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Research Article

Probiotics for Preventing Necrotizing Enterocolitis: A Meta-Analysis with Trial Sequential Analysis

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What is Known and Objective. The role of probiotics, especially the different genera of probiotics, in managing necrotizing enterocolitis (NEC) is controversial. Thus, we performed a meta-analysis with trial sequential analysis (TSA) to determine the efficacy and safety of probiotics for preventing NEC. *Methods*. Medline, Embase, CENTRAL, WorldCat, TROVE, DART-Europe, and CBM were searched from inception to May 2022. Two investigators independently screened the literature, extracted data, and assessed the quality of the included studies. Meta-analysis was performed using RevMan 5.4, and TSA was conducted using TSA 0.9 beta. *Results and Discussion*. Fifty-five studies involving 12897 newborns were eligible. The use of probiotics for preventing NEC reduced the incidence of NEC (RR 0.48, 95% CI 0.41 to 0.57, and $P < 0.05$) and sepsis (RR 0.77, 95% CI 0.64 to 0.94, and $P < 0.05$), the risk of mortality (RR 0.69, 95% CI 0.58 to 0.84, and $P < 0.05$), and shortened the average days of hospitalization (MD −3.12, 95% CI −4.98 to −1.26, and $P < 0.05$). However, subgroup analysis revealed that different genera of probiotics gave rise to different outcomes. In addition, TSA indicated that the cumulative z-curve crossed the traditional and trial sequential monitoring boundaries for benefit, providing firm evidence that multiple strains and *Lactobacillus* species of probiotics decreased the incidence of NEC. However, the current evidence was inconclusive for *Bifidobacterium* and *Saccharomyces* species. *What is New and Conclusions*. Probiotics are effective in preventing NEC and sepsis and could provide added benefits, including decreasing mortality and the number of days of hospitalization. However, considering the heterogeneity of probiotics regimens and the risk of selective reporting of RCTs, more high-quality clinical trials targeting different genera of probiotics with suitable doses and timing to prophylactic use of probiotics are needed to avoid overestimating the role of probiotics in preterm infants.

1. What Is Known and Objective

Necrotizing enterocolitis (NEC) is the leading cause of neonatal death but a poorly understood disease. It frequently occurs in preterm infants, especially those with very low birth weight. The mortality and morbidity in very low birth weight infants are 10–30% and 5–10%, while the mortality is as high as 30–50% in neonates with extremely low birth weight [1, 2]. As the most common gastrointestinal emergency in neonates, it is categorized into three stages according to clinical symptoms. The typical initial symptoms include feeding intolerance, increased gastric residuals, abdominal distension, and bloody stools, which rapidly deteriorate to intestinal perforation, peritonitis with or without pneumoperitoneum, systemic hypotension, and coagulopathy, resulting in ischemic necrosis (tissue death) of the intestinal mucosa [3]. Inflammatory reactions of neonates with NEC would cause delayed neurodevelopment in the neonate, and 25% of neonates with NEC would progress to brain malformation or serious neurodevelopmental problems [4, 5]. NEC increases the duration of intravenous nutrition in infants, potentially increasing the risk of infectious complications and extending the duration of hospitalization [6]. Therefore, early prevention and early diagnosis of NEC are crucial.

Probiotics are nonpathogenic strains of organisms that are beneficial to the host by modulating the intestinal microbiome and promoting mucosal barrier functions and resistance to pathogens in the gut [7]. Some studies have shown a significant reduction in the prevalence of NEC in preterm infants who receive preventive treatment with different probiotic strains. However, current evidence fails to recommend the routine clinical use of probiotics for preventing NEC in preterm infants because the safety of probiotics in preterm infants, such as whether probiotics will increase the incidence of infection or unexpected outcome, is inconclusive [8, 9]. Therefore, its validity and safety remain to be further verified [10]. This study aimed to evaluate the efficacy and safety of probiotics in preventing NEC using systematic review and meta-analysis methods. In addition, the study sought to overcome the limitation of traditional meta-analysis by performing a trial sequential analysis (TSA) to analyze whether the available sample will be sufficiently powered to support the results and provide firm and solid evidence for clinical practice.

2. Methods

2.1. Search Strategy. A highly sensitive search was performed in May 2022 using a combination of MeSH terms and keywords with no restriction by region or language. The main sources included Medline, Embase, Cochrane Central Register of Controlled Trials (CENTRAL), WorldCat, TROVE, DART-Europe, and CBM. In addition, reference lists of all full-text articles were hand searched for additional studies. The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines were used as a reporting framework for this systematic review and meta-analysis [11, 12].

2.2. Eligibility Criteria. All titles and abstracts retrieved from the searches were screened for relevance independently by two reviewers (Yang Zhang and Qiong Xu). The full text of all articles that appeared to meet the inclusion criteria based on reading the abstract were retrieved for further evaluation and validation according to predefined criteria. Discrepancies were resolved by asking a third independent reviewer (Chunlei Sun).

We predefined the inclusion criteria as follows: (1) randomized clinical trials (RCTs), which investigated the effect of probiotics (including all types of probiotics: multiple strains and different species of probiotics) in premature infants (including low birth weight and extremely low birth weight infants) with gestational age <37 weeks and/or body weight <2500 g; (2) enteral administration of any probiotics within the first 10 days of life and continued for at least seven days; and (3) any types of controls were considered admissible.

Trials with any of the following criteria were excluded: (1) nonrandomized or uncontrolled trials; (2) the literature had no clear definition of NEC while using different outcome parameters who are not in line with the objective of our meta-analysis; and (3) it combined the literature of other drug therapies.

2.3. Data Extraction. Data from the included studies were extracted and summarized independently by two reviewers (Yang Zhang and Qiong Xu) using a predesigned form and subsequently validated by another reviewer (Feng Zhang). The following data were extracted: first author and year of publication, characteristics of participants, experimental and control interventions, and the primary outcome.

2.4. Quality Assessment. The methodological quality of all included RCTs was assessed independently by two researchers (Yang Zhang and Qiong Xu), using the Cochrane risk of bias tool for RCTs [13]. Disagreements were settled by consulting the senior author (Chunlei Sun). Funnel plots were used to investigate publication bias. All authors had access to the study data and reviewed and approved the final manuscript.

