Systematic Review and Meta-Analysis

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Preventing necrotizing enterocolitis by food additives in neonates

A network meta-analysis revealing the efficacy and safety

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Abstract

Background: Necrotizing enterocolitis (NEC) is a serious multifactorial gastrointestinal disease which is often discovered in premature infants. Various additives have been used to prevent NEC; yet, their relative efficacy and safety remain disputed. This study aims to compare the efficacy and safety of 5 food additives, namely, probiotics, probiotics + fructo-oligosaccharides, pentoxifylline, arginine, and lactoferrin in preventing NEC in neonates.

Methods: Embase, PubMed, and Cochrane Library had been searched for all eligible randomized control trials. Odds ratios (ORs) were estimated for dichotomous data and mean differences with 95% credible intervals (CrIs) were estimated for continuous data. Surface under the cumulative ranking curve was used to rank efficacy and safety of the prevention methods on each endpoint.

Results: A total of 27 eligible studies with 4649 preterm infants were included in this network meta-analysis (NMA), and the efficacy and safety of 5 food additives were evaluated. Probiotic and arginine exhibited better preventive efficacy compared with placebo (OR = 0.50, 95% CrIs: 0.32–0.73; OR = 0.30, 95% CrIs: 0.12–0.73, respectively). Only probiotic achieved a considerable decrease in the risk of mortality compared to placebo (OR = 0.68, 95% CrIs: 0.46–0.98). NEC patients with lactoferrin appeared to have lower incidence of sepsis than those of placebo (OR = 0.13, 95% CrIs: 0.03–0.61) or probiotic (OR = 0.18, 95% CrIs: 0.03–0.83).

Conclusion: Based on this NMA, probiotics had the potential to be the most preferable additive, since it exhibited a significant superiority for NEC and mortality as well as a relatively balanced performance in safety.

Abbreviations: CrI = credible interval, NEC = necrotizing enterocolitis, NMA = network meta-analysis, OR = odds ratio, RCT = randomized control trial, SUCRA = surface under the cumulative ranking curve, VLBW = very low birth weight.

Keywords: efficacy, food additives, necrotizing enterocolitis, network meta-analysis, premature infants

1. Introduction

Necrotizing enterocolitis (NEC) is a severe multifactorial gastrointestinal disease discovered in premature infants. Intensive care, surgery intervention, and potent antimicrobial agents have been introduced in order to reduce its morbidity and mortality.[1,2] Over 85% of the NEC cases occur in newborns with very low birth weight (VLBW, birth weight <1500 g).[3] The average prevalence rate of NEC in VLBW preterm infants is approximately 7%, and 20% to 30% VLBW infants with NEC eventually experience fatal outcomes.[4,5] Besides that, NEC may cause long-term adverse effects on infants, including short bowel syndrome, intestinal stricture, and neuro-developmental retardation.[6] Moreover, there are several risk factors linked with NEC as well, including pathologic bacteria, gastrointestinal immaturity, excessive protein substrate in the intestinal cavity, and enteral feeding (especially formula feeding).

Probiotics are believed to be particularly beneficial to preterm infants, because microorganisms are able to regulate immune response, host metabolism, and produce antimicrobial substances.[2] Moreover, some microorganisms can reduce the potential growth of pathogenic bacteria, enhance antibiotic activities, increase the barrier function of the intestinal barrier, and promote the production of anti-inflammatory cytokines.[7] Besides, both lactoferrin and L-arginine are resistant to a wide range of antibiotics, which can help prevent intestinal infection.[8,9] Some studies also suggested that lactoferrin was able to prevent NEC. There were articles which studied immunoglobulin as an intervention for prevention of neonatal NEC, but nowadays, it seems to be replaced.[10,11] However, the current literature has not conclusively recommended an optimum prevention treatment for NEC in premature infants.

Editor: Jing Liu.

WY and WS are first co-authors.

Ethics statement: There work involves no human related experiments, patient consent, and ethical approval is not necessary.

