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Review

Bifidobacterium may benefit the prevention of necrotizing enterocolitis in preterm infants: A systematic review and meta-analysis

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ABSTRACT

Aim: A systematic review and meta-analysis was designed to evaluate the efficacy and safety of Bifidobacterium for preventing necrotizing enterocolitis (NEC) in preterm infants.

Methods: We searched the Cochrane Library, PubMed, EMBASE and Web of Science to December 2017. Risk ratio (RR) with 95% confidence intervals (CIs) were estimated to compare the outcomes of the groups. For the pooled RR estimating the incidence of NEC, we also performed subgroup analysis. Besides, sensitivity analysis was performed to examine the stability of the combined results. Two reviewers assessed trial quality and extracted data independently. The work has been reported in line with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and AMSTAR (Assessing the methodological quality of systematic reviews) Guidelines. All statistical analyses were performed using standard statistical procedures provided in Review Manager 5.2.

Results: Twenty four randomized, placebo-controlled studies (N = 6155 participants) were included in this analysis, of which twenty two studies were used for assessing the efficacy of Bifidobacterium for preventing NEC and seventeen for assessing the safety (sepsis and death). When comparing Bifidobacterium groups with control groups, the relative risk of developing NEC (RR 0.38, 95% CI 0.25–0.58; $P < 0.00001$) or death (RR 0.74, 95% CI 0.60–0.92; $P = 0.006$) was significantly lower in the Bifidobacterium groups. No significant difference in the incidence of sepsis was found (RR 0.87, 95% CI 0.73–1.03; $P = 0.11$). In addition, significant results for NEC were also found in all subgroups we made.

Conclusions: Bifidobacterium may have a beneficial effect and be safe in preventing necrotizing enterocolitis in preterm infants.

1. Background

Bifidobacterium, as one of the most commonly used probiotics, has many benefits in several fields. Recently, there were many reports about the benefits of Bifidobacterium in preventing necrotizing enterocolitis (NEC) in preterm infants [1–7]. NEC can be a devastating gastrointestinal disease, mostly on premature infants [8]. The morbidity and mortality which NEC results in are equal to other severe diseases that preterm infants experienced, especially in premature infants [9]. The majority of NEC in infants always occurs within the first few days after their birth. Among these NEC infants, more than 85% of them experienced very low birth weight less than 1500 g and gestation less than 32 weeks [10].

The cause and mechanism of NEC has not been completely understood to date. It may be resulted from an undue inflammatory response and subdued inflammatory control in the intestinal mucosa. In these infants, intolerance of feeding was also observed. In addition, NEC patients almost experienced abdominal distension and bloody stools within the first few days after their birth. To date, the straightforward pathomechanism still remains unclear and does not been well studied, in spite many studies about microbiology, molecular biology have been performed for the intestine of premature infants and infants with NEC [11]. According to the presumption which Claud EC and Walker WA raised, incidence of NEC in preterm infants may be multifactorial and inappropriate initial microbial colonization is one of important risk factors [12].

One experimental report from Athalye-Jape G et al., 2017 showed that probiotics could significantly reduce the incidence of NEC because

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Table 1
The characteristics of included studies for meta-analysis of efficacy and safety of Bifidobacterium.