2.5. Data Synthesis and Statistical Analysis. All data syntheses were conducted using RevMan 5.4 software. We performed the meta-analysis according to the recommendations in the Cochrane Handbook for Systematic Reviews of Interventions. Dichotomous data were pooled using risk ratios (RRs) and corresponding 95% confidence intervals (CIs). Continuous outcomes were measured using mean differences (MDs) and corresponding 95% CIs. Heterogeneity between studies was quantified using the I^2 statistic. A fixed-effect model was used to perform the meta-analysis if the $I^2 < 50\%$; otherwise, a random effects model was utilized. Since cumulative meta-analyses of RCTs are at risk of yielding random errors due to sparse data and repetitive testing of accumulating data, we performed TSA for the major outcomes using TSA 0.9 beta software to evaluate whether the present meta-analysis had sufficient sample size to reach firm conclusions about the effect of the interventions [14–18].

3. Results

3.1. Study and Quality Characteristics. A total of 1249 articles were initially identified, and 55 RCTs involving 12897 eligible infants were eventually [9, 19–40] included [41–72]. The PRISMA flow diagram is shown in Figure 1. The characteristics of the included studies are shown in Table 1.

The enrolled infants had a gestational age of <37 weeks, except in the study by Rueman et al., where the neonatal age was <72 h after birth. In addition, the birth weights of the newborns were <2500 g, except in the study by Arora et al., where the birth weight was not restricted but included newborns with gestational ages below 37 weeks; thus, we included the study. Probiotics used in this study included multiple strains, *Bifidobacterium* species, *Lactobacillus* species, and *Saccharomyces* species, and the course treatment was 7–14 days. All neonates in the included studies were preterm neonates with low birth weight, including extremely low birth weight infants.

The quality of the included studies varied due to different reasons at different stages of the studies. The risk of bias from selective reporting and some other potential sources of bias

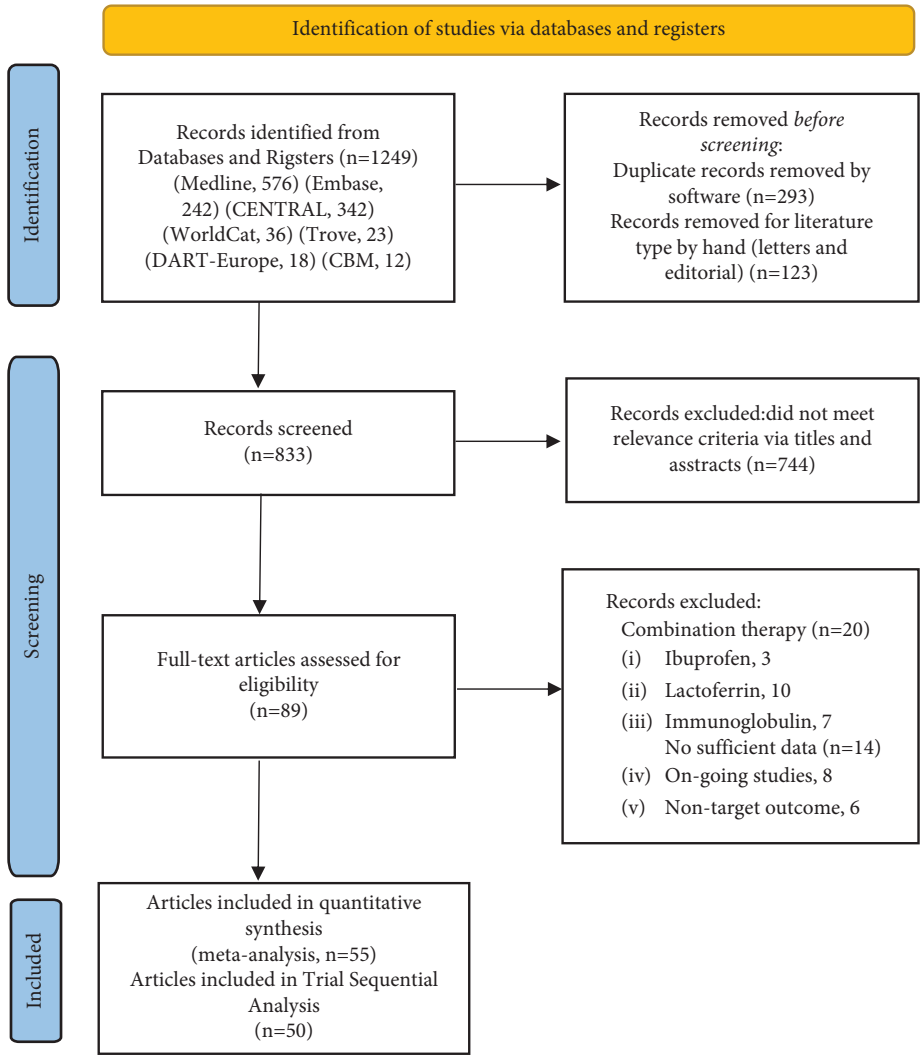

Figure 1: Flow diagram of the study selection process.

were unclear in most studies. Figure 2 shows the risk bias graph of the included studies.

3.2. Meta-Analysis and Trial Sequential Analysis Results. In total, all 55 included RCTs had data on the predefined clinical outcomes of interest suitable for quantitative comparison of probiotics versus placebo.

3.2.1. Incidence of NEC. Fifty RCTs reported the incidence of NEC. The rate in the probiotic group was 3.3%, compared with 7.0% in the placebo-controlled group. A fixed-effect meta-analysis showed a significantly lower incidence of NEC in the probiotic group than in the placebo group (RR 0.48, 95% CI 0.41 to 0.57, and $P < 0.05$, Figure 3). Given the different genera of probiotics used in the included studies, we conducted a subgroup analysis based on the species of probiotics used.

In the subgroup analysis by probiotic genus, 21 RCTs used composites of multiple strains, including *Lactobacillus* and *Bifidobacterium* with or without *Saccharomyces* species. The meta-analysis showed that the composite of multiple strains could reduce the incidence of NEC compared with placebo (RR 0.32, 95% CI 0.24 to 0.43, and $P < 0.05$), and the TSA result showed that the current studies' cumulative sample surpassed the Required Information Size (RIS = 4109). In addition, the cumulative *Z*-curve crossed the traditional and trial sequential monitoring boundaries for benefit (Figure 4), indicating firm evidence that multiple strains of probiotics can prevent the incidence of NEC and no further RCTs related to multiple strains were needed.

