic Mix	Preterm infants	NR	Yes (GRADE)	RR for NEC
	8 India	1314						RR for mortality
Sharifi-Rad et al. (a) 2020 [54]	14 UK	2988	< 32 week	*Bifidobacterium spp*	Very preterm or VLBW infants	< 1500	Yes (Cochrane) 29/56 high	RR for NEC
	12 UK	2761						RR for mortality
	12 UK	2761						OR for mortality
Sharif et al. (b) 2020 [42]	12 UK	2000	< 32 week	*Lactobacillus spp*	Very preterm or VLBW infants	< 1500	Yes (Cochrane) 29/56 high	RR for NEC
	12 UK	2000						RR for mortality
	12 UK	2000						OR for mortality
Sharif et al. (c) 2020 [42]	4 UK	621	< 32 week	*Sacchromyces spp*	Very preterm or VLBW infants	< 1500	Yes (Cochrane) 29/56 high	RR for NEC
	3 UK	534						RR for mortality
	3 UK	534						OR for mortality

Table 1 (continued)

Citation (First author et al., year)	No. of Studies in Meta-analysis/ Location	No. of Participants in Meta-analysis	Age (year)	Intervention	Health conditions	Weight	Quality Assessment Scale and Outcome	
Sharif et al. (d) 2020 [42]	2 UK	465	< 32 week	*Bacillus spp*	Very preterm or VLBW infants	< 1500	Yes (Cochrane) 29/56 high	RR for NEC
	2 UK	465						RR for mortality
	2 UK	465						OR for mortality
Gao et al. 2020 [28]	8 China	1100	< 37 weeks	*Saccharomyces boulardii*	ELBW	NR	Yes (Cochrane)	RR for NEC
	2 China	1100						RR for mortality
Liu et al. 2020 [55]	NR China	4686	< 37 weeks	*Lactobacillus spp*	Preterm infants	< 1500	NR	RR for NEC
								RR for mortality
Bi et al. (a) 2019 [26]	14 China	3561	NR	Probiotic Mix	Preterm infants	< 2500	Yes (cochrane) 21/34 high	RR for NEC
		3459						RR for mortality
Bi et al. (b) 2019 [26]	5 China	2210	NR	*Lactobacillus spp*	Preterm infants	< 2500	Yes (cochrane) 21/34 high	RR for NEC
		2036						RR for mortality
Bi et al. (c) 2019 [26]	6 China	2513	NR	*Bifidobacterium spp*	Preterm infants	< 2500	Yes (cochrane) 21/34 high	RR for NEC
		2328						RR for mortality
Bi et al. (d) 2019 [26]	1 China	244	NR	*Bacillus spp*	Preterm infants	< 2500	Yes (cochrane) 21/34 high	RR for NEC
		244						RR for mortality
Bi et al. (e) 2019 [26]	3 China	747	NR	*Sacchromyces spp*	Preterm infants	< 2500	Yes (cochrane) 21/34 high	RR for NEC
		660						RR for mortality
Jiao et al. (a) 2020 [30]	7 China	2429	< 34 week	*Bifidobacterium spp*	VLBW preterm infants	< 1500	Yes (Jadad score) 16/16 high	RR for NEC
Jiao et al. (b) 2020 [30]	3 China	1371	< 34 week	NR	VLBW preterm infants	< 1500	Yes (Jadad score) 16/16 high	RR for NEC
Jiao et al. (c) 2020 [30]	6 China	832	< 34 week	Probiotic Mix	VLBW preterm infants	< 1500	Yes (Jadad score) 16/16 high	RR for NEC
Jiang et al. (a) 2020 [11]	11 China	3083	< 37 weeks	Probiotic Mix	Premature infants	< 2500	Yes (Jadad score)	RR for NEC
	12 China	3175						RR for mortality
Jiang et al. (b) 2020 [11]	8 China	3222	< 37 weeks	*Lactobacillus spp*	Premature infants	< 2500	Yes (Jadad score)	RR for NEC
	7 China	2647						RR for mortality
Jiang et al. (c) 2020 [11]	6 China	2117	< 37 weeks	*Bifidobacterium spp*	Premature infants	< 2500	Yes (Jadad score)	RR for NEC
	6 China	2414						RR for mortality
Jiang et al. (d) 2020 [11]	2 China	479	< 37 weeks	*Sacchromyces spp*	Premature infants	< 2500	Yes (Jadad score)	RR for NEC
	2 China	479						RR for mortality
Zhu et al. 2019 [52]	24 China	6155	< 37 week	*Bifidobacterium*	Preterm infants	NR	Yes (cochrane) 12/24 high	RR for NEC
								RR for mortality