Study/Year	Country	Design	BW/GA	Probiotic Interventions	Dosage and Duration	P/C (No. of NEC stage ≥ 2)	P/C (total No.)	Outcomes	Jadad Score
Al-Hosni M et al., 2012	United States	Double blinded	501–1000 g	Lactobacillus rhamnosus GG, Bifidobacterium infantis	5×10^8; Enteral; 28 d	2/2	50/51	NEC; death; sepisis; apnea	2
Bin-Nun A et al., 2005	Israel	Single blinded	< 1500 g/ < 37WK	Bifidobacteria infantis; Streptococcus thermophiles; Bifidobacterium bifidus;	0.35×10^9 CFU of each, once daily from first feed to 36 wk corrected age	1/10	72/73	NEC; mortality; sepsis	3
Braga TD et al., 2011	Brazil	Double blinded	< 37 wk	Lactobacillus casei; Bifidobacterium	$3.5 \times 10^7$to 3.5×10^9 CFU daily; Since second day of life until 30 days	0/4	119/112	NEC; sepsis; death	5
Chou IC et al., 2010	China	Single blinded	< 37 wk	Lactobacillus acidophilus; Bifidobacteria infantis	10^9 CFU for each strain; Oral; until discharge	0/2	153/148	NEC, death, sepsis, hospitalization et al.	2
Chowdhury T et al., 2016	Bangladesh	Double blinded	< 33 W; < 1500 g	Capsule TS6 probiotic containing Bifidobacterium species, Lactobacillus	6×10^9	1/6	52/50	NEC, hospital stay	4
Costeloe K et al., 2016	England	Double blinded	23-30w	Bifidobacterium breve BBG-001	10^9; Oral; 36 wk PMA or discharge	61/66	650/660	ROP, death, NEC, sepsis	5
Deng JL et al., 2010	China	NR	< 37 wk	Bifidobacterium longum; Lactobacillus acidophilus; E. faecalis	For BW < 1500 g, 0.33×10^7 CFU and for BW > 1500 g, 0.5×10^7 CFU twice daily; For 14 days	NR	63/62	NEC	4
Dilli D et al., 2015	Turkey	Double blinded	32w 1500 g	Bifidobacterium lactis	5×10^9; Oral; NICU stay 34.5 (11)/37 (18)	2/18	100/100	Height, NEC, sepsis, mortality et al.	4
Fernandez-Carrocera LA et al., 2013	Mexico	Double blinded	26–36 wk	Lactobacillus A//RH/C/P; Bifidobacteria infantis; ST	1 g organisms daily; Since first feed until death or discharge	6/12	75/75	NEC; death; sepisis; apnea; weight	5
Hays S et al., 2016	France	Double blinded	37w	Bifidobacterium lactis and Bifidobacterium longum	10^9; Oral; 4–6wk	8/3	147/52	Weight, length, HC, NEC, sepsis et al.	4
Huang B et al., 2009	China	NR	28–32 wk	Bifidobacterium A	0.25×10^8 organisms twice daily, Started from day 7 of life to day 14	0/3	95/88	NEC; weight gain; gut colonization	3
Jacobs SE et al., 2013	Australia	Double blinded	32w 1500 g	Bifidobacterium infantis, Streptococcus thermophilus, Bifidobacteria lactis	3×10^8 of B. infantis, 3.5×10^8 of S. thermophiles, 3.5×10^8 of B. lactis; Oral; until discharge or term-corrected age	11/24	548/551	Sepsis, death, weight at 28 d, ROP, CLD, IVH	2
Kanic Z et al., 2015	Slovenia	Single blinded	33w 1500 g	Lactobacillus acidophilus, Enterococcus faecium, Bifidobacterium infantum	1.2×10^7; Enteral; until discharge	0/5	40/40	Hospitalization, sepsis, pneumonia, NEC, death	2
Kitajima H et al., 1997	Japan	NR	< 1500 g/23-33WK	Bifidobacterium breve	0.5×10^9 organisms once daily, from first feed for 28 d	0/0	45/46	Weight gain; sepsis; NEC; death et al.	4
Lin HC et al., 2008	Taiwan	Single blinded	< 34wk and < 1500 g	Bifidobacterium bifidus; Lactobacillus acidophilus	BBB 1×10^9 CFU/d and LB-A 1×10^9 CFU/d, for 6 wk	4/14	217/217	NEC; death; sepsis; weight gain	4
Lin HC et al., 2005	China	Single blinded	< 1500 g	Lactobacillus acidophilus; Bifidobacteria infantis	125 mg/kg LB-A and BI organisms twice daily from day 7 until discharge	2/10	180/187	NEC; death; sepsis	5
Mihatsch WA et al., 2010	Germany	Single blinded	< 30 wk	Bifidobacterium lactis	2×10^9 CFU/kg for 6 times a day, For the first 6 weeks of life	2/4	91/89	NEC; sepsis; death	5
Mohan R et al., 2006	Germany	NR	< 37 wk	Bifidobacterium lactis Bb12	1.6×10^9 CFU once daily from day 1 to day 34.8×10^9 CFU once daily from day 4 to day 21	2/1	21/17	NEC	4
Patole S et al., 2014	Australia	Double blinded	< 33 W	Bifidobacterium breve M-16 V	3×10^9; Oral; until 37 wk PMA	0/1	77/76	Discharge weight, death et al.	5
Saengtawesin V et al., 2014	Thailand	NR	< 33wk or1500 g	Lactobacillus acidophilus; Bifidobacterium bifidum	1×10^9 of each; 125 mg/kg/dose twice a day; until 6 weeks or discharge.	1/1	31/29	NEC stage ≥ 2; adverse effects	2
Samanta M et al., 2009	India	Double blinded	< 34wk and < 1500 g	Bifidobacterium infantis/bifidus/longum/acidophilus	2.5×10^9 CFU/d until discharge	5/15	91/95	NEC; sepsis; death	3
Stratiki Z et al., 2007	Greece	Single blinded	27–37 wk	Bifidobacterium lactis	Preterm formula 1×10^7 CFU/g started within 48 h to 30d	0/3	41/36	Sepsis; NEC; death; weight gain et al.	5
Totsu S et al., 2014	Japan	Double blinded	1500 g	Bifidobacterium bifidum	2.5×10^9; Enteral; until body weight reached 2000 g	0/0	153/130	Body weight, sepsis, death, NEC et al.	4

(*continued on next page*)

Table 1 (*continued*)

Study/Year	Country	Design	BW/GA	Probiotic Interventions	Dosage and Duration	P/C (No. of NEC stage ≥ 2)	P/C (total No.)	Outcomes	Jadad Score
Underwood MA et al., 2009	United States	Double blinded	< 35 wk	Lactobacillus-GG/acidophilus; Bifidobacterium longum/bifidus/infantis	1×10^{10} of LB-GG,LB-A,BB-LG, BBB, BI twice daily, Since first feed for 28 days or until discharge	1/1	31/29	NEC; weight gain	5

Note: NEC, necrotizing enterocolitis; CFU, colony forming units; GA, Gestational age; BW, birth weight; BB, Bifidobacterium breve; SB, Saccharomyces boulardii; BI, Bifidobacteria infantis; BBB, Bifidobacterium bifidus; LB-A, Lactobacillus acidophilus; LB-C, Lactobacillus casei; BB-LG, Bifidobacterium longum; CLD, chronic lung disease; ROP, retinopathy of prematurity; IVH, intraventricular hemorrhage.
The Bell staging criteria for NEC is as follows: STAGE I (Suspect): a. Any one or more historical factors producing perinatal stress; b. Systemic manifestations-temperature instability, lethargy, apnea, bradycardia; c. Gastrointestinal manifestations-poor feeding, increasing pregavage residuals, emesis (may be bilious or test positive for occult blood) mild abdominal distension, occult blood may be present in stool (no fissure); d. Abdominal radiographs show distension with mild ileus. STAGE II (Definite): a. Any one or more historical factors; b. Above signs and symptoms plus persistent occult or gross gastrointestinal bleeding; marked abdominal distension; c. Abdominal radiographs show significant intestinal distension with ileus; small bowel separation (edema in bowel wall or peritoneal fluid), unchanging or persistent "rigid" bowel loops, pneumatosis intestinalis, portal vein gas. STAGE III (Advanced): a. Any one or more historical factors; b. Above signs and symptoms plus deterioration of vital signs, evidence of septic shock or marked gastrointestinal hemorrhage; c. Abdominal radiographs may show pneumoperitoneum in addition to others listed in II c [15].

probiotics may improve immunity and inflammation, relieve tissue injury, strengthen gut barrier, and restrain intestinal dysbiosis [13]. Additionally, in recent years, increasing clinical trials have focused their interests on the efficacy of probiotics in preventing NEC in preterm infants. However, the dosage, duration, kind of bacterial strains and other informations lack homogeneity. Additionally, the adverse events of bifidobacterium using including alimentary infection, bacteria induced death or sepsis, have not been examined. Furthermore, one of the primary outcomes—incidence of NEC has not been detailed, comprehensively collected and analyzed.