Thirteen RCTs used *bifidobacterium* probiotics. The meta-analysis showed that the use of *bifidobacterium* significantly reduced the incidence of NEC (RR 0.67, 95% CI 0.51 to 0.88, and $P < 0.05$), and the TSA result demonstrated that 2731 (15.6%) of the RIS of 17535 patients accrued. However, the cumulative Z-curve only crossed the traditional boundary for benefit but did not cross the trial sequential monitoring boundary for benefit (Figure 4), revealing that more RCTs are needed to clarify the result of *bifidobacterium* species.

Table 1: Characteristics of the included studies.

Study ID	Sample size		Age (w or h); birth weight (g)	Probiotic formulations	Outcomes
	Experimental (probiotics)	Control (placebo)			
Reuman, 1986, America	15	15	<72 h; <2000 g	*Lactobacillus*	③④
Millar, 1993, England	10	10	33 w; <1500 g	*Lactobacillus*	②
Kitajima, 1997, Japan	45	46	<1500 g	*Bifidobacterium*	①
Dani, 2002, Italy	295	290	<33 w; <1500 g	*Lactobacillus*	①③
Costalos, 2003, Greece	51	36	28–32 w	*Saccharomyces*	①②
Lin, 2005, China	180	187	<1500 g	Multiple strains	①②③④
Bin-Nun, 2005, Israel	72	73	<1500 g	Multiple strains	①②③
Manzoni, 2006, Italy	39	41	<1500 g	*Lactobacillus*	①②③
Fuji, 2006, Japan	11	8	<34 w	*Bifidobacterium*	①②
Mohan, 2006, German	37	32	<37 w	*Bifidobacterium*	①
Stratiki, 2007, Greece	41	34	<37 w	*Bifidobacterium*	①②
Lin, 2008, Chinese Taiwan	217	217	<1500 g	Multiple strains	①②③④
Samanta, 2008, India	91	95	<32 w; <1500 g	Multiple strains	①②③④
Ke, 2008, China	438	446	<37 w	Multiple strains	①
Underwood, 2009, America	CUL:30 PBP:31	29	<35 w; <2000 g	CUL:*Lactobacillus* PBP:*Bifidobacteria*	①
Rouge, 2009, Spain	45	49	<32 w; <1500 g	Multiple strains	①②③④
Manzoni, 2009, Italy	151	153	<30 w; <1500 g	Multiple strains	①②③
Huang, 2009, China	95	88	28–32 w; <1500 g	*Bifidobacterium*	①
Awad, 2010, Pakistan	60	30	<37 w; <2500 g	*Lactobacillus*	①③
Mihatsch, 2010, German	91	89	<30 w; <1500 g	*Bifidobacterium*	①③
Ren, 2010, China	80	70	28–33 w; <2500 g	Multiple strains	①
Braga, 2011, Brasilia	119	112	<1500 g	Multiple strains	①③
Sari, 2011, Turkey	110	111	<33 w; <1500 g	*Lactobacillus*	①②③
Rojas, 2011, Colombia	176	184	<1500 g	*Lactobacillus*	①④
Romeo, 2011, Italy	A:83 B:83	83	<37 w; <2500 g	A:*Lactobacillus reuteri* B:*Lactobacillus rhamnosus*	④
Al-Hosni, 2012, America	50	51	<1000 g	Multiple strains	①③
Fernandez-Carrocera, 2013, Mexico	75	75	<1500 g	Multiple strains	①③④
Jacobs, 2013, Australia	548	551	<32 w; <1500 g	Multiple strains	①②③④
Serce, 2013, Turkey	104	104	<32 w; <1500 g	*Saccharomyces*	①③
Demirel, 2013, Turkey	135	136	<32 w; <1500 g	*Saccharomyces*	①②③④
Wang, 2013, China	121	118	<37 w	*Saccharomyces*	1
Saengtawesin, 2014, Thailand	31	29	<34 w; <1500 g	Multiple strains	①③
Oncel, 2014, Turkey	200	200	≤32 w; ≤1500 g	*Lactobacillus*	①③
Benor, 2014, Israel	25	33	<30 w; <1500 g	Multiple strains	①②③
Roy, 2014, India	11	11	<1000 g	Multiple strains	①
Patole, 2014, Australia	77	76	<33 w	*Bifidobacterium*	①③
Totsu, 2014, Japan	153	130	<1500 g	*Bifidobacterium*	①③
Hays, 2016, France	145	52	<30 w; <1500 g	*Bifidobacterium*	①③
Hua, 2014, China	119	138	<37 w	Multiple strains	①②③
Sinha, 2015, India	668	672	<2500 g	Multiple strains	①
Dilli, 2015, Turkey	100	100	<32 w; <1500 g	*Bifidobacterium*	①③
Dutta, 2015, India	38	35	27–33 w	Multiple strains	①②③

Table 1: Continued.

Study ID	Sample size		Age (w or h); birth weight (g)	Probiotic formulations	Outcomes
	Experimental (probiotics)	Control (placebo)			
Kanic, 2015, Slovenia	40	40	<33 w; <1500 g	Multiple strains	①②③
Tewari, 2015, India	121	123	<34 w	*Bifidobacterium*	①③
Costeloe, 2016, England	650	660	<30 w	*Bifidobacterium*	①③
Chowdhury, 2016, England	52	50	<35 w; <1500 g	Multiple strains	①④
Xu, 2016, China	51	49	30–37 w; 1500–2500 g	*Saccharomyces*	①②④
Hernandez Enriguez, 2016, Mexico	24	20	<34 w; <1500 g	*Lactobacillus*	①②
Arora, 2017, India	75	75	<34 w; no restricted	Multiple strains	①②③④
Singh Dongol, 2017, Nepal	37	35	32.6 ± 2.2 w; <2000 g	*Lactobacillus*	①
Shashidhar, 2017, India	52	52	31.2 ± 2.1 w; 750–1499 g	Multiple strains	①③④
Wejryd, 2018, Sweden	68	66	<28 w; <1000 g	*Lactobacillus*	①③
Cui, 2019, China	45	48	30–37 w; 1500–2000 g	*Lactobacillus*	①②④
Kaban, 2019, Indonesia	47	47	28–34 w; 1000–1800 g	*Lactobacillus*	①②③④
Oshiro, 2019, Japan	17	18	24–31 w; <1500 g	*Bifidobacterium*	②

Note. (1) outcomes: ①: incidence of necrotizing enterocolitis; ②: incidence of sepsis; ③: mortality; ④: average days of hospitalization. (2) age: w = weeks (gestational age); h = hours (after birth age).

