The role of probiotics in preventing necrotizing enterocolitis and reducing mortality in neonates: a meta-analysis

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Abstract

Background: Probiotics are gradually being used as a supplementation to prevent necrotizing enterocolitis (NEC) and reduce mortality in neonates. We performed an updated meta-analysis to systematically evaluate the efficacy and safety of prophylactic probiotic supplementation for preventing NEC. **Methods:** The databases including PubMed, EMBASE, and China National Knowledge Infrastructure were used to search the relevant articles. The latest retrieval date was up to May 2019. Two reviewers independently screened literature, extracted data, and assessed the risk of bias of the included studies. The meta-analysis was performed using Stata version 10.0. **Results:** Finally, a total of 74 studies containing – cases and – controls were included. The results showed that the probiotics could significantly reduce the incidence of NEC (stage II or more) (OR=0.435, 95% CI=0.357-0.530, $p<0.001$), the overall mortality (OR=0.630, 95% CI=0.491-0.808, $p<0.001$), and NEC-related mortality (OR=0.639, 95% CI=0.423-0.966, $p=0.034$). **Conclusion:** This meta-analysis indicates that the use of probiotics can effectively reduce the occurrence of NEC and mortality in neonates. These data provide the possible information for the clinical treatment.

Background

Necrotizing enterocolitis (NEC) is one of the potentially catastrophic illness of neonate with a high morbidity of up to 10% and a mortality of 20–30% in developed countries ^{1–3}. It usually has the clinically significant long- and short-term consequences. Nearly one-third to one-half of NEC patients require surgery ⁴. The infant recovering from NEC may have a 25% chance of microcephaly and serious neurodevelopmental delays ⁵. Additionally, the financial cost of NEC is substantial with the annual cost from \$500 million to \$1 billion in the United States ¹.

The etiology of NEC is not fully understood and various mechanisms have been considered to contribute to its development. Its incidence is relatively rare in unfed newborns. The risk factors include fetal distress and perinatal events after births, such as hypothermia, congenital heart disease, and sepsis ⁴. The main factors thought to be involved in the pathogenesis of NEC are intestinal immaturity, enteral feeds, the intestinal microbiome, inflammation, and local ischaemia and/or reperfusion injury ⁶.

Some dietary has been suggested to prevent the incidence of NEC. Lactoferrin, is found in human breast milk. As a glycoprotein, it can participate the formation of innate immune response and reduce the sepsis in infants after oral human lactoferrin ⁷. A randomized controlled trial (RCT) showed that bovine lactoferrin reduced the occurrence of NEC and the risk of death ⁸. Recently, the effect of probiotics on the prevention of NEC has attracted many attentions. A lot of RCTs and cohort studies have reported that the supplementation of probiotics can reduce the incidence of NEC ⁹. However, the inadequate sample size limits their value. There is still no sufficient evidence to recommend the routine administration of probiotics. Moreover, the optimum probiotic species or strains, duration of treatment, and its interaction with diet are also undetermined ⁹. Meta-analysis is a widely used statistical method in medical studies, particularly for a topic that are being extensively studied with controversial results¹⁰. Thus, we performed a meta-analysis of studies examining the role of probiotics in preventing NEC and decreasing mortality to provide a more comprehensive assessment.

Methods

Identification and eligibility of relevant studies

To identify studies eligible for inclusion in this meta-analysis, three online electronic databases (PubMed, EMBase, and China National Knowledge Infrastructure) were searched (the last search update was 2019 May). The following key words were used in the literature search: probiotics, necrotizing enterocolitis, necrotizing enterocolitis, or NEC. Reference lists from identified articles and potentially relevant review articles were also screened to identify additional studies. Studies met the following inclusion criteria: the intervention was the probiotic supplementation to the intervention group, the control was a control or placebo group that did not receive probiotic supplementation, and the outcomes included NEC (stage $\geq$ 2)¹¹, mortality, or NEC-related mortality. The participants included both of preterm and term neonates.

Data extraction

Based on the inclusion criteria, two reviewers (Yu-ping Pan and A-na Hou) independently extracted information from all eligible publications. Disagreements were resolved through discussion until the two reviewers reached a consensus. The following data were included from each study: first author's last name, publication year, country, probiotic strains, and number of NEC, mortality, or NEC-related mortality between cases and controls.