Previous systematic reviews and meta-analysis have shown that probiotics could reduce the incidence of NEC and death. But their studies included many kinds of strains as well as bacteria categories. Few rounded analyses assessed the efficacy and safety of bifidobacterium for preventing NEC in preterm infants.

In this present analysis, twenty four randomized, placebo-controlled studies including 6155 participants were included to demonstrate whether bifidobacterium are effective in reducing incidence of NEC. In addition, we also assessed the safety of bifidobacterium when preventing NEC in preterm infants.

2. Methods

2.1. Criteria for considering studies

2.1.1. Including criteria

(1) Studies are designed as blinded randomized, controlled trials (RCTs); (2) Included neonates with gestational age (GA) < 37 weeks; (3) Included neonates received bifidobacteria treatment for preventing NEC; (4) Outcomes including NEC Stage ≥ II (Bell staging criteria), sepsis and death were reported; (5) The incidence of outcomes was given.

2.1.2. Excluding criteria

(1) Experimental trials on animals or non-human studies; (2) Non-placebo-controlled studies were excluded; (3) Abstracts, letters, editorials, expert opinions, reviews, case reports were excluded; (4) Studies without sufficient data or did not meet our including criteria were excluded.

2.2. Types of outcome measures

2.2.1. The primary outcomes

Incidence of NEC Stage ≥ II (Bell staging criteria) in both of two groups.

2.2.2. Secondary outcomes

Incidence of sepsis and death in both of two groups.

2.3. Search strategy

We searched the Cochrane Library, PubMed, EMBASE and Web of Science till December 2017. Our searching terms and procedures were as follows: (1)"preterm infants"; OR "neonates"; (2) "necrotizing enterocolitis" OR "sepsis" OR "death"; (3) "bifidobacterium" OR "bifidobacteria" OR "probiotics". The retrieval scope includes all titles and abstracts and these terms above was searched in titles and abstracts. Other related terms, including references of some literature we encountered, were also searched. The language we searched was English. Two readers independently screened the titles and abstracts of each study initially acquired for further excluding and including. Once relevant studies meeting our criteria became certain, the full texts were obtained for further evaluation.

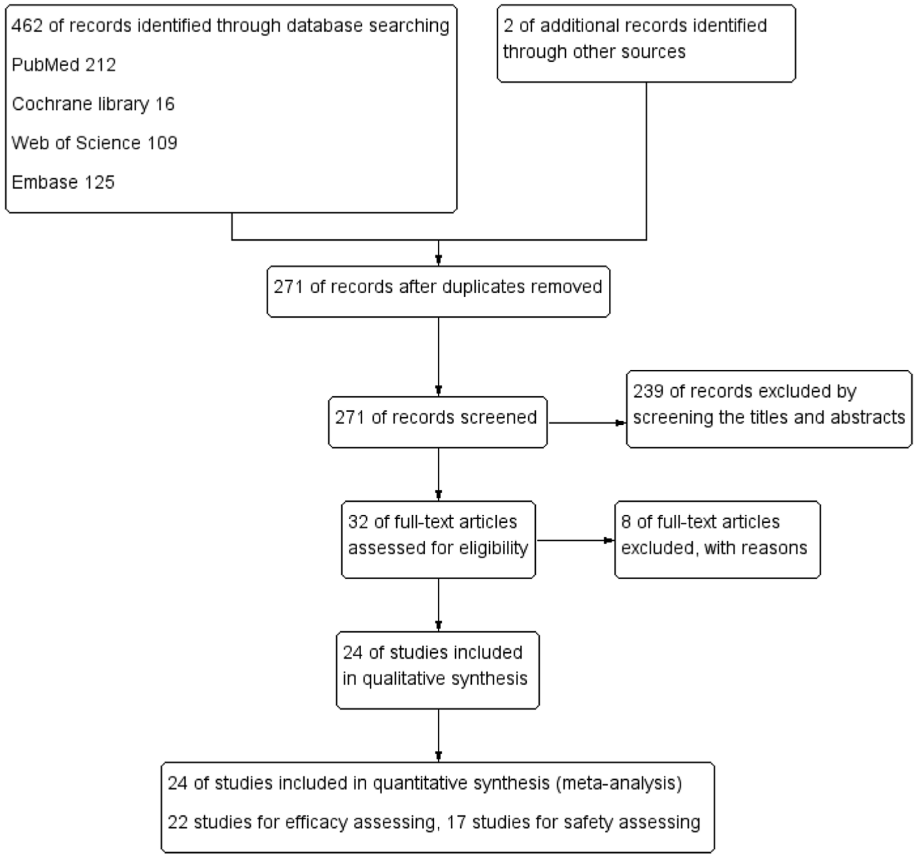

Fig. 1. Flow diagram following the PRISMA template of the search strategy for efficacy and safety of Bifidobacterium in preventing necrotizing enterocolitis (NEC) in preterm infants.

2.4. Quality assessment

Two reviewers assessed the quality of each study using the previously validated 5-point Jadad scale [14] and the total scores of each study were displayed in the characteristics table (Table 1). Studies with scores of 3 or more were considered high quality. In addition, the risk of bias for each RCT and the risk of bias across all RCTs were evaluated and shown with figures generated by RevMan 5.2 software [15].