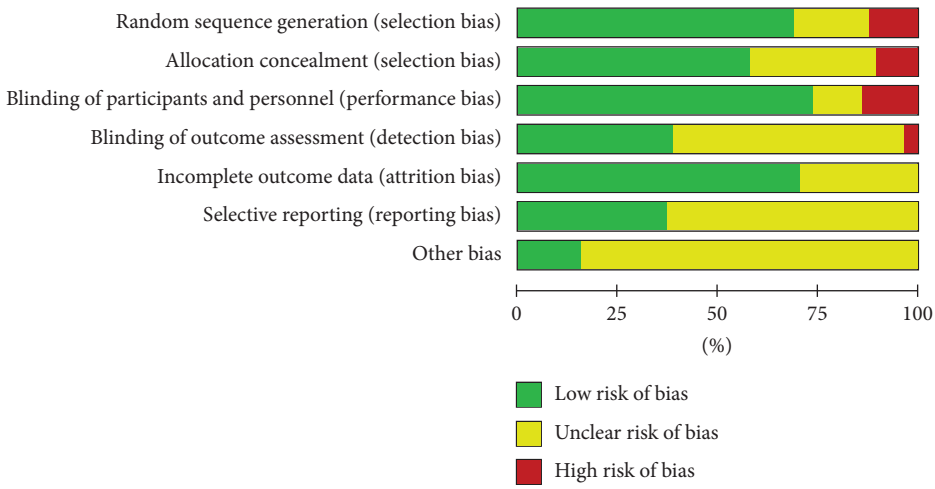

Figure 2: Risk of bias graph of the included randomized controlled trials on the efficacy and safety of probiotics in preventing necrotizing enterocolitis in infants.

Eleven RCTs provided data on *Lactobacillus* species. The meta-analysis showed that lactobacillus species could significantly reduce the incidence of NEC (RR 0.51, 95% CI 0.35 to 0.74, and $P < 0.05$). In addition, the TSA result showed that the cumulative Z-curve crossed the traditional and trial sequential monitoring boundaries for benefit (Figure 4), providing strong evidence for *Lactobacillus* species in preventing the incidence of NEC. Although it did not reach the RIS, to some extent, there is no need to conduct more RCTs to verify the result.

As for the five RCTs about *Saccharomyces* species, the meta-analysis showed that there was no significant difference in the incidence of NEC compared with placebo. In the TSA, the cumulative Z-curve neither crossed the traditional nor the trial sequential monitoring boundary for benefit, indicating that the current evidence is inconclusive for *Saccharomyces* species (Figure 4).

3.2.2. Incidence of Sepsis. Twenty-six RCTs reported the incidence of sepsis. The incidence of sepsis in the probiotic group was 14.6%, compared with 18.6% in the placebo group. The random effects meta-analysis model showed that probiotics could slightly reduce the incidence of sepsis (RR 0.77, 95% CI 0.64 to 0.94, and $P < 0.05$, Figure 5). In the subgroup analysis, multiple strains, *Lactobacillus*, and *Saccharomyces* species failed to significantly reduce the incidence of sepsis except *Bifidobacterium* species (RR 0.39, 95% CI 0.23 to 0.68, and $P < 0.05$, Figure 5).

3.2.3. Mortality. Thirty-five RCTs reported mortality. The mortality rate in the probiotic group was 3.5%, compared with 5.1% in the control group mortality. A fixed-effect meta-analysis model showed that probiotics significantly decreased mortality, with a difference of statistical significance (RR 0.69, 95% CI 0.58 to 0.84, and $P < 0.05$, Figure 6). In the subgroup analysis, 16 RCTs reported on multiple strains and showed that multiple strains probiotics could significantly reduce the mortality rate (RR 0.56, 95% CI 0.42 to 0.76, and $P < 0.05$). *Bifidobacterium* species, *Lactobacillus* species, and *Saccharomyces* species alone had the trend to reduce the mortality compared with the control group, but the difference was not statistically significant.

3.2.4. Average Days of Hospitalization. Sixteen RCTs reported the days of hospitalization, and random effects meta-analysis showed that probiotics statistically significantly reduced the days of hospital stay compared with placebo (MD −3.12, 95% CI −4.98 to −1.26, and $P < 0.05$, Figure 7). However, there was no significant difference in shortening the days of hospitalization by the genus of probiotics used, except for probiotics using multiple strains.

3.3. Reporting Bias Analysis. The funnel plot based on the incidence of NEC showed that the distribution of the studies on both sides of the funnel was not quite symmetrical, 23 studies on the left, and 17 on the right (Figure 8), suggesting the possibility of publication bias.

4. Discussion

To the best of our knowledge, similar systematic reviews have been topic, but this is the first meta-analysis to incorporate TSA to investigate the effect of probiotics for NEC to obtain a more robust conclusion. The data from 55 trials, including more than 10000 preterm infants, demonstrated that probiotics could reduce the incidence of NEC, decrease the risks of sepsis and mortality, and shorten the days of hospitalization. Probiotics appear to be one of the best strategies for preventing NEC. Nevertheless, the primary challenge in expanding their application is the heterogeneity of the genus of the probiotics used in RCTs. Thus, we conducted subgroup analyses by the genus of probiotics supplementation. Meanwhile, we used the TSA in our meta-analysis to handle problems with multiplicity by considering both risks of random and systematic errors.