Table 1 (continued)

Citation (First author et al., year)	No. of Studies in Meta-analysis/ Location	No. of Participants in Meta-analysis	Age (year)	Intervention	Health conditions	Weight	Quality Assessment Scale and Outcome	
Thomas et al. 2017 [43]	23 UK	7325	NR	Probiotic Mix	VLBW infant, ELBW infants	< 1500	Yes (Jadad score) 23/23 high	RR for NEC
Dermyshi et al. 2017 [27]	44 (30 RCT and 14 OBZ) China	22,401	< 34 week	NR	Preterm Infants	< 1500	*Yes (GRADE, NOS)*	RR for NEC
	27 RCT China	8156						RR for ortality
Sun et al. 2017 [40]	25 Australia	8492	< 32 week	Probiotic Mix	Preterm infants	< 1500	Yes (Physiotherapy Evidence Database tool)	RR for NEC
	21 Australia	7332						RR for mortality
Rees et al. 2017 [41]	19 UK	3975	< 34 week	Probiotic Mix	Preterm infants	< 1500	NO	RR for NEC
								RR for mortality
Thomas et al. (a) 2017 [43]	23 UK	7325	NR	Probiotic Mix	VLBW infant	< 1500	Yes (Jadad score) 4/5 high	RR for NEC
	22 UK	6954						RR for all cause mortality
Thomas et al. (b) 2017 [43]	5 UK	1596	NR	Probiotic Mix	ELBW infants	< 1000	Yes (Jadad score) 4/5 high	RR for NEC
	4 UK	1121						RR forall cause mortality
Thomas et al. (c) 2017 [43]	10 UK	4642	NR	Probiotic Mix	VLBW infant	< 1500	Yes (Jadad score) 4/5 high	RR for NEC related mortality
Chang et al. (a) 2017 [48]	12 Taiwan	2889	< 34 week	Probiotic Mix	Preterm very low birth weight	< 1500	Yes (Jadad score) 25/25 high	OR for NEC
	10 Taiwan	2867						OR for mortality
Chang et al. (b) 2017 [48]	5 Taiwan	1646	< 34 week	*Lactobacillus spp*	Preterm very low birth weight	< 1500	Yes (Jadad score) 25/25 high	OR for NEC
	3 Taiwan	701						OR for mortality
Chang et al. (c) 2017 [48]	6 Taiwan	2244	< 34 week	*Bifidobacterium spp*	Preterm very low birth weight	< 1500	Yes (Jadad score) 25/25 high	OR for NEC
	6 Taiwan	2244						OR for mortality
Chang et al. (d) 2017 [48]	3 Taiwan	566	< 34 week	*Sacchromyces spp*	Preterm very low birth weight	< 1500	Yes (Jadad score) 25/25 high	OR for NEC
	2 Taiwan	479						OR for mortality
Deshpande et al. 2017 [38]	23 Australia	4783	< 37 week	Probiotic Mix	Preterm neonates in low-income and medium-income countries	< 2500	Yes (GRADE)	RR for mortality
Sawh et al. (a) 2016 [49]	37 Canada	10,520	< 37 weeks	Probiotic Mix	Preterm infants	< 2500	Yes (Cochrane)	RR for NEC
	29 Canada	9507						RR for mortality
Sawh et al. (b) 2016 [49]	25 Canada	6587	< 37 weeks	Probiotic Mix	VLBW Infants	< 2500	Yes (Cochrane)	RR for NEC
Sawh et al. (c) 2016 [49]	6 Canada	1618	< 37 weeks	Probiotic Mix	ELBW Infants	< 2500	Yes (Cochrane)	RR for NEC
Billimoria et al. 2016 [46]	30 USA	8000	< 37 week	Probiotic Mix	Preterm infants	< 2500	NO	OR for NEC
	24 USA	7739						OR for mortality