2.5. Data extraction

The qualified data for the combined analysis for evaluating and assessing the efficacy and safety of Bifidobacterium intervention in reduce incidence of NEC were independently extract and collected by two reviewers, and disagreement was resolved by their discussion. The extracted contents included study demographics, published years, trial design, gestational age or birth weight of preterm infants, probiotic intervention regimens, number of NEC Stage ≥ II (according to the Bell staging criteria [15]) and total number in both of two groups, Outcomes and Jadad Score of each study using a standardized form. When the incidence of NEC Stage ≥ II, sepsis or death was only presented with percentages, we convert the proportion to the specific number of people for coalescent analysis. In addition, some stages of NEC were merged and split to adapt to analyzing the data. The Bell staging criteria [15] for NEC is three stages: STAGE I, Suspect; STAGE II, Definite; STAGE III, Advanced. The detail description was offered in Table 1.

Data collected were input into RevMan 5.2 software for analysis [16].

2.6. Statistical analysis

The data we collected was pooled analyzed in meta-analysis using standard statistical procedures provided in the statistical soft of RevMan 5.2 [16]. Results of comparing the outcomes of the groups were displayed with RR with its 95% CI. The between-study heterogeneity was assessed by the chi-square-based Q statistical test [17]. The heterogeneity analysis results were displayed with p value and I^2 statistic, which ranged from 0% to 100%. When $p \leq 0.10$ was deemed to represent significant heterogeneity [18,19], pooled risk ratio (RR) was estimated using a random-effect model (the DerSimonian and Laird method [20]). Inversely, if heterogeneity analysis results were not significant ($p > 0.10$), a fixed effects model (the Mantel–Haenszel method [21]) was used. The effects of outcome measures were considered to be statistically significant if RRs 95% CI did not overlap with 1.

We performed subgroup analysis for the pooled results of NEC according to Bacterium (Bifidobacterium or Involve Bifidobacterium), Jadad score (Score 1–2, 3–4 or 5), Blind methods (Single blind or Double blind). Besides, sensitivity analysis was performed to examine the stability of the combined results. The work has been reported in line with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and AMSTAR (Assessing the methodological quality of systematic reviews) Guidelines.

3. Results

3.1. Retrieval results and study characteristics

By screening the titles and abstracts, 239 of studies were excluded preliminary then 32 of studies were chosen from 271 of records. After reading the full texts of the 32 studies, eight studies were excluded further (two studies for lack of available data [10,22], three studies for not bifidobacterium [23–25], two study for just review [8,26] and one study for animal experimental study [13]), eventually a total of 24 studies (N = 6155 participants) were included in this meta-analysis, of which twenty two studies were used for assessing the efficacy of Bifidobacterium for preventing NEC and seventeen for assessing the safety (sepsis and death). Of the included studies, twelve studies were designed as double blinded randomized, controlled trials and seven studies were designed as single blinded randomized, controlled trials. The sample sizes ranged from 38 to 1310 patients. Ten studies used only bifidobacterium to prevent NEC in preterm infants, twelve studies used multiple probiotics involving bifidobacterium in the trials (Table 1).

The detail search process and summary of studies were showed in study flow diagram (Fig. 1). The other study characteristics of each study were showed in Table 1.

3.2. Quality assessment

As Table 1 showing, there are five studies with a Jadad score of 2, three studies with a Jadad score of 3, eight studies with a Jadad score of 4 and 5 respectively. According to our definition for good quality, approximately 80% studies experienced good quality.

In addition, to further identify the risk of bias of our including studies, we generated risk of bias graphs. The risk of bias for each RCT is presented as percentages across all included studies in Fig. 2, and the risk of bias item for each included study is displayed in Fig. 3. The risk of bias graphs indicated generally good methodological quality. Blinding issues were low risk of bias in these studies. High risk of bias was mainly about attribution and reporting bias, which had not been stated in some studies [27–32]. Unclear risks of bias was mainly observed in allocation concealment, attribution bias and other bias.

3.3. Efficacy of bifidobacterium

Twenty two RCTs were deemed available for meta-analysis to evaluate the efficacy of Bifidobacterium for preventing NEC in preterm infants [1,3,4,6,27–44]. In these studies, incidence of NEC Stage ≥ II (according to the Bell staging criteria) were used as the outcome measurement assessing the preventing effect of Bifidobacterium in NEC of preterm infants. In study of Kitajima H et al., 1997 and study of Totsu S et al., 2014 [3,43], the incidence of NEC Stage ≥ II in both control and experimental groups were 0, thus we eliminated them in the statistics list. The total incidence of NEC was 5.75% (320 infants experienced NEC in 5568 infants). As Fig. 4 showing, compared bifidobacterium groups with control groups in the incidence of NEC (defined as NEC Stage ≥ II according to the Bell staging criteria in original research), significant result was found with pooled RR of 0.38 (95% CI 0.25–0.58; $P < 0.00001$). The pooled analysis was estimated using random-effect models because significant heterogeneity ($p = 0.02$ and $I^2 = 43\%$) between studies was found.

3.4. Safety of bifidobacterium

3.4.1. Sepsis

Seventeen studies were used for the analysis of sepsis in supplement of bifidobacterium for preventing NEC in preterm infants [3,4,7,27–29,33–35,37–43,45]. The total incidence of sepsis was 19.5% (786 infants experienced sepsis in 4034 infants). As shown in Fig. 5, compared bifidobacterium groups with control groups in the incidence of sepsis when using supplement of bifidobacterium to prevent NEC in preterm infants, no significant difference in the incidence of sepsis was found (RR 0.87, 95% CI 0.73–1.03; $P = 0.11$). This result indicated that the supplement of bifidobacterium did not increase the risk of infection or sepsis of preterm infants. Considering the significant heterogeneity ($p = 0.03$ and $I^2 = 43\%$) between studies, random-effect models was used to estimate the combined results of sepsis.