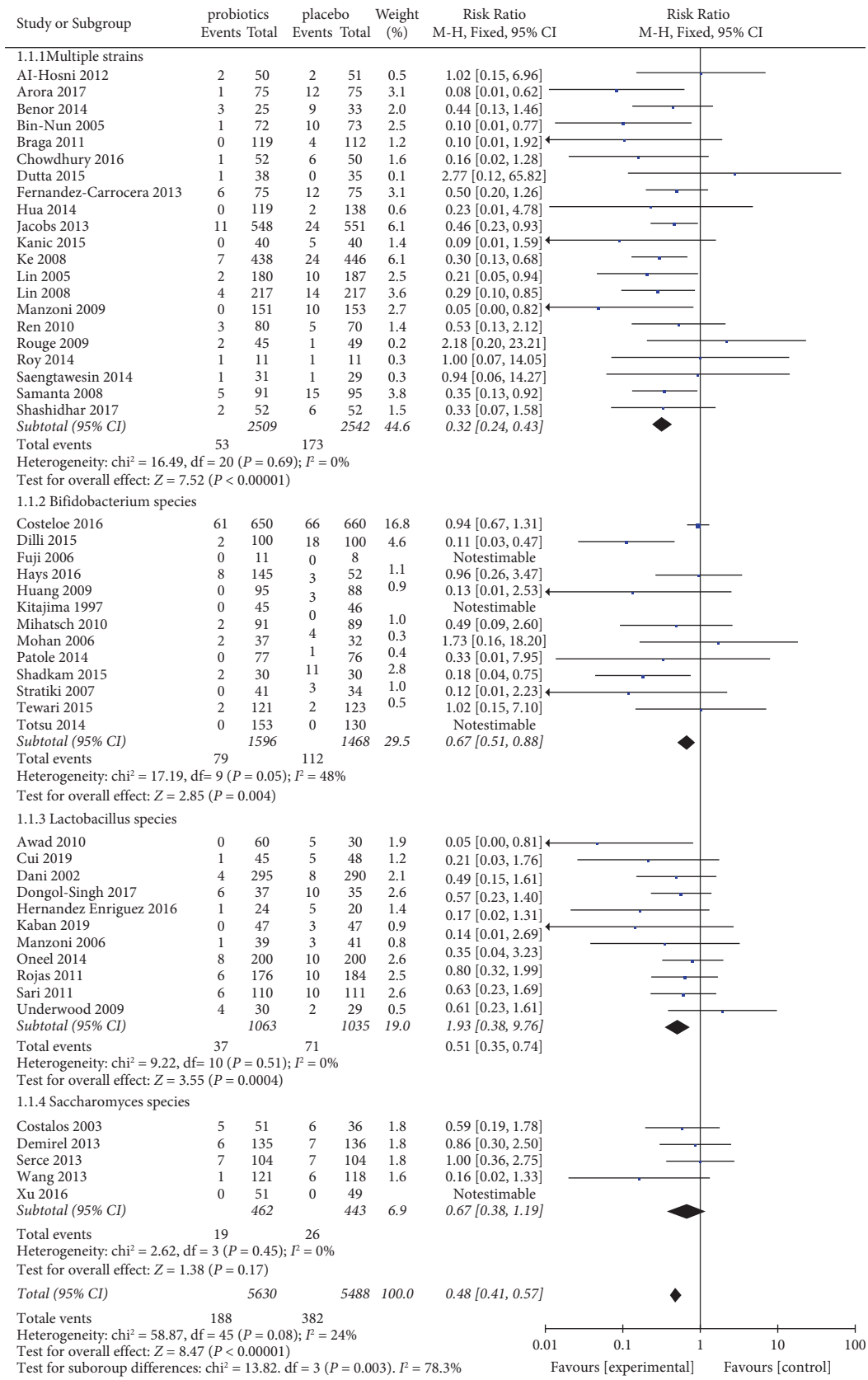

Figure 3: Forest plot of the meta-analysis of the incidence of necrotizing enterocolitis.

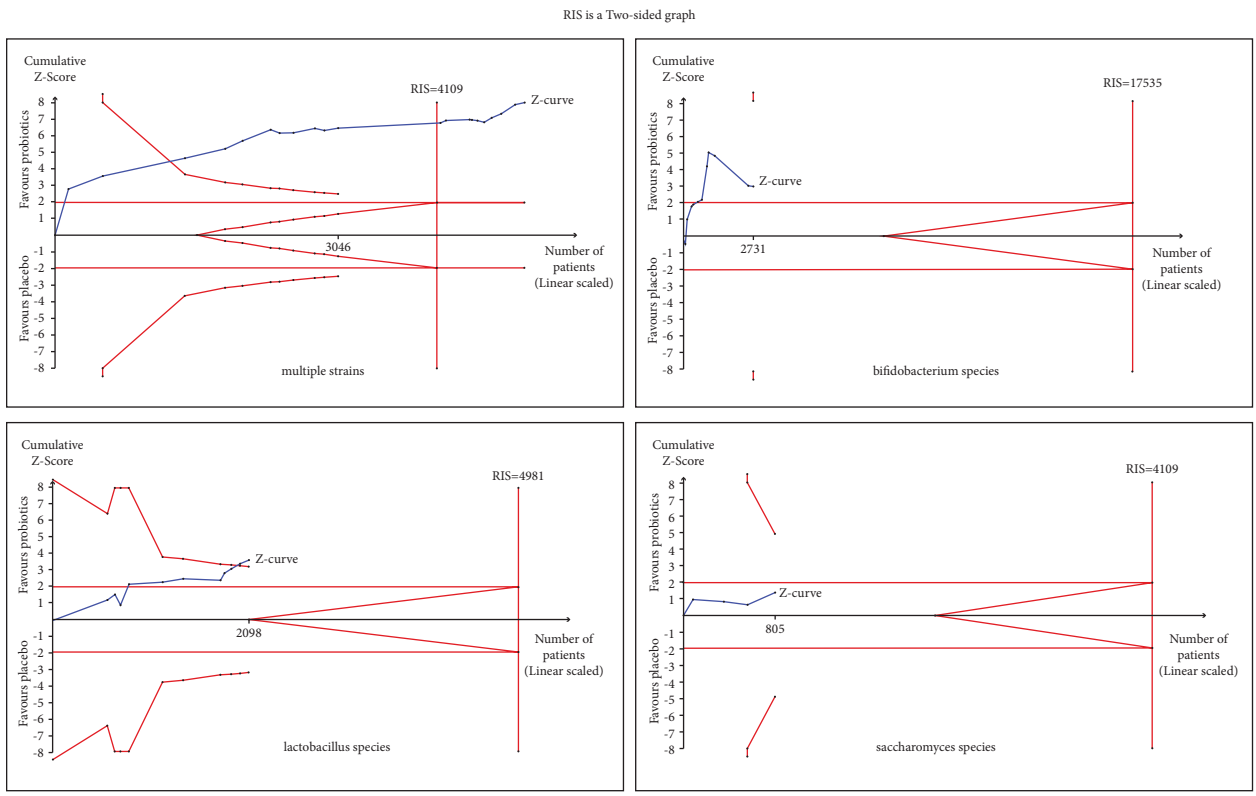

Figure 4: Trial sequential analysis of probiotics for incidence of necrotizing enterocolitis.