Table 1 (continued)

Citation (First author et al., year)	No. of Studies in Meta-analysis/ Location	No. of Participants in Meta-analysis	Age (year)	Intervention	Health conditions	Weight	Quality Assessment Scale and Outcome	
Aceti et al. (a) 2015 [8]	9 Italy	1815	< 37 week	*Lactobacillus spp*	Preterm infants	NR	Yes (GRADE) 7/26 high	RR for NEC
Aceti et al. (b) 2015 [8]	13 Italy	1024	< 37 week	*Bifidobacterium spp*	Preterm infants	NR	Yes (GRADE) 7/26 high	RR for NEC
Aceti et al. (c) 2015 [8]	11 Iataly	2979	< 37 week	Probiotic Mix	Preterm infants	NR	Yes (GRADE) 7/26 high	RR for NEC
Lau et al. 2015 [9]	20 New Jersy	5982	< 37 week	Probiotic Mix	VLBW Infants	< 1500	NO	RR for NEC
	16 New Jersy	4450						RR for mortality
Olsen et al. 2016 [50]	12 Denmark	10,800	< 37 weeks	Probiotic Mix	Preterm infants	< 2500	Yes (NOS)	RR for NEC
	9 Denmark	8139						RR for mortality
AlFaleh and Anabrees 2014 [7]	24 Saudi Arabia	5529	< 37 week	Probiotic Mix	Preterm infants	< 2500	Yes (The Cochrane Collaboration and theNeonatal Review) 11/24	RR for NEC
	17 Saudi Arabia	2755						RR for mortality
Yang et al. (a) 2014 [34]	27 China	6655	< 37 weeks	NR	Preterm infants	NR	Yes (Jadad score)	RR for NEC
	14 China	3583						RR for mortality
Wang et al. 2012 [10]	20 China	3816	< 34 week	Probiotic Mix	Preterm very low-birth-weight	< 1500	Yes (Jadad score) 20/20 high	RR for NEC
	13 China	3090						RR for mortality
AlFaleh and Bassler 2010 [56]	9 Saudi Arabia	1425	< 37 week	Probiotic Mix	Preterm infants	< 2500	Yes (The Cochrane Collaboration and theNeonatal Review) 2/9	RR for NEC
Guthmann et al. 2010 [45]	8 Germany	1918	< 37 week	Probiotic Mix	Preterm infants	NR	Yes (Cochrane)	RR for NEC
	8 Germany	1918						RR for mortality
Szajewska et al. 2010 [51]	3 Poland	293	< 37 week	*Bifidobacterium animalis ssp. lactis*	Preterm infants	< 2500	Yes	RR for NEC
								RR for mortality
AlFaleh et al. (a) 2010 [44]	NR Germany	2331	< 37 week	Probiotic Mix	Preterm infants	< 2500	NR	RR for mortality
AlFaleh et al. (a) 2010 [44]	NR Germany	1476	< 37 week	Probiotic Mix	Preterm infants	< 2500	NR	RR for mortality
AlFaleh and Bassler 2010 [56]	9 Saudi Arabia	1425	< 37 week	Probiotic Mix	Preterm infants	< 2500	Yes (The Cochrane Collaboration and theNeonatal Review) 2/9	RR for NEC

Abbrevations: LBW Low birth weight, *VLBW* Very low birth weight, *ELBW* Extremely low birth weight, *NR* Not reported, *NEC* necrotizing enterocolitis, *RR* Relative risk, *OR* Odds Ratio