3.4.2. Death

After eliminated the study of Patole S et al., 2014 [7] and Saengtawesin V et al., 2014 [45] for the incidence of death in both control and experimental groups were 0, fifteen studies were included for analysis of death [1,3,4,27–29,33–35,37,39–43]. The total incidence of sepsis was 6.4% (325 infants experienced sepsis in 5053 infants). As Fig. 6 showing, compared bifidobacterium groups with control groups in the incidence of death, significant result was found with pooled RR of 0.74 (95% CI 0.60–0.92; $P = 0.006$). The combined result indicated that the supplement of bifidobacterium played a significant role in reducing the risk of death in preterm infants. For that significant heterogeneity ($p = 0.14$ and $I^2 = 29\%$) between studies was found, fixed-effect models was used to estimate the combined results of death.

3.5. Subgroup and sensitivity analysis

Subgroup analysis was conducted to find the efficacy of bifidobacterium for preventing NEC in preterm infants further. Statistically significant effect of bifidobacterium was observed in subgroups of bifidobacterium (RR 0.48; 95% CI 0.24, 0.95; $P = 0.04$) and involve bifidobacterium (RR 0.31; 95% CI 0.21, 0.46; $P < 0.00001$); Jadad score 1–2 (RR 0.41; 95% CI 0.22, 0.76; $P = 0.005$), Jadad score 3–4 (RR 0.26; 95% CI 0.16, 0.41; $P < 0.00001$) and Jadad score 5 (RR 0.73; 95% CI 0.55, 0.97; $P = 0.03$); single blind (RR 0.21; 95% CI 0.11, 0.41; $P < 0.00001$) and double blind (RR 0.49; 95% CI 0.30, 0.81; $P = 0.005$) (Table 2).

We also performed sensitivity analysis to examine the stability of the combined results of the efficacy of bifidobacterium for preventing NEC and to identify the source of heterogeneity by omitting any single study. Sensitivity analysis showed that the combined results of the

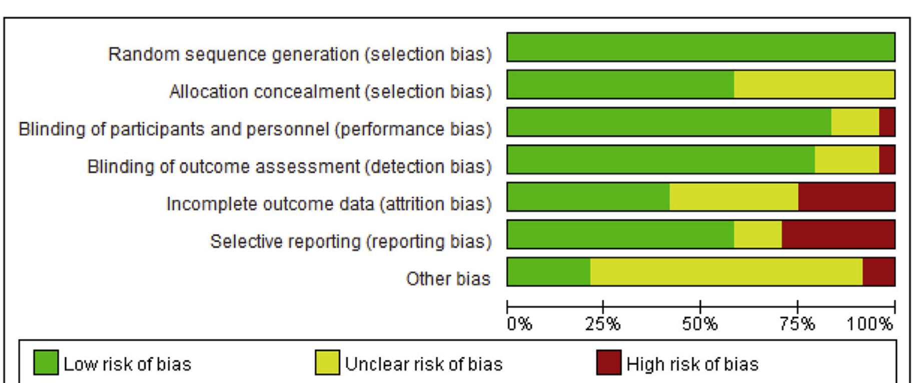

Fig. 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

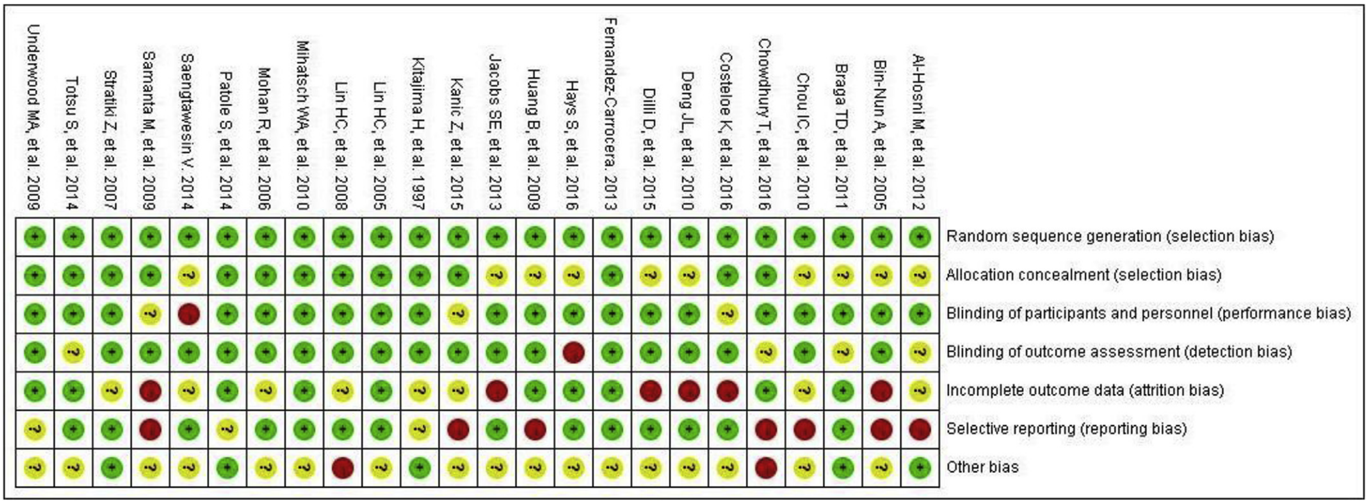

Fig. 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

efficacy of bifidobacterium had high stability by omitting each single study and no particular study significantly affect the pooled results, for that the pooled RRs ranged from 0.36 (95% CI 0.23, 0.56; $P < 0.00001$) after omitting the first study of Al-Hosni M et al., 2012 [27] to 0.37 (95% CI 0.24, 0.57; $P < 0.00001$) after omitting the last study of Underwood MA et al., 2009 [44]. Only one particular study, Costeloe K et al., 2016 [1], affect the heterogeneity results, for it changed to $p = 0.59$ and $I^2 = 0\%$ when the study was excluded from the meta-analysis seriatim (Table S1).