The multiple strains combining the *Bifidobacterium* and *Lactobacillus* species with or without the *Saccharomyces* species could significantly reduce the incidence of NEC and mortality and even decrease the days of hospitalization. However, there was no significant effect on preventing the incidence of sepsis, which seems to be an obstacle against the application of prophylactic probiotics preparations in preterm infants to decrease NEC induced by sepsis by administering probiotics strains. Whether the complex of multiple strains increases the risk of infection warrants further verification [73–75]. The *Bifidobacterium* species reduced the incidence of NEC and sepsis but did not decrease mortality. Interestingly, probiotics containing the *Bifidobacterium* species seem effective in preventing NEC-related infection. By contrast, the *Lactobacillus* species could only decrease the incidence of NEC but had no effect on the incidence of sepsis and mortality.

The European Society for Paediatric Gastroenterology, Hepatology, and Nutrition panel recommended in 2020 that probiotics such as *Bifidobacterium* could reduce the risk of NEC in preterm infants [76]. However, a multicenter randomized controlled study found that early administration of *Bifidobacterium bifidum* (BBG-001) did not reduce the risk of NEC and sepsis in preterm infants [9]. Hence, international guidelines or policy statements do not recommend the unconditional use of probiotics combinations or single strains of probiotics in preterm infants [77]. As a result, the optimal probiotic composition or combination could not be determined reliably by analyzing existing trial data. In addition, most probiotics preparations circulating in the market did not meet drug standards, and unregulated use beyond the instructions would be a potential safety hazard.

Previous studies have shown that after taking probiotics, the body could produce short-chain fatty acids and organic acids to stimulate peristalsis of the large intestine, thus relieving constipation. The *Lactobacillus, Bifidobacterium*, and *Saccharomyces* species in the ileum could maintain the balance of intestinal microflora and promote the growth of normal intestinal flora and the secretion of intestinal mucosal immunoglobulin A [78, 79]. Meanwhile, *Saccharomyces* species could inhibit the overbreeding of pathogenic intestinal bacteria, increase intestinal permeability, promote the gut immune response, improve the intestinal barrier of the gut, and reduce inflammation [80, 81]. Surprisingly, the included trials about the *Saccharomyces* species revealed no effect on NEC, sepsis, and mortality. *Saccharomyces* species, such as yeast differ from the *Bifidobacterium* and *Lactobacillus* species, which are bacteria. The merit of *Saccharomyces* is that it can combine antibiotics to treat infections in preterm infants [82]. Of course, the mechanisms need to be deeply elucidated to support the clinical applicability of the *Saccharomyces* species.

It is worth mentioning that our study focused on the average days of hospitalization as one of the outcomes. This outcome was chosen because the development of NEC increases the chance of undergoing surgical treatment leading to a prolonged duration of intravenous nutrition in infants, potentially increasing the risk of infectious complications and prolonging the length of hospitalization. Besides, the