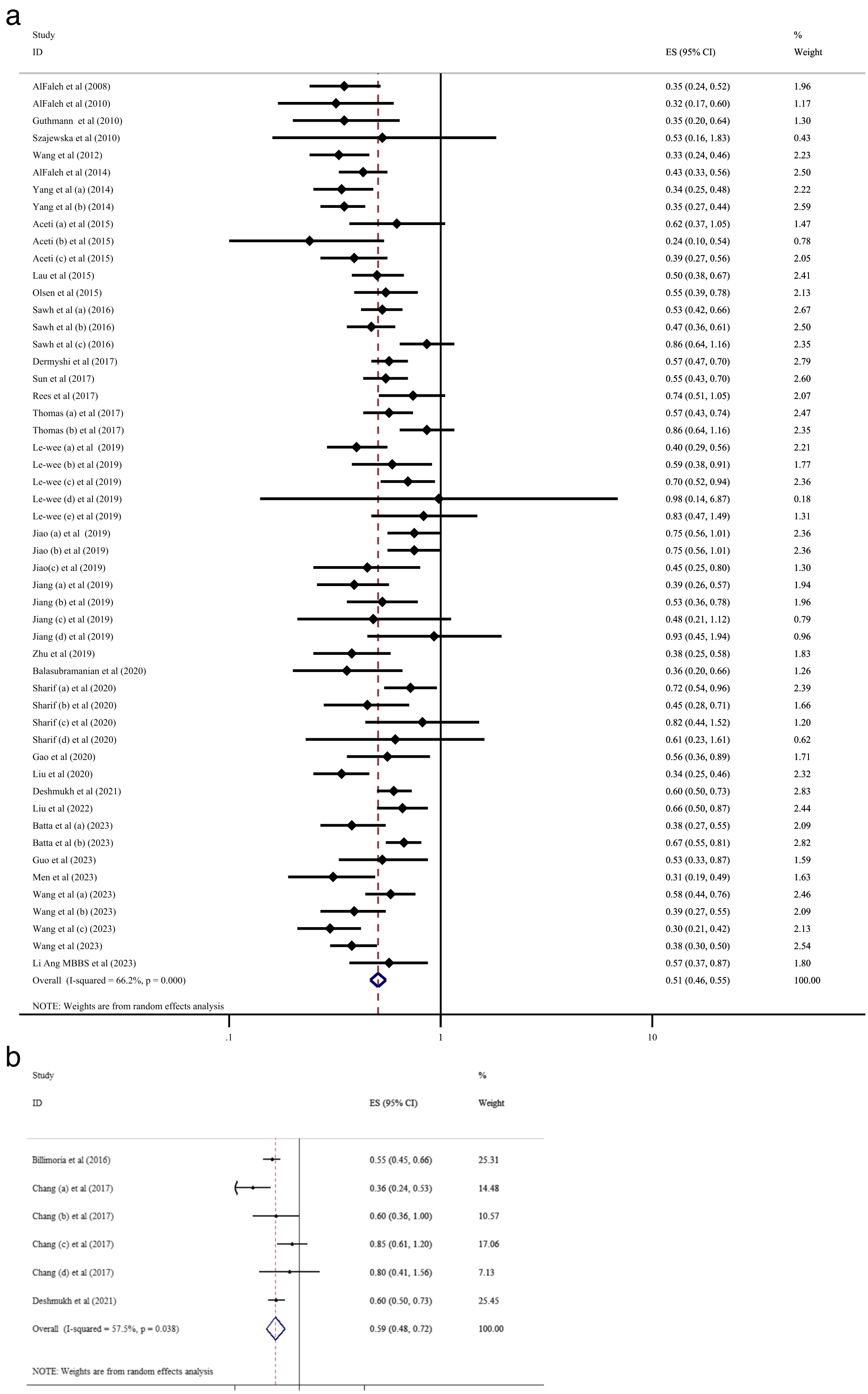

Fig. 2 **A** The Forest plot of the efficacy of probiotics supplementation in prevention of NEC according to relative risk (RR) analysis. **B** The Forest plot of the efficacy of probiotics supplementation in prevention of NEC according to odds ratio (OR) analysis

Table 2 Subgroup analyses for the effects of probiotics on necrotizing enterocolitis and mortality