4. Discussion

Nowadays, the incidence of NEC is becoming one of common complications of preterm infants and is a devastating gastro-intestinal emergency because of its significant mortality (25%) and morbidity [46]. The economic cost of NEC treatment is high, with an average cost of $500 000 per infant in the United States. Though great advances in neonatal care have been achieved, NEC has directly resulted in substantial morbidity, mortality and health-care costs in many years. The economic burden of NEC in the United States has been estimated as high as several billions USD per year, which is approximately 20% of the costs for Neonatal Intensive Care Units in the country [47]. Up to now, the underlying mechanism or pathogenesis remains poorly understood and it is preferred to such a view that NEC is a multifactorial disease. Recently, many researches focused the factor of NEC on the role of gut microbiota and its manipulations, such as the use of probiotics, on disease and health status. The higher risk in premature infants is attributed to the immature development of the immune system, intestinal epithelial barrier function, gut motility and regulation of the microvascular circulation [11].

Study or Subgroup	Bifidobacterium Events	Bifidobacterium Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Al-Hosni M, et al. 2012	2	50	2	51	3.7%	1.02 [0.15, 6.96]
Bin-Nun A, et al. 2005	1	72	10	73	3.4%	0.10 [0.01, 0.77]
Braga TD, et al. 2011	0	119	4	112	1.8%	0.10 [0.01, 1.92]
Chou IC, et al. 2010	0	153	2	148	1.7%	0.19 [0.01, 4.00]
Chowdhury T, et al. 2016	1	52	6	50	3.3%	0.16 [0.02, 1.28]
Costeloe K, et al. 2016	61	650	66	660	14.5%	0.94 [0.67, 1.31]
Deng JL, et al. 2010	1	63	8	62	3.3%	0.12 [0.02, 0.95]
Dilli D, et al. 2015	2	100	18	100	5.6%	0.11 [0.03, 0.47]
Fernandez-Carrocera. 2013	6	75	12	75	9.0%	0.50 [0.20, 1.26]
Hays S, et al. 2016	8	147	3	52	6.4%	0.94 [0.26, 3.42]
Huang B, et al. 2009	0	95	3	88	1.8%	0.13 [0.01, 2.53]
Jacobs SE, et al. 2013	11	548	24	551	11.0%	0.46 [0.23, 0.93]
Kanic Z, et al. 2015	0	40	5	40	1.9%	0.09 [0.01, 1.59]
Lin HC, et al. 2005	2	180	10	187	5.2%	0.21 [0.05, 0.94]
Lin HC, et al. 2008	4	217	14	217	7.7%	0.29 [0.10, 0.85]
Mihatsch WA, et al. 2010	2	91	4	89	4.5%	0.49 [0.09, 2.60]
Mohan R, et al. 2006	2	21	1	17	2.7%	1.62 [0.16, 16.37]
Samanta M, et al. 2009	5	91	15	95	8.6%	0.35 [0.13, 0.92]
Stratiki Z, et al. 2007	0	41	3	36	1.8%	0.13 [0.01, 2.36]
Underwood MA, et al. 2009	1	31	1	29	2.1%	0.94 [0.06, 14.27]
Total (95% CI)		**2836**		**2732**	**100.0%**	**0.38 [0.25, 0.58]**
Total events	109		211			

Heterogeneity: Tau² = 0.29; Chi² = 33.37, df = 19 (P = 0.02); I² = 43%
Test for overall effect: Z = 4.51 (P < 0.00001)

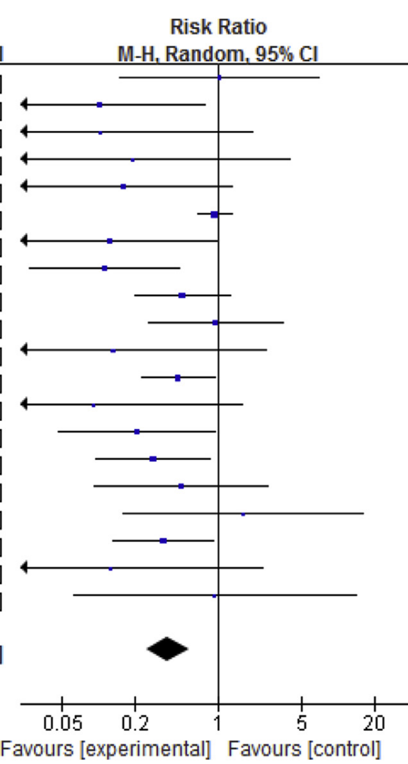

Fig. 4. Forest plot of the efficacy of Bifidobacterium for preventing NEC in preterm infants.