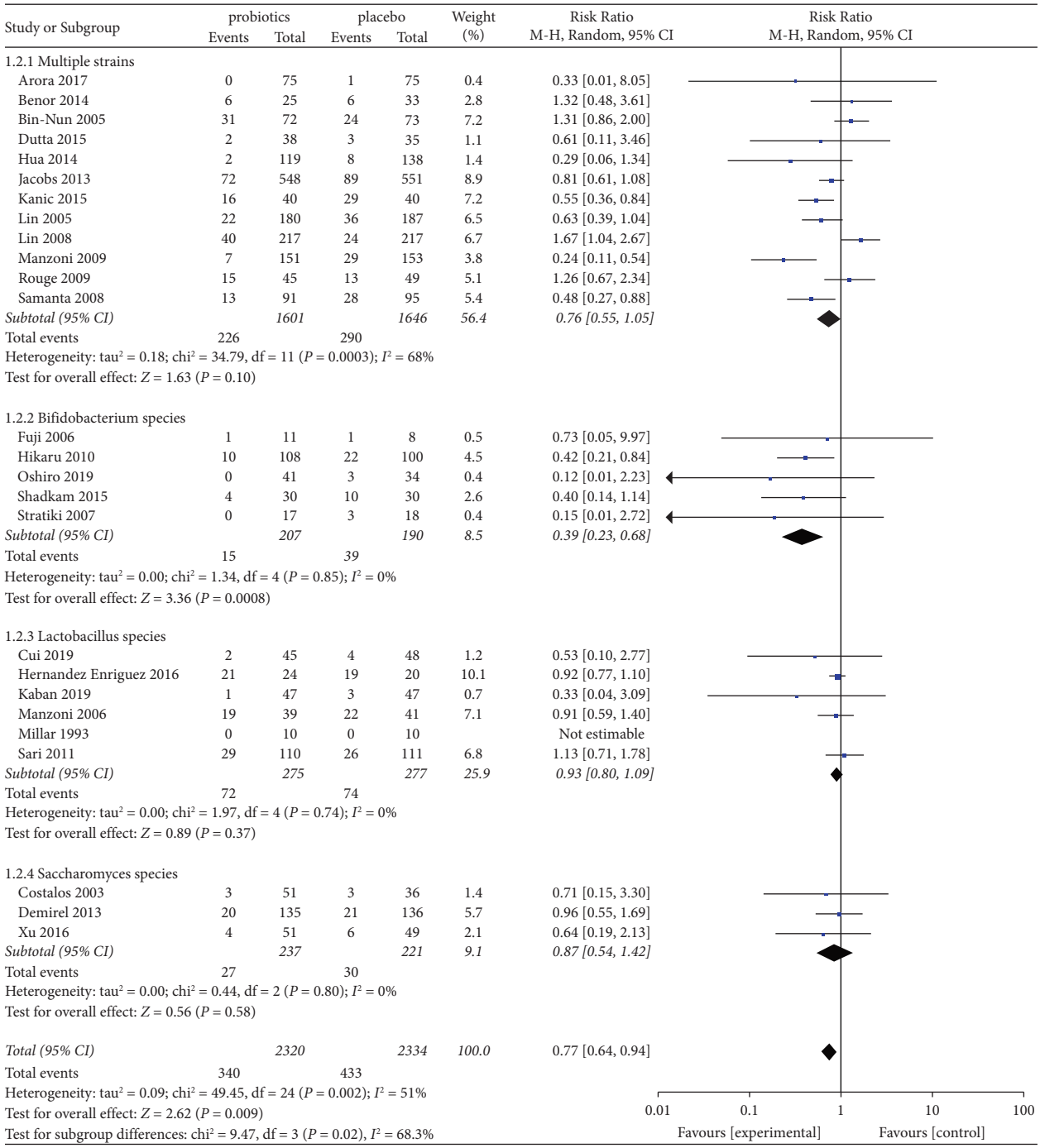

Figure 5: Forest plot of incidence of sepsis.

financial cost of NEC is substantial, with the total annual cost of caring for affected infants in the United States estimated between $500 million and $1 billion. The total mean cost of care over five years for a child with short-bowel syndrome is approximately $1.5 million [83, 84]. Unfortunately, our meta-analysis showed no significant difference in shortening the days of hospitalization, except for the multiple strains.

As mentioned above, up to now, there is no consensus on the optimal strain, dose, and timing of probiotic administration for NEC prevention, so further confirmatory results are needed to promote the clinical applicability of probiotics. Therefore, we recommend conducting more prospective multicenter studies to guarantee the efficacy of different genera of probiotics, the appropriate dose and right timing for prophylactic use of probiotics, and different feeding methods. Future clinical trials ought to strive to guarantee double-blinded interventions and the primary outcomes, including NEC, neurological damage, and duration of hospitalization. In addition, we should pay more attention towards investigating the interactions

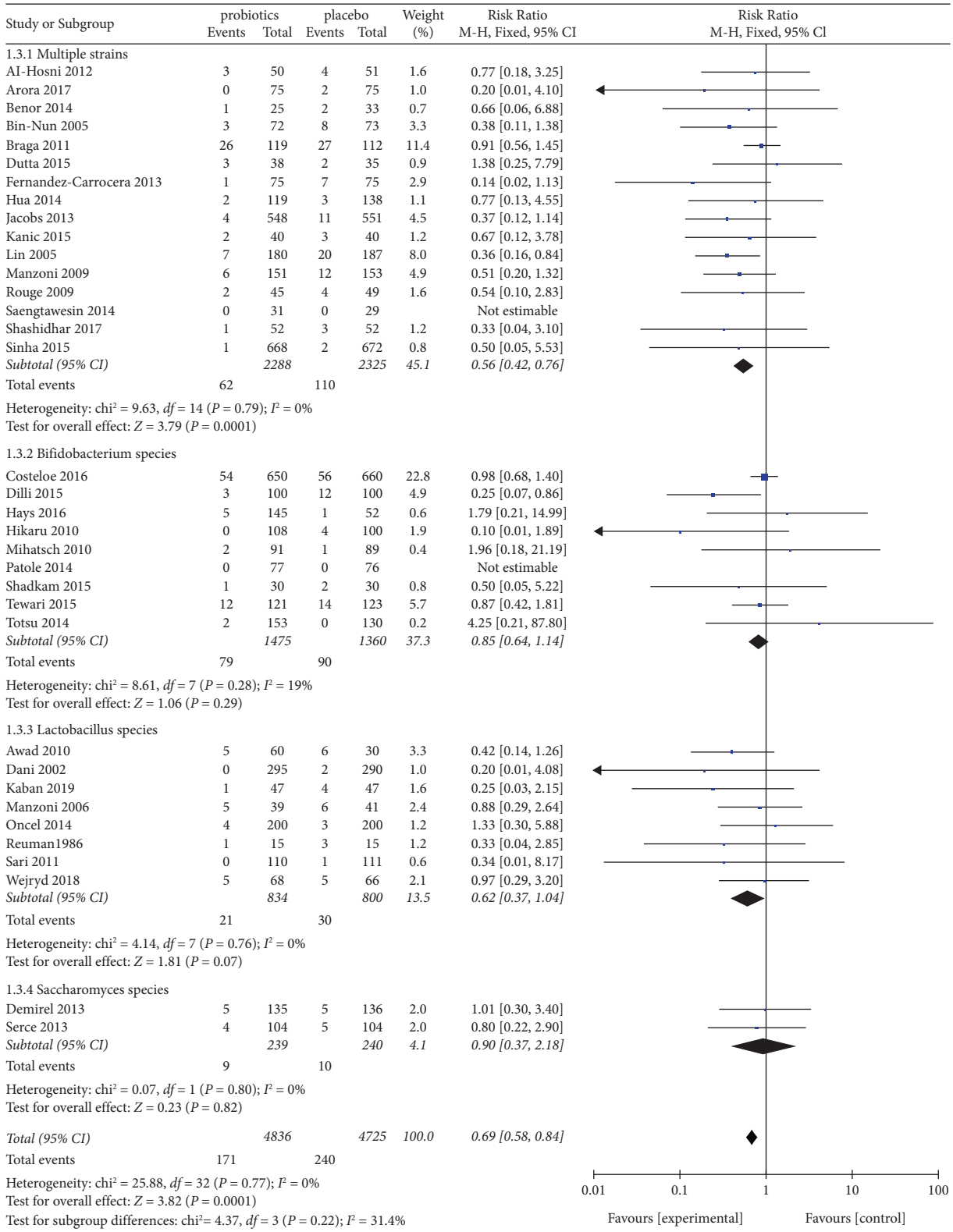

Figure 6: Forest plot of mortality.

between the interventions and the other enteral supplementation received. Alternatively, we could explore whether the synbiotics are superior to probiotics or prebiotics.