The authors have no funding and conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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Medicine (2017) 96:21(e6652)

Received: 13 November 2016 / Received in final form: 27 March 2017 / Accepted: 29 March 2017

http://dx.doi.org/10.1097/MD.0000000000006652

Since there is an increasing demand for reviewing and disclosing the relative efficacy and safety of the above therapies, a thorough review and network meta-analysis (NMA) may help clinicians achieve the objectives. Therefore, we designed this study in order to discover whether these additives exhibited equivalent efficacy and safety with respect to NEC prevention in neonatology. A total of 6 additives were researched in the current literature, including probiotics, pentoxifylline, lactoferrin, probiotics + fructo-oligosaccharides, and arginine. The relative efficacy and safety of the above therapies were evaluated by using the following endpoints: NEC incidence, all-cause mortality, NEC related mortality, sepsis, and hospitalization days.

2. Material and methods

2.1. Study design and selection strategy

Embase, PubMed, and Cochrane Library had been searched for all eligible randomized control trials (RCTs). Besides that, trial databases of the main regulatory agencies were also searched to identify relevant studies published before June 5, 2016. The following key terms and their synonyms were used to find relevant studies: "necrotizing enterocolitis," "infants," "newborn," "probiotics," "anti-bacterial agents," "pentoxifylline," "laparotomy," "arginine," "lactoferrin," "fructo-oligose," and "randomized control trials."

2.2. Inclusion criteria

We have considered a large scale of studies and all the relevant researches have to meet the following conditions for inclusion: all the trials should be designed as RCT; research subjects in our study must be newborns; all trials should include at least one of the following endpoint: NEC incidence, all-cause mortality, mortality related to NEC, sepsis, and hospitalization days; primary trials should contain enough information or data for NMA.

2.3. Exclusion criteria

Studies should be eliminated if they had any of the following situations: studies focusing on feeding rate or studies without additives; duplicated studies from the same cohort; meeting abstract, meta-analysis, and case reports.

2.4. Outcome measures and data extraction

In our study, NEC incidence was considered as the primary outcome since it was investigated by the majority of trials. Secondary outcomes included all-cause mortality, sepsis, NEC-related mortality and hospitalization days. Two investigators extracted the corresponding data independently. Once there were disagreements, a further discussion was implemented. We extracted the following data from eligible studies: the first author's name, publication year, study design, sample size, intervention method of addictive, gestational age, birth weight, delivery pattern, Apgar score, as well as necessary data about 5 outcomes. The statistics of outcomes, including NEC, all-cause mortality, NEC-related mortality, sepsis, and hospitalization were also extracted from eligible studies. The Jadad scale system was used to assess the risk of bias in included studies.

2.5. Statistical analysis

A Bayesian NMA was performed to obtain estimates for primary and secondary outcomes in order to compare their efficacy and safety for preventing NEC. STATA version 13.1 (Stata Corp, College Station, TX) and WinBUGS 1.4.3 (MRC Bio-statistics Unit, Cambridge, UK) were used to perform statistical analysis. For continuous outcomes, mean differences with their 95% credible intervals (CrIs) were estimated. For dichotomous outcomes, odds ratios (ORs) with their 95% CrIs were calculated. Surface under the cumulative ranking curve (SUCRA) was applied to rank all of the above therapies with respect to each endpoint. The larger the SUCRA value was, the better the performance of treatment presented. Finally, potential publication bias was assessed by comparison-adjusted funnel plots.

3. Results

3.1. Study selection

The process of study selection is displayed in Fig. 1. First, a total of 1531 records were identified using the searching strategy as mentioned, among which 563 came from PubMed and 968 were retrieved from Embase. Then 1184 studies were removed after reviewing the titles and abstracts and another 320 studies were also excluded because of insufficient information or irrelevant comparisons. Finally, we included 27 RCTs which were subject to full-text review and data extraction.[12–38] All the 27 eligible studies were published between 1999 and 2016.

3.2. Characteristics of included studies

The baseline characteristics of RCTs included in the NMA are summarized in Table 1. A total of 4649 preterm infants from 27 studies were involved (the sample size of trials ranged from 37 to 585 participants), and the outcome of NEC incidence was assessed by all included studies. The majority of included studies were designed as double blinding RCTs while only 1 study was single-blinded.[25] All of the included studies compared study additive with placebo in order to determine their relative efficacy or safety. The network structure of evidence with respect to each endpoint can be illustrated in Fig. 2. Moreover, the quality of included studies was overall medium-high using the Jadad Scale