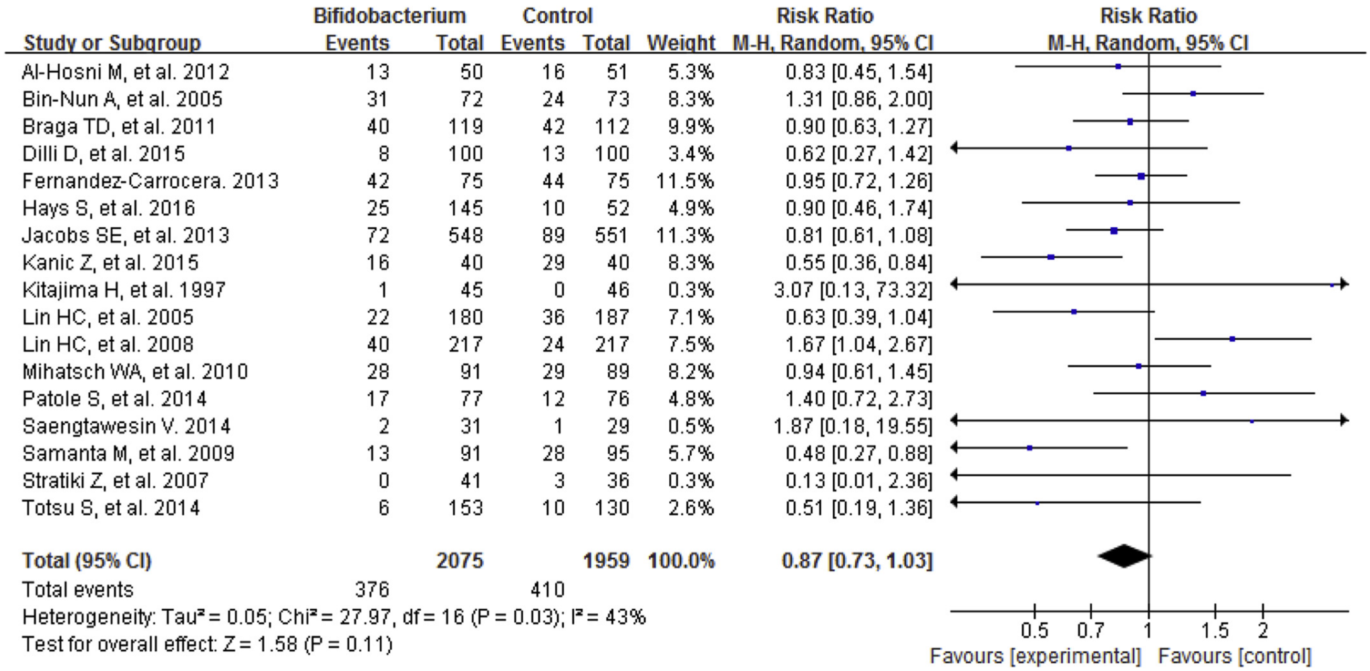

Fig. 5. Forest plot for evaluating the incidence of sepsis comparing bifidobacterium groups with control groups in preterm infants.

This review found twenty two RCTs of bifidobacterium for prevention of NEC and identified seventeen studies for assessing the safety (sepsis and death) in preterm infants. We perform this meta-analysis for purpose of demonstrating the efficacy and safety of Bifidobacterium in preventing NEC in preterm infants. Our analysis results statistically support the conclusions that the supplement of bifidobacterium could reduce the incidence of NEC compared with control groups, with pooled RR of 0.38 (95% CI 0.25–0.58; $P < 0.00001$). In addition, to assess the safety of bifidobacterium used in preterm infants, we compared and evaluated the incidence of sepsis (RR 0.87, 95% CI 0.73–1.03; $P = 0.11$) and death (RR 0.74, 95% CI 0.60–0.92; $P = 0.006$) of infants. The results showed that using bifidobacterium did not increase the risk of sepsis and may reduce the risk of death of infants, which to a certain extent indicated the safety of bifidobacterium. Subgroup analysis also demonstrated statistically significant effect of bifidobacterium in preventing NEC in preterm infants. Sensitivity analysis showed high stability of the efficacy of bifidobacterium. According to the present analysis and researches, the probiotics were proven to be beneficial to reduction of incidence of NEC and death of infants. No increasing of incidence of sepsis was found for probiotics groups. Thus infants, including premature infants that meet our including criteria, without excluding criteria should use probiotics to prevent the incidence of NEC. In addition, a cost-benefit study would be designed further to evaluate this possibility in the future to evaluate the efficacy and benefits of probiotics in this aspect.

Nevertheless, there were several limitations for this meta-analysis. The main limitation is the inconsistency of dosage and duration of bifidobacterium supplement. The bacterial strains of included studies