Statistical analysis

All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant. The meta-analysis was performed using Stata version 10.0 (Stata Corp., College Station, TX). The strength of the association between the supplementation of probiotics and NEC was measured by odd ratios (ORs) with 95% confidence intervals (CIs). Pooled effect sizes across studies were performed by a random effect model, which evaluates the likely effect size across different populations and takes heterogeneity across studies into account. This model is different from a fixed effects model, which evaluates the most likely effect size from multiple studies by hypothesizing that they are sampled from a single population, but the model can be biased by high heterogeneity across studies¹².

The degree of heterogeneity between studies was determined by Q-statistic^{13,14}. $P > 0.05$ for the Q-test indicated a lack of heterogeneity and $p < 0.05$ indicated heterogeneity. I^2 is the proportion of observed variance in effect sizes attributable to the true differences among studies. Conventional interpretations of I^2 define limits for low ($<25\%$), moderate ($\sim 50\%$), and high ($>75\%$) heterogeneity¹⁵.

An estimate of publication bias was performed by visual inspection of a funnel plot in which the standard error of log (OR) of each study was plotted against its log (OR). An asymmetric plot indicated possible publication bias, and the degree of asymmetry was tested using Egger's test ($p < 0.05$ indicated significant publication bias)¹⁶.

Sensitivity analysis was performed to assess the potential influences of a single study on the pooled effect size. It was performed by omitting single studies one at a time for each meta-analysis to screen for

significant alterations to pooled effect size.

Results

After the removal of overlapping articles and those that did not meet the inclusion criteria, a total of 68 articles were finally included ^{8,17–83}. Seventy three studies with 8472 cases and 9431 controls investigated probiotics for the prevention of NEC. Forty studies with 6410 cases and 7717 controls investigated probiotics for the reducing of mortality. Seventeen studies with 3004 cases and 3002 controls investigated probiotics for the reducing of NEC-related mortality. The key characteristics of the studies were showed in Table 1.

Quantitative synthesis and heterogeneity analysis

We analyzed 70 studies regarding the role of probiotics in preventing NEC after three studies were removed without the available calculated data (Table 2). The pooled results indicate that there was an association between probiotics and the occurrence of NEC under random effects model (OR = 0.435, 95% CI = 0.357–0.530, $p < 0.001$) (Fig. 1). The heterogeneity test showed that the degree of heterogeneity was statistically significant ($p = 0.006$; $I^2 = 32.5\%$).

A total of 38 studies explored the association between probiotics and incidence of mortality after removing two studies without the available calculated data (Table 2). The pooled results indicate that there was an association between probiotics and the risk of mortality under random effects model (OR = 0.630, 95% CI = 0.491–0.808, $p < 0.001$) (Fig. 2). The heterogeneity test showed that the degree of heterogeneity was statistically significant ($p < 0.001$; $I^2 = 52.0\%$).

There were 16 studies concerning the association of probiotics with the risk of NEC-related mortality after one study was removed without the available calculated data (Table 2). The meta-analysis showed that the supplementation of probiotics could decrease the incidence of NEC-related mortality under random effects model (OR = 0.639, 95% CI = 0.423–0.966, $p = 0.034$) (Fig. 3). The heterogeneity test showed that there was no significant heterogeneity ($p = 0.525$; $I^2 = 0.0\%$).

Cumulative meta-analysis

Cumulative meta-analysis was performed according to published date in chronological order using the random model. No continuous trend toward a significant association with a more narrowing 95% CI was presented as studies published by year.

Sensitivity analysis

Sensitivity analysis was performed for each meta-analysis to assess the influence of every single study. Corresponding pooled ORs showed no significant change when one study was removed at a time from each meta-analysis, indicating that these results are stable and reliable.

Publication bias

The funnel plots were generated to assess potential publication bias (Fig. 4–6). Egger's tests were used to supply statistical evidence for funnel plot symmetry. The results showed that there was publication bias existing in the studies about NEC incidence and overall mortality ($p < 0.001$ and $p = 0.023$, respectively), while the result of NEC-related mortality did not show any evidence of publication bias ($p = 0.621$).