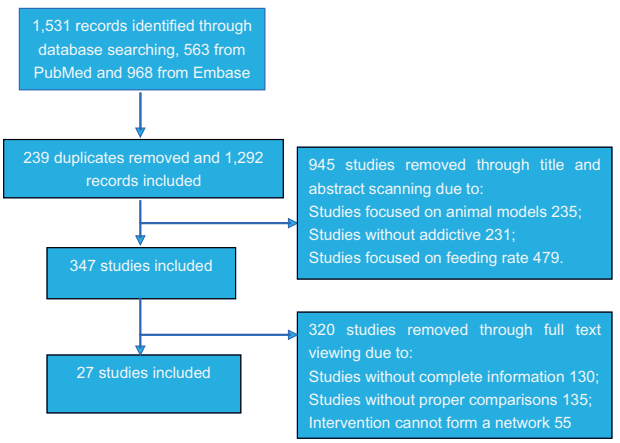

Figure 1. Flowchart of study selection.

Table 1

Characteristics of included studies.

Author	Design	Group	Type & dose	Start time & duration	Size (n)	Male, %	Cesarean	Gestational age, wk	Birth weight, g	Feeding, %	Milk source
Sherman (2016)	RCT, DB	Lactoferrin	Talactoferrin; 300 mg/kg/d	Within 24 h of birth; 28 d	60	55	49	28 ± 7	1152 ± 206	H/F	M
		PCB	Placebo		60	60	48	28 ± 7	1143 ± 220	H/F	M
Shabaan (2015)	RCT, DB	Pentoxifylline	Pentoxifylline; intravenously 5 mg/kg/h for 6 h	Simultaneously with the first dose of antibiotics; 6 successive days	60	57	32	30.2 ± 2.5	1404 ± 417	–	–
		PCB	Placebo		60	73	32	30.1 ± 2.2	1370 ± 471	–	–
Totsu (2014)	RCT, DB	Probiotic	Bifidobacterium bifidum OLB6378; 500 mg, bid	Within 48 h after birth; until the bodyweight reached 2000 g	153	57	91	29 ± 3	1016 ± 289	H/F	NS
		PCB	Placebo		130	55	103	28 ± 3	998 ± 281	H/F	NS
Patole (2014)	RCT, DB	Probiotic	Bifidobacterium breve M-16V; 1.5 × 10^9 CFU/d to 3 × 10^9 CFU/d	First feeding; until the corrected age 37 wk	77	58	58	29 ± 3	1090 ± 170	H (99)/F (1)	M/D
		PCB	Placebo		76	54	49	28 ± 2	1025 ± 135	H	M/D
Oncel (2014)	RCT, DB	Probiotic	Lyophilized L reuteri; 100 million CFU/d (5 drops)	First feeding; until discharge	200	54	163	28 ± 2	1071 ± 274	H (17)/F (16)/Mixed (67)	M
		PCB	Placebo		200	49	152	27 ± 3	1048 ± 298	H (13)/F (11)/Mixed (76)	M
Manzoni (2014)	RCT, DB	Lactoferrin	Bovine lactoferrin; 100 mg/d	In the first 48 h; until day 30 (45 for neonates <1000 g at birth)	247	52	194	29.7 ± 2.5	1158 ± 251	H (28)/F (19)/Mixed (54)	M
		PCB	Placebo		258	52	196	29.4 ± 3.1	1118 ± 259	H (31)/F (17)/Mixed (52)	M
Benor (2014)	RCT, DB	Probiotic	Lactobacillus acidophilus + bifidobacteria lactis; 2 × 10^10 CFU/d	At 0 to 72 h after birth; until the infant was discharged home	25	62	–	24.5 ± 2.7	1105 ± 267	H/F	M