This study had some limitations. First, some studies had an unclear risk of bias in different domains, while others had a high risk of bias in at least one domain. Notably, most of the included studies with a high risk of bias did not show significant effects of

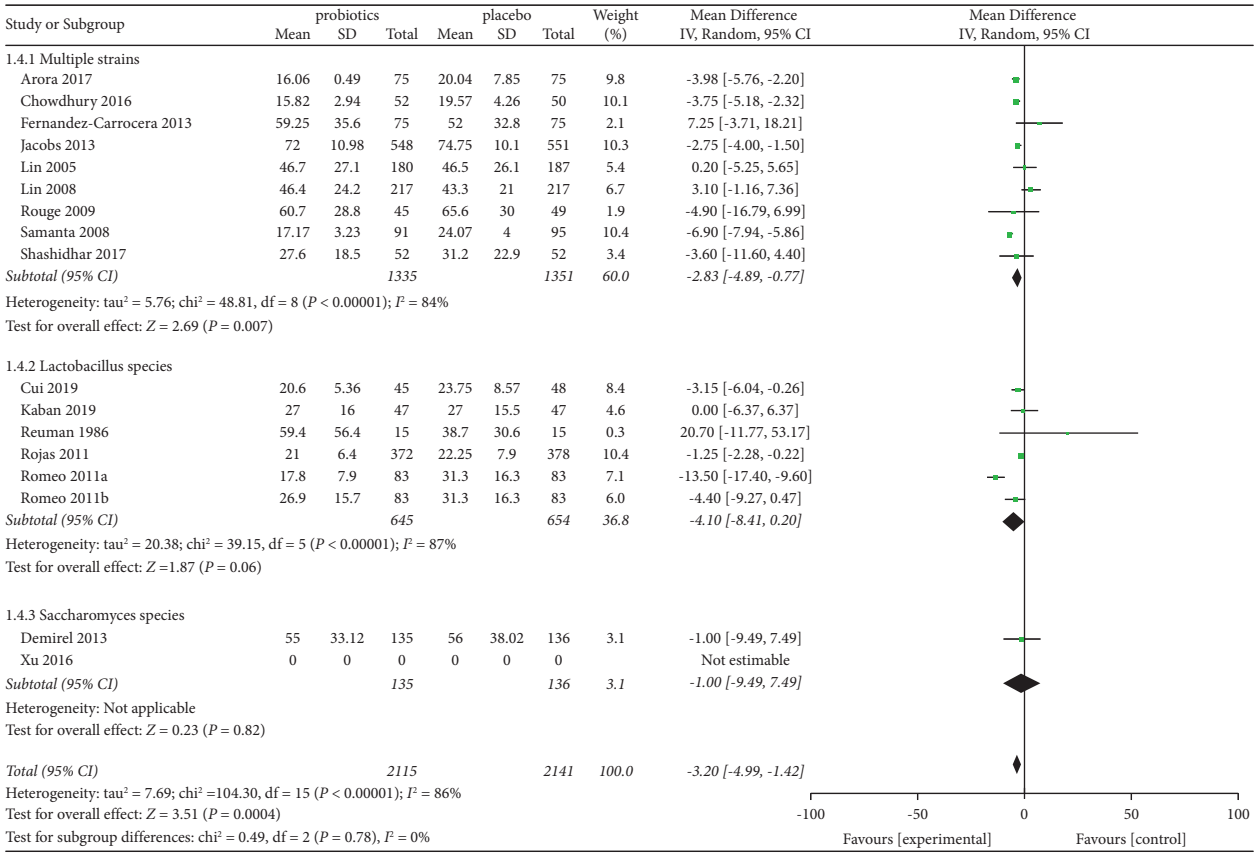

Figure 7: Forest plot of average days of hospitalization.

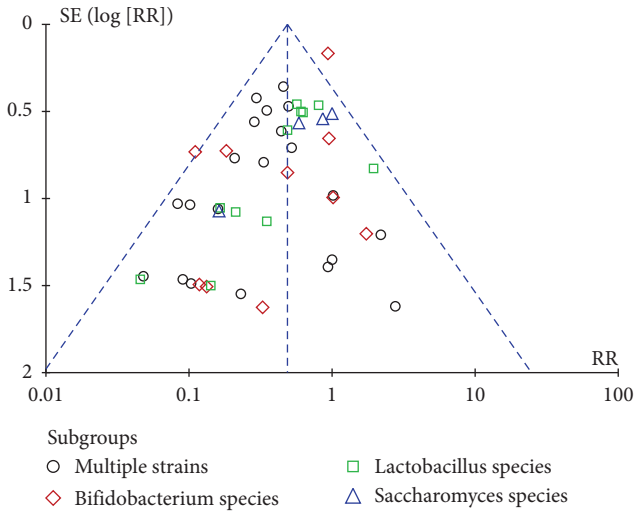

Figure 8: Funnel plot of the incidence of necrotizing enterocolitis.

probiotics in preventing NEC. In addition, the incidence of NEC was not the primary outcome in several included studies, which seemed to have weakened the strength of the evidence. Furthermore, some clinical trials were not registered, so it is unclear whether there is a risk of selective reporting. Moreover, the probiotics genus, feeding methods, dosage, and course of treatment might have had some impact on the results. Finally, whether clinical decision-making should apply probiotic supplementation is complicated and should consider other factors, such as methodological quality, types of preterm infants, setting, and other practices such as feeding types of enteral supplementation and use of antibiotics.

5. What Is New and Conclusions

In summary, the current evidence shows that using probiotics to prevent NEC could effectively reduce the incidence of NEC, sepsis, and mortality and shorten the days of hospitalization. However, due to the limitations of the study, the current study is not enough to support the routine treatment of probiotics in preterm infants. The above conclusion needs to be further confirmed by more high-quality RCTs, especially those using the *Bifidobacterium* species and the *Saccharomyces* species. Only the precise probiotics strain proven effective in clinical trials could be further recommended in clinical practice.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the supplementary materials.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Supplementary Materials

The supplementary material, including search strategy in Embase and risk of bias summary. (*Supplementary Materials*)

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