Discussion

The present meta-analysis included 73 studies to explore the association between probiotics and the incidence of NEC, overall mortality, and NEC-related mortality. The final pooled results showed that the supplementation of probiotic bacteria could effectively prevent the occurrence of NEC, overall mortality, and NEC-related mortality. Cumulative meta-analysis and sensitivity analysis strengthened the validity of the results. There were significant heterogeneity in the meta-analysis of the role of probiotics in the incidence of NEC and overall mortality. It may be due to the differences in species, dose and time of administration, complications, *et al*⁸⁴. The results need to be interpreted cautiously. Additionally, there are many reviews and meta-analysis to investigate the association between probiotic supplementation and the occurrence of NEC and mortality. To some extent, our present meta-analysis seems superfluous and unnecessary. However, after screening the databases in English and Chinese, our pooled analysis included a total of 73 studies with 8472 cases and 9431 controls investigating probiotics for the prevention of NEC, 40 studies with 6410 cases and 7717 controls investigating probiotics for the reducing of mortality, and 17 studies with 3004 cases and 3002 controls investigating probiotics for the reducing of NEC-related mortality. The results with the large sample size can provide more reliable reference for the future study.

Previously, several meta-analysis reported the association between probiotics and NEC. Zhu *et al.* found that bifidobacterium may benefit the prevention of NEC in preterm infant⁸⁵. Thomas *et al.* reported that the usage of probiotics could reduce the incidence of NEC and mortality in very low-birth-weight infants⁸⁶. The similar results were found in very preterm infants^{84,87}. Thus, our present meta-analysis included both of preterm and term neonates.

The mechanism by which probiotics reduce the incidence of NEC may be to prevent the growth of bacteria and the production of bacterial toxin by cleaning intestinal bacteria⁸⁸. The recent study has reported that inflammation is one of the most important factors in the process of NEC. The activation of Toll-like receptor 4 (TLR4) inflammatory signaling pathway can give rise to the excessive inflammatory response⁸⁹. Moreover, another study reported that the 72-h survival rate of experimental NEC in the formula-fed group was 56.3%, but it increased to 86.7% after administrating *Bifidobacterium adolescentis*. The improvement might be related to the alteration of TLR4 expression⁹⁰. In addition, *Bifidobacterium* can inhibit the secretion of proinflammatory cytokine to enhance intestinal barrier function⁹¹. The administration of *Bifidobacterium* significantly reduced the occurrence and development of NEC or mortality⁸⁵. However, the suitable strain types, dosage, and forms (i.e., breast milk, preterm formula, and formula) are still undermined. A randomised controlled phase 3 trial did not support the routine use of probiotic *Bifidobacterium breve* BBG-001 for prevention of NEC, suggesting that this bifidobacterium alone was not the optimal agent to use⁵⁹. Another study showed that a small dosage of probiotics ($<10^9$ CFU/d) significantly reduced the incidence of NEC and

mortality and shorten hospital stay ⁸⁷, while the other study suggested that probiotics were more effective when taken in breast milk or breast milk plus formula form, at a dosage of $<10^9$ CFU/d, and containing multiple strains ⁸⁷. However, a randomized clinical trial reported that the incidence of NEC was similar between single strain group and multispecies probiotic group (0% vs. 2.2%) ⁹². Presently, no sufficient evidence recommended the optimal administration of probiotics in the clinical prevention of NEC. More studies are needed to explore the effects of probiotic in the risk of NEC and death.

There are several potential limitations to the present study. First, there was significant heterogeneity in the analyses. The pooled results need to be interpreted cautiously. There are other unknown aspects partially contributing to heterogeneity. Second, due to the lack of sufficient sample size, we did not performed the subgroup by types of probiotic strain and feeding to analyze the effects of bacterium in detail. Finally, we did not consider the impacts of neonatal birth status, weight and other complications on the outcomes.

Conclusion

In summary, our findings showed that the supplementation of probiotic bacteria could effectively prevent the occurrence of NEC, overall mortality, and NEC-related mortality. Cumulative meta-analysis and sensitivity analysis strengthened the validity of the results. More relevant studies are needed to validate our finding and provide the reference for clinical prevention and treatment of NEC.

Abbreviations

NEC: Necrotizing enterocolitis; *RCT*: randomized controlled trial; *ORs*: Odds ratios; *CI*s: confidence interval.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YQP participated in the design of the study and performed the statistical analysis. ANH and XDX exacted the key information and drafted the manuscript. JHF conceived the study and participated in its design and coordination. All authors contributed toward the drafting and revising of the final manuscript.