		PCB	Placebo		33	36	–	29.5 ± 2.6	1080 ± 237	H/F	M
Akin (2014)	RCT, DB	Lactoferrin	Oral lactoferrin; 200 mg/d	Within the first 72 h of life; until death or discharge	25	48	22	29.5 ± 1.6	1307 ± 262	H (64)/Mixed (36)	M
		PCB	Placebo		25	44	23	30.3 ± 2.5	1290 ± 347	H (52)/Mixed (48)	M
Akdag (2014)	RCT, DB	Pentoxifylline	Pentoxifylline; 6 mg/kg/h IV, over 4 h/d	Three consecutive days	51	57	38	31 ± 5	1490 ± 990	–	–
		PCB	Placebo		51	65	35	31 ± 4	1410 ± 900	–	–
Serce (2013)	RCT, DB	Probiotic	Saccharomyces boulardii; 50 mg/kg every 12 h	First feeding; until discharged	104	51	84	28.8 ± 2.2	1126 ± 232	H/F	NS
		PCB	Placebo		104	56	92	28.7 ± 2.1	1162 ± 216	H/F	NS
Polycarpou (2013)	RCT, DB	Arginine	L-Arginine supplementation; 1.5 mmol/kg/d	The 3rd day of life; until the 28th day of life	40	42	30	29.2 ± 0.37	1168 ± 109	H (18)/F (82)	M
		PCB	Placebo		43	44	32	28.8 ± 0.44	1127 ± 118	H (12)/F (88)	M
Fernández-Carrocera (2013)	RCT, DB	Probiotic	Lactobacillus acidophilus + lactobacillus rhamnosus + lactobacillus casei + lactobacillus plantarum + bifidobacteruim infantis + streptococcus thermophillus; 1 g/d	First feeding	75	–	–	31.2 ± 2.8	1090 ± 239	H (21)/Mixed (79)	M
		PCB	Placebo		75	–	–	31 ± 2.5	1170 ± 238	H (15)/Mixed (85)	M
Al-Hosni (2012)	RCT, DB	Probiotic	Lactobacillus rhamnosus GG + Bifidobacterium infantis; 500 million CFU and 500 million CFU qd	First feeding; until discharge or until 34 wk postmenstrual age	50	44	22	25.7 ± 1.4	778 ± 138	H	NS
		PCB	Placebo		51	55	30	25.7 ± 1.4	779 ± 126	H	NS
Sari (2011)	RCT, SB	Probiotic	Lactobacillus sporogenes; 350,000,000 CFU/d	First feeding; until discharged	110	54	74	29.5 ± 2.4	1231 ± 262	H (42)/Mixed (58)	NS
		PCB	Placebo		111	56	84	29.7 ± 2.4	1278 ± 282	H (54)/Mixed (46)	NS