	Effect size, *n*	RR (95% CI)	*P*-value	I^2 (%)	P-heterogeneity
Probiotics on NEC					
Overall	52	0.51 (0.46, 0.55)	< 0.001	66.2	< 0.001
Age (week)					
Very preterm or extremely preterm (< 32)	16	0.61 (0.53, 0.69)	< 0.001	61.5	< 0.001
Preterm (32–37)	30	0.45 (0.41, 0.50)	< 0.001	59.1	< 0.001
NR	6	0.54 (0.40, 0.72)	< 0.001	67.0	0.010
Weight (gram)					
< 1500	17	0.58 (0.51, 0.66)	< 0.001	64.5	< 0.001
1500–2500	20	0.53 (0.46, 0.60)	< 0.001	55.9	0.001
NR	15	0.40 (0.35, 0.45)	< 0.001	33.5	0.100
Type of strain probiotics					
Lactobacillus	6	0.49 (0.40, 0.60)	< 0.001	34.5	0.178
Bifidobacterium	9	0.53 (0.42, 0.68)	< 0.001	61.0	0.009
Saccharomyces	4	0.72 (0.54, 0.96)	0.024	0.0	0.560
Bacillus	2	0.67 (0.28, 1.60)	0.368	0.0	0.669
Enterococcus	1	0.31 (0.19, 0.50)	< 0.001	-	-
Multi-strain probiotics	25	0.49 (0.44, 0.55)	< 0.001	71.0	< 0.001
NR	5	0.50 (0.37, 0.66)	< 0.001	83.1	< 0.001
Probiotics on NEC related mortality					
Overall	44	0.72 (0.68, 0.76)	< 0.001	30.2	0.033
Age (week)					
Very preterm or extremely preterm (< 32)	10	0.80 (0.73, 0.87)	< 0.001	0.0	0.873
Preterm (32–37)	9	0.69 (0.64, 0.74)	< 0.001	36.1	0.029
NR	5	0.77 (0.60, 0.98)	0.037	43.5	0.120
Weight(gram)					
< 1500	14	0.76 (0.71, 0.82)	< 0.001	13.7	0.303
1500–2500	20	0.70 (0.64, 0.77)	< 0.001	34.9	0.063
NR	10	0.66 (0.58, 0.75)	< 0.001	18.4	0.269
Type of strain probiotics					
Lactobacillus	5	0.70 (0.55, 0.88)	0.003	52.6	0.077
Bifidobacterium	6	0.77 (0.67, 0.88)	< 0.001	0.0	0.667
Saccharomyces	4	1.11 (0.72, 1.72)	0.636	0.0	0.943
Bacillus	2	0.86 (0.53, 1.40)	0.534	0.0	0.944
Multi-strain probiotics	24	0.70 (0.65, 0.75)	< 0.001	40.3	0.020
NR	3	0.71 (0.58, 0.86)	< 0.001	49.0	0.141

Abbrevations: *NR* Not reported, *NEC* necrotizing enterocolitis, *RR* Relative risk

imputed studies (RR: 0.66; 95% CI: 0.61, 0.72, $P < 0.05$), and finding did not alter (Supplementary Figure 2).

Discussion

This umbrella review of meta-analysis studies indicates that probiotic supplementation can significantly reduce the incidence of NEC and related mortality in preterm or very preterm infants. Subgroup analyses suggest that multi-strain probiotics are particularly effective in reducing the incidence of NEC and related mortality, especially in preterm infants aged 32–37 weeks and those with a birth weight between 1500 and 2500 g. It is important to note that the conclusions drawn for these subgroups may not be fully generalizable or robust due to the sample size limitations. More detailed, stratified analyses, particularly for very low birth weight or extremely preterm infants, would be valuable for understanding the impact of probiotics on these specific subgroups. We recommend that future studies focus on including these more granular data to enhance the precision and applicability of probiotic recommendations for these vulnerable populations. Potential sources of heterogeneity in the pooled