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Not applicable

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Tables

Table 1. Baseline characteristics of qualified studies in this meta-analysis.

Author	Year	Country	Probiotic composition	NEC stage ≥ 2 in probiotic group, (%)	NEC stage ≥ 2 in control group, (%)	All cause mortality in probiotic group, (%)	All-cause mortality in control group, (%)	NEC-related mortality in probiotic group, (%)	NEC-related mortality in control group, (%)
Dani	2002	Italy	LRh	4/295	8/290	0/295	2/290	2/295	0/290
Costalos	2003	Greece	SB	5/51	6/36				
Bin-Nun	2005	Israel	Bbi, BI, ST	1/72	10/73	3/72	8/73	0/72	3/73
Lin	2005	China	LA, BI	2/180	10/187	7/180	20/187		
Manzoni	2006	Italy	LRh	1/39	3/41	5/39	6/41		
Stratiki	2007	Ireland	BLa	0/41	3/34				
Samanta	2008	India	BI, BBi, BLo, LA	5/91	15/95	4/91	14/95		
Lin	2008	Taiwan	BBi, LA	4/217	14/217	2/217	9/217	2/217	3/217
Rouge	2009	Spain	LRh, BLo	2/45	1/49	4/49	2/45		
Samanta	2009	UK	BI, LA	5/91	15/95	4/91	14/95		
Manzoni	2009	Italy	LRh	0/151	3/153	6/151	4/153		
Mark	2009	US	LA, BLo, BLa	1/31	2/29				
Underwood	2009	US	LA	1/30	1/29				
Underwood	2009	US	LA, BLa	1/31	1/29				
Mihatsch	2010	Germany	BLa	2/91	4/89	2/91	1/89	1/91	0/89
Chou	2010	US	BLa, LA	0/153	2/148	8/153	24/148		
Hu	2010	China	BLa, LRb, ST	0/30	1/30				
Hikaru	2010	US	BLa			0/108	4/100		
Deng	2010	China	BLa, LA, ETf	1/62	8/62				
Sari	2011	Turkey	LS	6/110	10/111	3/110	4/111	0/110	1/111
Braga	2011	Brazil	LC, BBr	0/119	4/112	26/119	27/112		
Al-Hosni	2012	US	LRh, BI	2/50	2/51	3/50	4/50		
Rojas	2012	Columbia	LRe	6/176	10/194				
Zhou	2012	China	BLa, LA, ET, BC	3/75	7/50				
Jacobs	2013	Australia	BI, ST, BLa	11/548	24/551	27/548	28/551	4/548	11/551
Demirel	2013	Turkey	SB	6/135	7/136	5/135	5/136		
Serce	2013	Turkey	SB	7/104	7/104	5/104	4/104	3/104	3/104
Fernandez-Carrocer	2013	Mexico	LA, LRh, LC, LP, BI, ST	6/75	12/75	1/75	7/75		
Wang	2013	China	BLa, LA	1/120	17/120	16/120	0/120	5/120	0/120
Liao	2013	China	BLo, LA, ETf	0/61	3/61				
Li	2013	China	BLa	0/15	2/15				
Wu	2013	China	BLa, LRh	3/45	12/52	2/45	4/52	0/45	3/52
Lin	2013	China	BS, ETf	0/65	4/55				
Lu	2013	China	NS	2/84	9/84				
Totsu	2014	Japan	BBi	0/153	0/130	0/130	2/153		
Patole	2014	Australia	BBr	0/77	1/76	0/76	0/77		
Oncel	2014	Turkey	LRe	8/200	10/200	15/200	15/200	3/200	4/200
Saengtawesin	2014	Thailand	BBi, LA	1/31	1/29	0/29	0/31	0/29	0/31
Zeng	2014	China	BLa, LA, ETf	2/65	9/63				
Li	2014	China	BLa, LA, ETf	2/46	10/46			1/46	4/46
Manzoni	2014	Ireland	LRh	0/238	5/247	18/238	15/247	9/238	10/247
Liu	2014	China	BLa, LA, ETf	1/45	5/45				
Mao	2014	China	BS, ETf	1/44	6/44				
Zhang	2014	China	CB, BLa	1/115	5/115			0/115	1/115