(*continued*)

Table 1

(continued).

Author	Design	Group	Type & dose	Start time & duration	Size (n)	Male, %	Cesarean	Gestational age, wk	Birth weight, g	Feeding, %	Milk source
Braga (2011)	RCT, DB	Probiotic	Lactobacillus casei + Bifidobacterium breve; 3.5 × 10^7–3.5 × 10^9 CFU/d	The second day of life; until 30 d of life	119	49	64	29.5 ± 2.5	1195 ± 206	H	M/D
		PCB	Placebo		112	49	55	29.2 ± 2.6	1151 ± 225	H	M/D
Mihatsch (2010)	RCT, DB	Probiotic	Bifidobacterium lactis;12 billion CFU/kg/d	Within the first hours of life; for the first 6 wk of life	91	60	64	26.6 ± 1.8	856 ± 251	H (18)/F (62)/Mixed (21)	M
		PCB	Placebo		89	53	61	24.1 ± 2.3	871 ± 287	H (17)/F (73)/Mixed (10)	M
Awad (2010)	RCT, DB	Probiotic	Living *Lactobacillus* acidophilus; 6 × 10^9 CFU bid	Day 1 of age; until discharge	60	52	30	31.1 ± 3.7	2280 ± 800	H (7)/F (7)/Mixed (86)	NS
		Probiotic	Killed *Lactobacillus acidophilus*; 6 × 10^9 CFU bid	Day 1 of age; until discharge	60	53	38	34.9 ± 3.5	2260 ± 980	H (17)/F (7)/Mixed (76)	NS
		PCB	Placebo		30	40	20	36.0 ± 2.9	2660 ± 890	H (22)/F (7)/Mixed (70)	NS
Adel (2010)	RCT, DB	Pentoxifylline	Pentoxifylline; 5 mg/kg/h for 6 h	For 6 successive days	17	53	–	35.9 ± 4.0	2470 ± 890	–	–
		PCB	Placebo		20	55	–	36.0 ± 3.1	2210 ± 590	–	–
Underwood (2009)	RCT, DB	Probiotic + FOS	*Lactobacillus* + inulin; 5 × 10^8 organisms	For 28 d or until hospital discharge	30	70	–	29.5 ± 2.6	1394 ± 356	H (69)/F (31)	NS
		Probiotic + FOS	lactobacillus + *B. longum* + B. bifidum + B. infantis + inulin; 5 × 10^8 organisms	For 29 days or until hospital discharge	31	61	–	30.2 ± 2.4	1461 ± 372	H (67)/F (33)	NS
		PCB	Placebo		29	66	–	29.3 ± 2.6	1393 ± 363	H (61)/F (39)	NS
Samanta (2009)	RCT, DB	Probiotic	Bifidobacteria infantis + bifidobacteria bifidum + bifidobacteria longum + lactobacillus acidophilus; each 2.5 billion CFU bid	First feeding; until discharge	91	–	42	30.1 ± 1.6	1172 ± 143	H	NS
		PCB	Placebo		95	–	47	30.1 ± 1.6	1210 ± 143	H	NS
Rouge (2009)	RCT, DB	Probiotic	Bifidobacterium longum BB536 + Lactobacillus rhamnosus GG; 10^8 lyophilized cells per unit	First feeding; until discharge	45	62	28	28.1 ± 1.9	1115 ± 251	H	M/D
		PCB	Placebo		49	53	35	28.1 ± 1.8	1057 ± 260	H	M/D
Manzoni (2006)	RCT, DB	Probiotic	Lactobacillus casei subspecies rhamnosus; 6 × 10^9 CFU/d	First 3 d; for 6 wk or until discharge	39	51	–	29.6 ± 5	1212 ± 290	H	M/D
		PCB	Placebo		41	51	–	29.3 ± 4	1174 ± 340	H	M/D
Bin-Nun (2005)	RCT, DB	Probiotic	Bifidobacteria infantis + streptococcus thermophilus + bifidobacteria bifidus; 10^9 CFU/d	On similar days of life; until 36 wk postconceptual age	72	61	–	29.8 ± 2.6	1115 ± 262	H (58)/F (25)/Mixed (17)	M
		PCB	Placebo		73	51	–	29.3 ± 4.3	1111 ± 278	H (64)/F (23)/Mixed (12)	M
Costalos (2003)	RCT	Probiotic	Saccharomyces boulardii; 50 mg/kg every 12 h	First feeding; 30 d	51	47	–	31.1 ± 0.8	1651 ± 681	H	NS
		PCB	Placebo		36	64	–	31.8 ± 0.8	1644 ± 408	H	NS
Dani (2002)	RCT, DB	Probiotic	Lactobacillus GG; 6 × 10^9 CFU/d	First feeding; until discharge	295	46	225	30.8 ± 2.4	1325 ± 361	H (63)/F (36)/Mixed (1)	M/D
		PCB	Placebo		290	52	239	30.7 ± 2.3	1345 ± 384	H (64)/F (34)/Mixed (2)	M/D
Amin (2002)	RCT, DB	Arginine	L-Arginine; 1.5 mmol/kg/d	First 3 d of life; 28 d	75	61	–	27.4 ± 0.3	952 ± 25	H/F	M
		PCB	Placebo		77	56	–	27.6 ± 0.2	955 ± 20	H/F	M
Lauterbach (1999)	RCT, DB	Pentoxifylline	Pentoxifylline; 5 mg/kg/h for 6 h	6 successive days	40	58	–	31.6 ± 2.9	1690 ± 396	–	–
		PCB	Placebo		38	53	–	32.8 ± 3.0	1749 ± 476	–	–

For design column, DB = double-blinded, RCT = randomized controlled trial, SB = single-blinded. For feeding column, F = formula, H = human milk. For milk source column, D = donor, M = mother, NS = not specified.