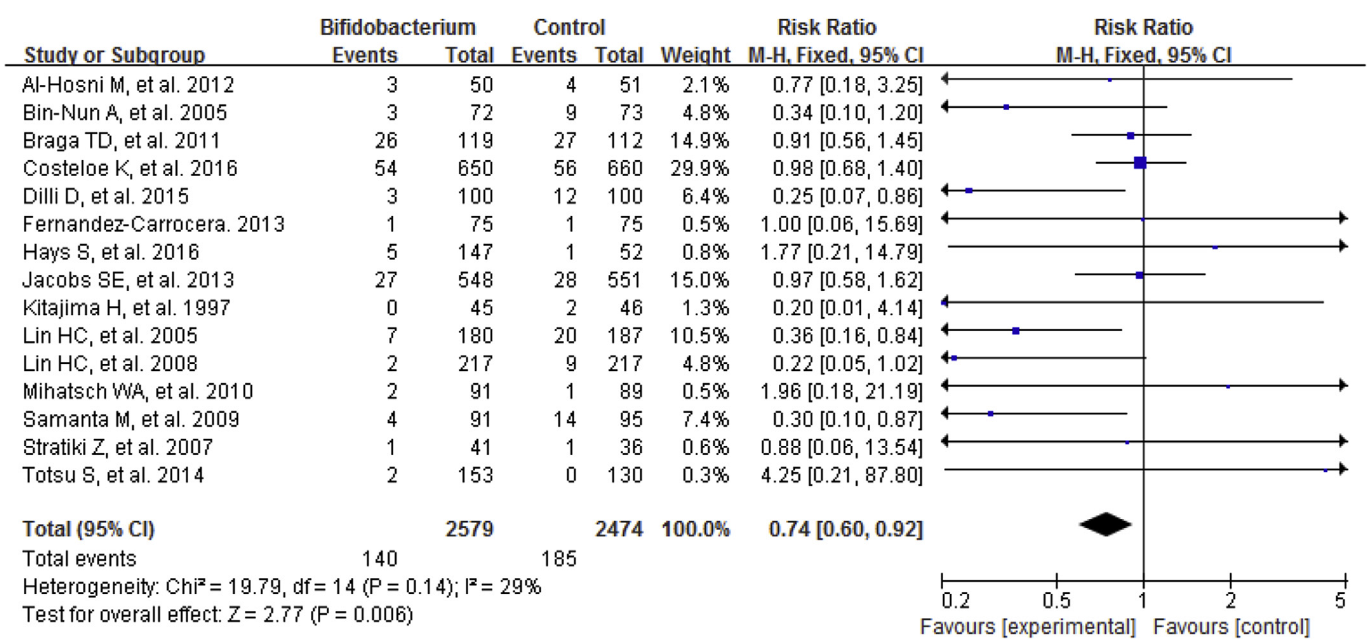

Fig. 6. Forest plot for evaluating the incidence of death comparing bifidobacterium groups with control groups in preterm infants.

Table 2
The pooled results of subgroups for the efficacy of Bifidobacterium preventing NEC.

Subgroups	Number of studies	Number of participants	Pooled results			Heterogeneity		
			RR	95% CI	*P* value	I^2	*p* value	Analytical effect model
Bacterium								
Bifidobacterium	8	2373	0.48	0.24, 0.95	0.04	54%	0.03	Random-effect model
Involve Bifidobacterium	12	3195	0.31	0.21, 0.46	< 0.00001	0%	0.68	Fixed-effect model
Jadad score								
Score 1-2	4	1581	0.41	0.22, 0.76	0.005	0%	0.52	Fixed-effect model
Score 3-4	9	1612	0.26	0.16, 0.41	< 0.00001	18%	0.28	Fixed-effect model
Score 5	7	2375	0.73	0.55, 0.97	0.03	33%	0.18	Fixed-effect model
Blind								
Single blind	7	1584	0.21	0.11, 0.41	< 0.00001	0%	0.90	Fixed-effect model
Double blind	10	3038	0.49	0.30, 0.81	0.005	49%	0.04	Random-effect model

NEC, necrotizing enterocolitis; RR, risk ratio; CI, confidence interve.

includes Bifidobacterium breve, Bifidobacteria infantis, Bifidobacterium bifidus, Bifidobacterium longum, as well as other probiotics such as Lactobacillus acidophilus, Lactobacillus casei, Saccharomyces boulardii, Enterococcus faecium, Streptococcus thermophilus, Lactobacillus rhamnosus. The dosage and duration ranged from 0.35×10^9 CFU once daily from first feed to 36 weeks corrected age to 1 g organisms daily, since first feed until death or discharge. In the study of Kitajima H et al., 1997 [3], Bifidobacterium breve was used with a dosage of 0.5×10^9 organisms once daily, from first feed for 28 days. Besides, Lin HC et al., 2005 used 125 mg/kg of Lactobacillus acidophilus and Bifidobacteria infantis organisms twice daily from day 7 until discharge [40]. The second limitation is the variation of gestational age or birth weight of preterm infants, for that the incidence of NEC or sepsis, death of preterm infants also is associated with gestational age or birth weight of preterm infants. In this meta-analysis, the majority of studies defined preterm infants as birth weight < 1500 g or gestational age < 37 weeks. However, in the study of Al-Hosni M et al., 2012 [27], their included preterm infants with their birth weight ranging from 501 g to 1000 g. Stratiki Z et al. [42] studied preterm infants with gestational age of 27–37 weeks. Finally, the incidence of NEC or sepsis, death of preterm infants were also influenced by other multiple factors, such as the health agency infants receiving therapy and hospitalization time, which should also be taken into consideration.

Our search strategy for this review was comprehensive, broad and systematic, with hand searching some references of included studies and previous systematic reviews. As considerable heterogeneity in the study design of the included trials, especially in the probiotic strains employed and daily dose of organisms administered, further more studies with single probiotic strains are needed. Besides, considering many studies designed with small sample size, further studies with sufficient sample size should be performed to demonstrate the efficacy and safety of bifidobacteria for preventing NEC in preterm infants. With further studies completed and available, more clinically convincing results may be drawn later.

5. Conclusion

This systematic review has shown that bifidobacteria may have a role in preventing NEC in preterm infants. Our meta-analysis has found that supplement of bifidobacteria could reduce the incidence of NEC Stage ≥ II (according to the Bell staging criteria) and death. And, what deserved to be mentioned is that the supplement of bifidobacteria does not increase the incidence of sepsis of preterm infants. On the basis of our analysis, bifidobacterium may have a beneficial effect and be safe in preventing NEC in preterm infants.

Abbreviations

NEC, necrotizing enterocolitis; RR, Risk ratio; CI, confidence intervals; RCT, randomized controlled trials; GA, gestational age; CFU, colony forming units; BW, birth weight; BB, Bifidobacterium breve Saccharomyces boulardii; BI, Bifidobacteria infantis; BBB, Bifidobacterium bifidus; LB-A, Lactobacillus acidophilus; LB-C, Lactobacillus casei; BB-LG, Bifidobacterium longum; CLD, chronic lung disease; ROP, retinopathy of prematurity; IVH, intraventricular hemorrhage.

Ethical approval

Ethical Approval is not applicable.

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Author contribution

The authors on this paper all participated in study design. All authors read, critiqued and approved the manuscript revisions as well as the final version of the manuscript. Also, all authors participated in a session to discuss the results and consider strategies for analysis and interpretation of the data before the final data analysis was performed and the manuscript written. All authors have the appropriate permissions and rights to the reported data.

Conflicts of interest

The authors declare no relevant conflict of interest.

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Consent for publication

Not applicable. Our manuscript contains none of individual person's data.

Availability of data and materials

Data and materials of this analysis were extracted from the original researches which were referenced in this article.

Provenance and peer review

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijsu.2018.11.026.

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