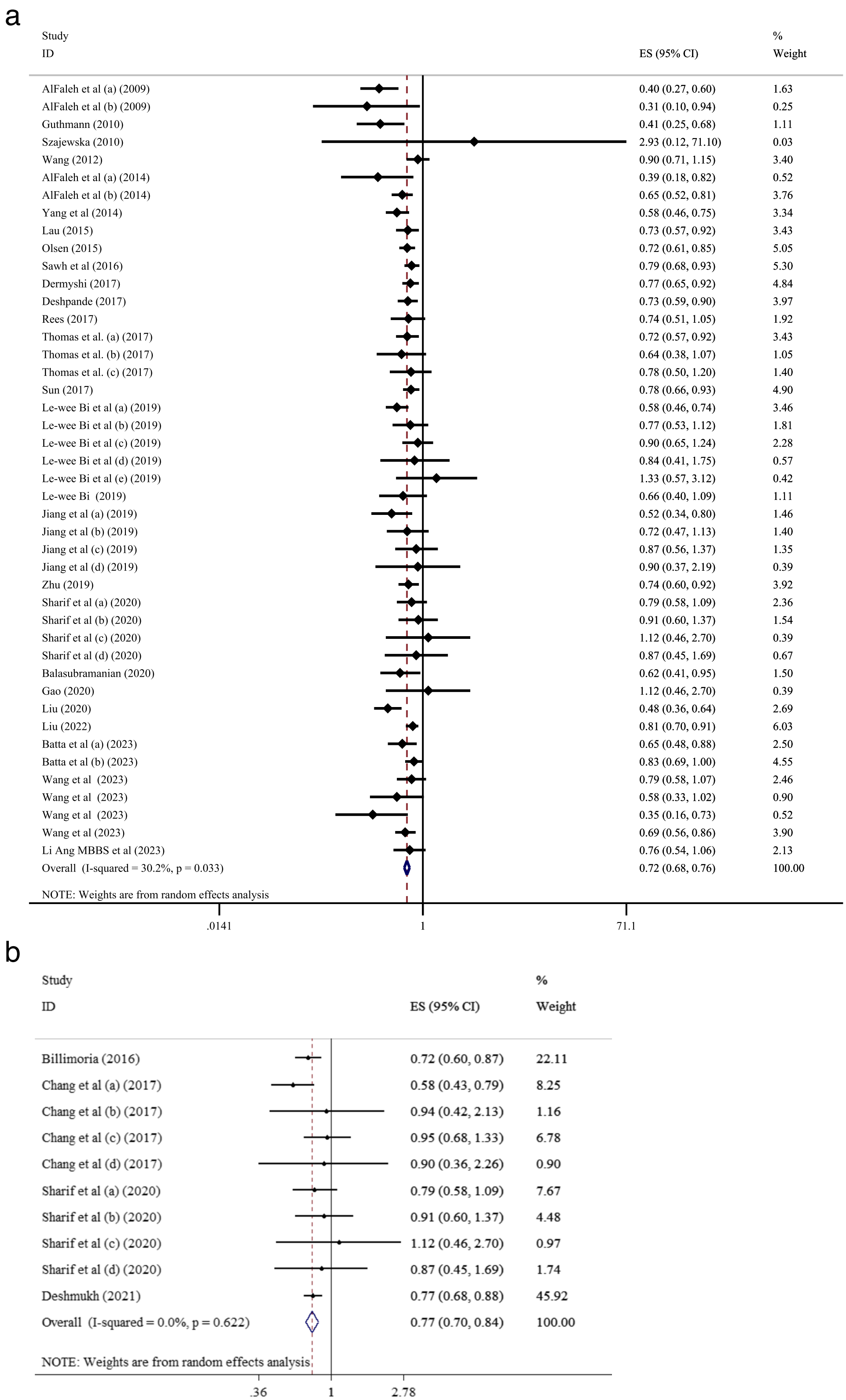

Fig. 3 **A** The Forest plot of the efficacy of probiotics supplementation on mortality related to NEC according to relative risk (RR) analysis. **B** The Forest plot of the efficacy of probiotics supplementation on mortality related to NEC according to odds ratio (OR) analysis

analyses include gestational age, birth weight, and probiotic strains. However, due to limited data on the dose and duration of probiotic supplementation, subgroup analyses based on these factors could not be conducted. Dosing and duration of probiotic supplementation were inconsistent, potentially leading to heterogeneity. Moreover, neonatal care practices, including feeding strategies (breast milk vs. formula), antibiotic use, and NICU protocols, vary by region, further contributing to variability in probiotic efficacy. To reduce heterogeneity and enhance the clinical applicability of probiotic supplementation, future trials should aim to establish standardized probiotic protocols. Specifically, consensus on the optimal probiotic strains, combinations, and dosages is needed to maximize efficacy while minimizing variability. Additionally, harmonization of NICU protocols, including feeding strategies and antibiotic stewardship, may help standardize probiotic interventions. Individualized probiotic therapy based on gestational age, birth weight, and gut microbiota composition should also be explored to optimize clinical outcomes. The high quality of the systematic reviews included in this study, as assessed by the AMSTAR checklist, supports the validity and safety of probiotic use for preterm infants globally. While we employed funnel plots and statistical tests to assess publication bias, it is important to acknowledge that this bias could still affect the balance and generalizability of our findings. However, the results of trim and fill analysis suggested that the significance of our findings is robust, even when adjusting for potential publication bias.