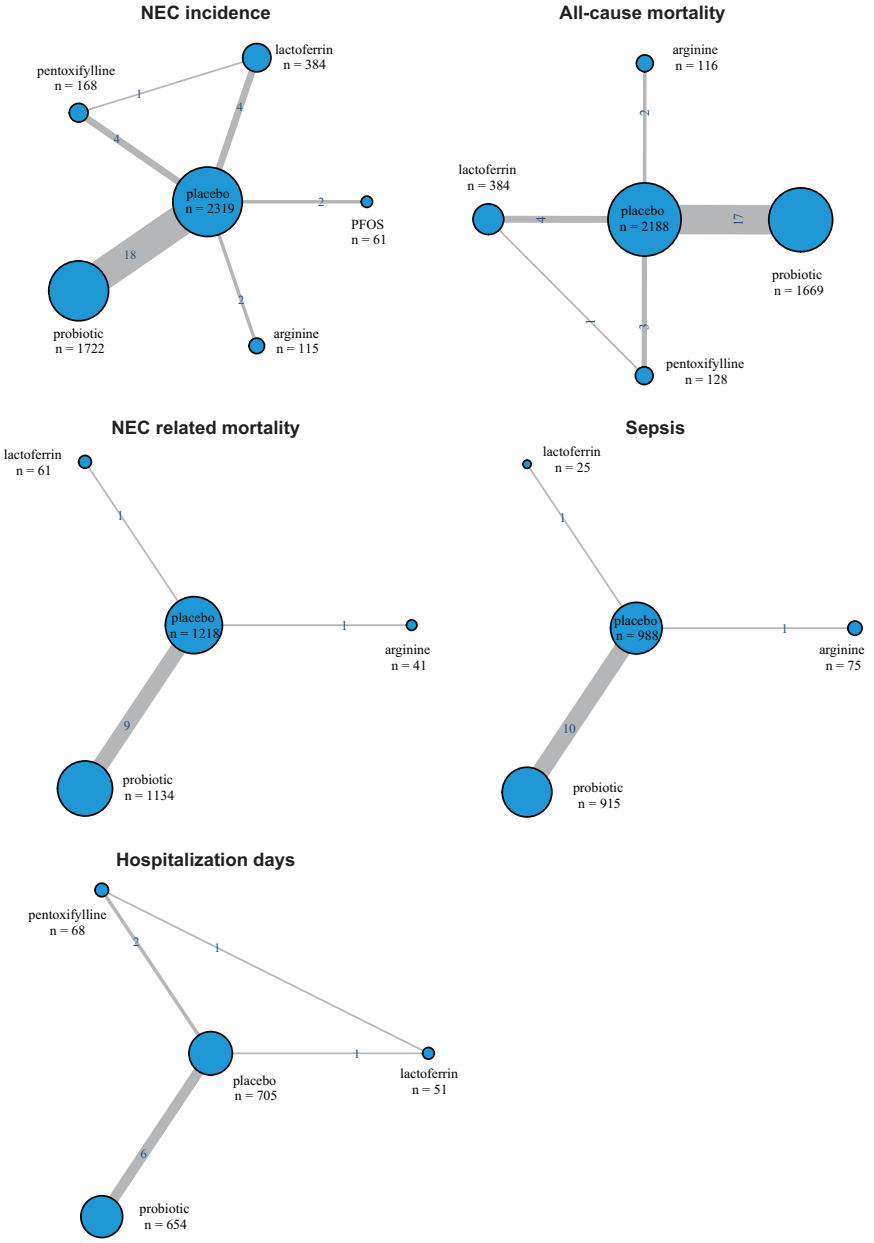

Figure 2. Network plot of randomized controlled trials comparing different addictive agents for necrotizing enterocolitis prevention. The width of the lines is proportional to the number of trials comparing each pair of treatments with numbers on the lines illustrating the exact number. The size of circles represents the cumulative number of patients for each intervention.

that incorporates whether the design of each study used any appropriate randomization techniques, whether an appropriate blinding procedure was introduced, and whether the study disclosed any information about withdrawals.

3.3. Network meta-analysis results for NEC incidence and mortality

As shown in Table 2 and Fig. 3, premature infants fed with probiotics or arginine exhibited significantly lower risk of NEC

Table 2

Network meta-analysis results for NEC incidence and all-cause mortality.

Mortality	Placebo	**0.50 (0.32, 0.73)**	0.59 (0.23, 1.51)	0.45 (0.19, 1.04)	0.98 (0.08, 11.25)	0.30 (0.12, 0.73)	NEC
	0.68 (0.46, 0.98)	Probiotic	1.22 (0.44, 3.42)	0.93 (0.37, 2.36)	2.05 (0.17, 23.81)	0.63 (0.23, 1.68)	
	0.84 (0.35, 1.92)	1.23 (0.48, 3.10)	Pentoxifylline	0.75 (0.23, 2.51)	1.67 (0.13, 20.91)	0.51 (0.14, 1.90)	
	0.52 (0.23, 1.23)	0.76 (0.32, 1.97)	0.63 (0.21, 1.93)	Lactoferrin	2.14 (0.16, 29.67)	0.68 (0.19, 2.29)	
	–	–	–	–	Probiotic + FOS	0.31 (0.02, 4.26)	
	1.60 (0.55, 5.21)	2.36 (0.76, 8.08)	1.93 (0.50, 8.25)	3.10 (0.78, 12.68)	–	Arginine	

FOS = fructo-oligosaccharides, NEC = necrotizing enterocolitis.
Bold type indicates significant values.