Dilli	2015	Turkey	BLa	2/100	18/100	3/100	12/100		
Hays	2015	France	BLa, BLo	8/145	3/52	1/52	5/145		
Kanic	2015	Germany	Bla, LA, ETf	0/40	5/40	2/40	3/40		
Van Niekerk	2015	UK	LRh, BI	1/54	4/56	2/54	5/56	0/54	3/56
Costeloe	2016	UK	BBr	61/650	66/660	54/650	56/660	9/650	14/660
Bian	2016	China	BLa	0/20	1/19				
Costeloe	2016	England	BBr	47/724	58/462	24/724	33/462		
hays	2016	France	BLa	2/50	3/52				
hays	2016	France	BLo	1/48	3/52				
hays	2016	France	BLa, BLo	5/47	3/52				
Xu	2016	China	CD	0/51	0/49				
Chowdhury	2016	UK	BLa, LA	1/52	6/50				
Wang	2016	China	BS, ET	2/70	10/70				
Mo	2017	China	BLa	0/21	0/20				
Güney-Varal	2017	Turkey	LRh, LC, LP, BBa	0/70	4/40	1/70	9/40	0/70	3/40
Uberos	2017	Spain	LRh	2/53	16/175	2/33	34/175		
Uberos	2017	Spain	BBi, LA	2/33	16/175	0/33	34/175		
Meyer	2017	New Zealand	LA and lactoferrin	4/396	15/439	39/396	36/439		
Li	2017	China	BLa, LA, ETf	1/10	3/10				
Xu	2017	China	BLa, LRb, ST	4/90	9/90				
Singh	2017	Austria	LRh	6/37	10/35				
Nie	2017	China	BLa, LA, ETf, BC	1/57	7/57	0/57	4/57		
Wen	2017	China	BLa, LRb, ST	1/35	7/35				
Ding	2017	China	BLa, LRb, ST	1/31	5/39	0/31	2/39		
Deng	2018	China	BLa, LA, ETf	2/102	10/100				
Shi	2018	China	CB	3/48	9/42				
Xu	2018	China	BS, ETf	9/247	15/191				
Kane	2018	US	LRh	33/197	45/443	13/197	17/443		
Sharpe	2018	Australia	NS	7/457	37/1334	11/457	101/1334		
Zhang	2018	China	BS, ETf	1/40	3/40				

Note: LA, *Lactobacillus acidophilus*; BLa, *Bifidobacterium lactis*; BI, *Bifidobacterium infantis*; BBr, *Bifidobacterium breve*; BBi, *Bifidobacterium bifidum*; BBa, *Bifidobacterium animalis*; BLo, *Bifidobacterium longum*; LRb, *Lactobacillus bulgaricus*; LRh, *Lactobacillus rhamnosus*; ST, *Streptococcus thermophiles*; LRe, *Lactobacillus reuteri*; LC, *Lactobacillus casei*; ET, *Enterococcus*; ETf, *Enterococcus faecalis*; BS, *Bacillus subtilis*; CB, *Clostridium butyricum*; BC, *Bacillus cereus*; CB, *Clostridium butyricum*; CD, *Clostridium difficile*; SB, *Saccharomyces boulardii*; NS, Not stated in original study.

Table 2. Summarized RRs with 95% CIs for the association between probiotics and NEC, overall mortality, and NEC-related mortality.

Pooled analysis	n	RR	95% CI	p _z	I ² (%)	p _h
NEC incidence	70	0.435	0.357-0.530	<0.001	32.5	0.006
All cause mortality	38	0.630	0.491-0.808	<0.001	52.0	<0.001
NEC-related mortality	16	0.639	0.423-0.966	0.034	0.0	0.525

Note: n, the number of studies; p_z , p value for association test; p_h , p value for heterogeneity test.

Figures

Figure 1

Forest plot of the association between probiotics and NEC under random model.

Figure 2

Forest plot of the association between probiotics and overall mortality under random model.

Figure 3

Forest plot of the association between probiotics and NEC-related mortality under random model.

Figure 4

Funnel plot analysis on the detection of publication bias in the association between probiotics and NEC.

Figure 5

Funnel plot analysis on the detection of publication bias in the association between probiotics and overall mortality.

Figure 6

Funnel plot analysis on the detection of publication bias in the association between probiotics and NEC-related mortality.

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