The greater effectiveness of multi-strain probiotics is likely due to the more diverse bacterial composition they provide. Network meta-analysis indicated that single probiotics have limited efficacy in preterm infants, while combinations, especially those containing *Lactobacillus* or *Bifidobacterium*, offer optimal benefits [57]. Among single probiotics, *Lactobacillus* and *Bifidobacterium* had the more promising impacts on NEC, possibly because they are the most common species in the gut microbiota [58]. Conditioned media from *Bifidobacterium infantis* and *Lactobacillus acidophilus* have been shown to differentially modulate inflammation in immature human intestinal epithelial cells. The combined use of these probiotics resulted in a more pronounced anti-inflammatory effect, suggesting strain-specific interactions that enhance their protective properties against NEC [59]. In the subgroup analysis based on birth weight, most studies involving infants weighing less than 1500 g used single-strain probiotics, while those with a birth weight of 1500–2500 g used multi-strain probiotics. This may explain the greater benefit observed in the latter group. Regarding gestational age, very preterm infants (born at less than 32 weeks) experience significant gastrointestinal immaturity, including increased intestinal permeability, which is often referred to as a “leaky gut” [60]. This underdevelopment facilitates the translocation of pathogenic bacteria, raising the risk of NEC [61]. Therefore, more improving effect in preterm infants aged 32–37 weeks may be attributed to the more mature intestinal barrier in these infants [61].

Several potential mechanisms explain the beneficial effects of probiotics on NEC. Preterm infants often have a less diverse gut microbiota, dominated by pathogenic bacteria such as *Enterobacteriaceae* and *Clostridium* species [62], which can trigger immune responses and inflammation, leading to NEC [63]. Probiotic supplementation has been proposed as a strategy to normalize bacterial colonization, thereby reducing the incidence of NEC [64]. They also modulate inflammatory responses by influencing mucosal dendritic cells and inducing T-reg cells, which secrete anti-inflammatory cytokines [65, 66]. Probiotics inhibit key inflammatory pathways, such as those involving nuclear factor-kappa-B (NF-κB) and Mitogen-activated protein kinase (MAPK) [67, 68], some of which are mediated by short-chain fatty acids (SCFAs) produced by *Lactobacillus* and *Bifidobacterium* [69, 70]. Furthermore, probiotics promote intestinal growth and barrier function in preterm infants, stimulating mucin production and enhancing tight junctions between epithelial cells [71, 72]. It has been shown that *Lactobacillus* can induce production of mucins, resulting in augmentation of the mucous layer in the gut [73]. Also, probiotics have a strengthening effect on tight junctions between intestinal epithelial cells [74]. Probiotics can also reduce exposure to toxins by increasing GI motility [75]. Specific probiotic strains function also must be noted. *Lactobacillus* spp. contribute to intestinal barrier integrity by upregulating tight junction proteins such as like ZO-1, Claudin-1, and Occludin [76] and producing antimicrobial peptides (bacteriocins) [77], which help limit pathogen invasion. They also enhance the production of short-chain fatty acids (SCFAs), such as butyrate, which provide energy for enterocytes and have anti-inflammatory properties through inhibiting the activation of the toll like receptor (TLR)−4/NF-κB signaling pathway [78]. *Bifidobacterium* spp. modulate immune responses by increasing the production of anti-inflammatory cytokines like interleukin-10 (IL-10) while reducing pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and IL-6, thereby promoting a balanced immune environment [79]. Moreover, *Bifidobacterium* species are predominant in the intestines of breastfed infants and metabolize human milk oligosaccharides, SCFAs like acetate. This metabolic activity acidifies the intestinal environment, inhibiting pathogen growth and promoting the development of regulatory T cells, which are essential for immune tolerance [80]. *Saccharomyces boulardii*, a probiotic yeast, prevents pathogen adhesion to the intestinal epithelium, neutralizes bacterial toxins, and enhances mucosal immunity [81, 82]; however, its efficacy in NEC prevention appears to be lower compared to bacterial probiotics, as suggested by our findings.