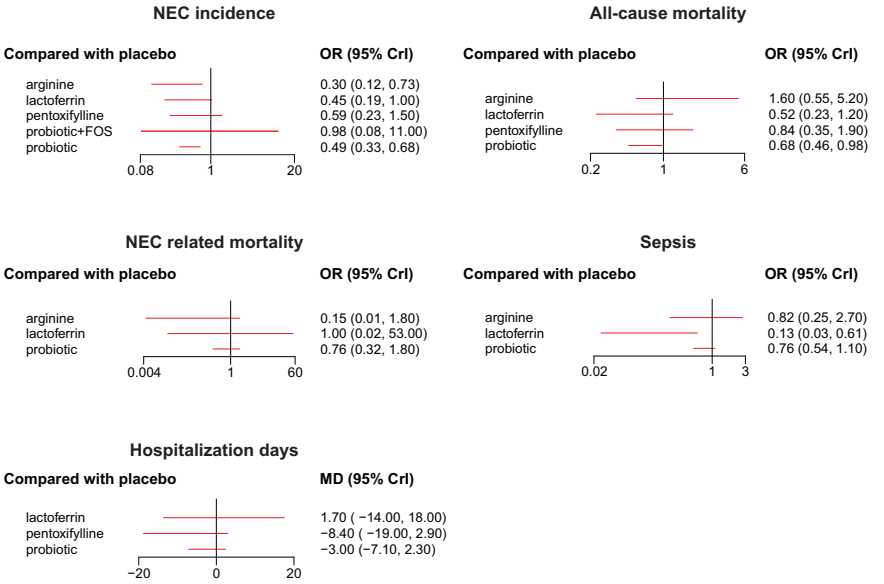

Figure 3. Forest plots for network comparison of necrotizing enterocolitis prevention under 5 endpoints. 95% CrI = 95% credible interval, OR = odds ratio.

incidence compared to those with placebo (probiotics: OR = 0.50, 95% CrIs: 0.32–0.73; arginine: OR = 0.30, 95% CrIs: 0.12–0.73). Besides that, preterm infants with probiotics also appeared to have significantly reduced risk of mortality compared to those with placebo (OR = 0.68, 95% CrIs: 0.46–0.98).

3.4. Network meta-analysis results for NEC-related mortality, sepsis, and hospitalization days

As for the endpoint of NEC-related mortality, no significant difference was found between food additives and placebo, as well as among different additives (Table 3). Lactoferrin was associated with a decrease in the risk of sepsis compared to placebo (OR = 0.13, 95% CrIs: 0.03–0.61), as well as probiotics (OR = 0.18, 95% CrIs: 0.03–0.83). Similar to the results of NEC-related mortality, food additives revealed no remarkable difference in hospitalization days mutually or compared to placebo (Table 4).

3.5. Ranking of 6 food additives and cluster analysis

The SUCRA value for each food additive implied their potential rankings for each outcome (Table 5, and Fig. S1, http://links.lww.com/MD/B693). Arginine and lactoferrin exhibited the highest SUCRA values with respect to NEC incidence (SUCRA = 0.850 and 0.640, respectively). However, the performance of arginine was compromised by its worst SUCRA ranking under the outcome of mortality (SUCRA = 0.118), while lactoferrin appeared to have the highest SUCRA value (SUCRA = 0.855). Cluster analysis was performed in order to categorize the above 6 food additives into distinctive groups (Fig. 4). The 2-dimensional graph indicated that lactoferrin had relatively stable performance with respect to almost all of the outcomes. Since there were no substantial asymmetry patterns in the funnel plots (Fig. 5), we concluded that no significant publication bias was presented in our study.

4. Discussion

Since NEC is a major challenge in neonatology, which has ongoing adverse effects on preterm infants, preventing this disease in preterm infants has been strongly advocated, and thus far enormous efforts have been made to unfold its pathogenesis. Several food additives have been introduced to reduce the incidence and mortality of NEC.[39,40] For the aim of understanding relative efficacy and safety of different food additives, our researchers conducted the first and most comprehensive NMA in this area. In this NMA, we involved 27 eligible studies with 4649 preterm infants.