The long-term implications of administering probiotics to preterm infants, including potential permanent alterations to the microbiome, remain uncertain. Notably, a study by Jacobs et al. found comparable rates of survival without major neurodevelopmental impairment among subjects enrolled in the ProPrems trial, suggesting no significant long-term adverse effects [83]. While probiotics are Generally Recognized as Safe (GRAS) (https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras), concerns persist regarding their use in extremely low birth weight infants. The lack of FDA-regulated pharmaceutical-grade products in the United States, coupled with conflicting data on safety and efficacy, underscores the need for caution. Current evidence does not support the routine, universal administration of probiotics to preterm infants, particularly those with a birth weight of less than 1000 g [84]. Moreover, cases of sepsis involving *Bifidobacterium longum* have been documented in preterm infants receiving probiotic therapy. For instance, a case series reported bacteremia in three preterm infants associated with *Bifidobacterium longum* supplementation [85]. Given the limited data on the long-term effects of probiotic supplementation, future research should prioritize extended follow-up studies. These studies should assess potential benefits and risks, including neurodevelopmental outcomes, metabolic health, and immune function, to inform neonatal probiotic supplementation strategies effectively.

Despite the significant protective effects of probiotics against NEC and NEC-related mortality, our findings should be interpreted in light of the heterogeneity observed across studies. While subgroup analyses based on gestational age, study population, birth weight, and probiotic strain helped identify potential sources of variability, heterogeneity remained high in certain subgroups, particularly those involving multi-strain probiotics. This suggests that differences in probiotic composition and host characteristics may contribute to the observed variability. As the limitations, due to not reporting the dose, treatment protocol, and duration in some included studies, subgroup analysis based on them was not performed. The included studies did not consistently report detailed information on the specific dosage, duration of administration, or feeding regimens used across individual trials. These factors likely play a crucial role in determining probiotic efficacy but remain a source of uncertainty due to the lack of standardization. Future research should aim to establish optimal probiotic strains, dosages, and treatment durations to maximize clinical benefits while minimizing heterogeneity in study outcomes. As another limitation, results of subgroup analysis may not be fully generalizable or robust due to the sample size limitations. Another key limitation of our umbrella meta-analysis is the lack of detailed data on clinical factors such as comorbidities, the use of antibiotics, and ventilator support, all of which could influence the effectiveness of probiotics in preventing necrotizing enterocolitis (NEC). These factors may significantly alter the outcomes of probiotic supplementation. Future studies should aim to report and account for these clinical variables to provide a clearer understanding of how probiotics function in different clinical contexts, especially in high-risk populations such as premature or critically ill infants.

As strengths, the results of our study by summarizing all previous studies with conflicting results can provide evidence for prescribing probiotics to prevent NEC. The high quality of included studies also confirms the validity of the obtained results. In addition, our protocol is registered in PROSPERO.

Conclusion

Probiotic supplementation can be recognized as a NEC preventing approach in preterm and very preterm infants. As well, probiotics can reduce all case and NEC-related mortality in preterm and very preterm infants. Multi-strain probiotics have a better preventive effect on NEC compared to single-strain probiotics. While multi-strain probiotics show promise in preventing NEC and related mortality, key questions remain regarding the optimal strains, dosage, and duration. Future research should focus on large-scale trials to determine the most effective regimens and explore safety profiles, particularly for vulnerable populations, to guide clinical practice.

Supplementary Information

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Supplementary Material 1. The search strategy and results of publication bias.

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Not applicable.

Authors' contributions
Jiaju Han, Yufeng Ren came up with the idea for the study and designed the search strategy. Peini Zhang searched databases, while Chengfeng Fang, Leilei Yang, and Shenkang Zhou were responsible for selecting the studies and extracting data. Jiaju Han and Zhiqing Ji carried out statistical analyses. Yufeng Ren and Peini Zhang wrote the initial draft of the manuscript. The manuscript was then critically revised by Yufeng Ren, and all authors approved the final version.

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Data availability
Information about the data and analysis performed in the present study is available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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