In our study, arginine and probiotic were more favorable than others since preterm infants with these 2 food additives exhibited a significantly reduced risk of NEC. However, the performance of arginine was compromised by its performance under the outcome of mortality, while probiotic was still superior to other additives under this endpoint. Platelet-activating factor and nitric oxide played major roles in the etiopathogenesis of NEC. Nitric oxide was synthesized from the amino acid arginine through nitric oxide synthases.[9] Many animal models suggested that suppressing nitric oxide might increase the area of intestinal damage

Table 3

Network meta-analysis results for sepsis and NEC related mortality.

Sepsis	Placebo	0.79 (0.34, 1.75)	0.96 (0.02, 41.26)	0.15 (0.00, 1.62)	NEC-related death
	0.76 (0.54, 1.08)	Probiotic	1.22 (0.02, 57.97)	0.19 (0.01, 2.39)	
	0.13 (0.03, 0.61)	**0.18 (0.03, 0.83)**	Lactoferrin	0.14 (0.00, 15.18)	
	0.82 (0.25, 2.72)	1.08 (0.31, 3.71)	6.17 (0.89, 45.60)	Arginine	

NEC = necrotizing enterocolitis.
Bold type indicates significant values.

Table 4

Network meta-analysis results for hospitalization days.

Placebo			
−3.01 (−7.14, 2.34)	Probiotic		
−8.44 (−18.80, 2.93)	−5.52 (−16.99, 6.40)	Pentoxifylline	
1.67 (−13.61, 17.51)	4.50 (−11.64, 20.78)	10.04 (−6.49, 26.52)	Lactoferrin

significantly.[41,42] For this mechanism, arginine might function effectively in preventing NEC in preterm infants. The gastrointestinal tract of preterm infants often exhibited abnormal bacterial colonization and inadequate immune defenses.[4] This kind of deficiency could be offset by probiotics, and our conclusions appear to support this hypothesis.

However, selecting an appropriate food additive merely based on its efficacy for preventing NEC may lead to biased results. Therefore, we included several safety outcomes in order to provide clinicians with more informative conclusions. Arginine for infants should be used with caution because excessive arginine ingestion may generate more nitric oxide, and this may cause adverse effects on infants.[9] The potential harm of arginine to infants appeared to be supported by our SUCRA results in which arginine exhibited worse performance than placebo with respect to the outcome of mortality. Although some studies proposed that the adverse effects of arginine could be reduced if the corresponding dose was reduced,[43] more evidence should be disclosed to verify its safety in infants. On the other hand, preterm infants with probiotics exhibited a significantly decreased risk of NEC and mortality, and probiotics also performed relatively well for NEC-related mortality, sepsis, and hospitalization days. Thus, probiotics may be a better option for preterm infants.

Nevertheless, several limitations were likely to affect the validity of our conclusions. First, the nature of systematic review and NMA did not enable us to adjust for a few confounding factors. In this study, there were several inevitable confounding factors, including gestational age, birth weight, various ways of using probiotics, umbilical channeling, and stage of NEC. The specific type, dose, start time, and treatment duration of probiotics were not unified, which could affect the results. As the ways of taking drugs, umbilical vein catheterization and umbilical artery catheterization may also have some influence. The stage of NEC involved in our study was generally II or above. It was difficult to establish more detailed stage subgroups, because some original trials didn't report certain numbers of each stage. Another important confounding factor can be feeding method. Mother's milk, which is regarded as a fundamental nutritional source for neonates, can reduce the risk of NEC, but donor breast milk may not work this way, in which the

Table 5

SUCRA values for the treatments under 5 endpoints.

Addictive	NEC	Mortality	NEC-related mortality	Sepsis	Hospitalization
Placebo	0.133	0.308	0.276	0.140	0.251
Probiotic	0.595	0.714	0.455	0.504	0.591
Pentoxifylline	0.469	0.506	–	–	0.889
Lactoferrin	0.640	0.855	0.385	0.983	0.270
Probiotic + FOS	0.314		–	–	–
Arginine	0.850	0.118	0.883	0.373	–

FOS = fructo-oligosaccharides, NEC = necrotizing enterocolitis.

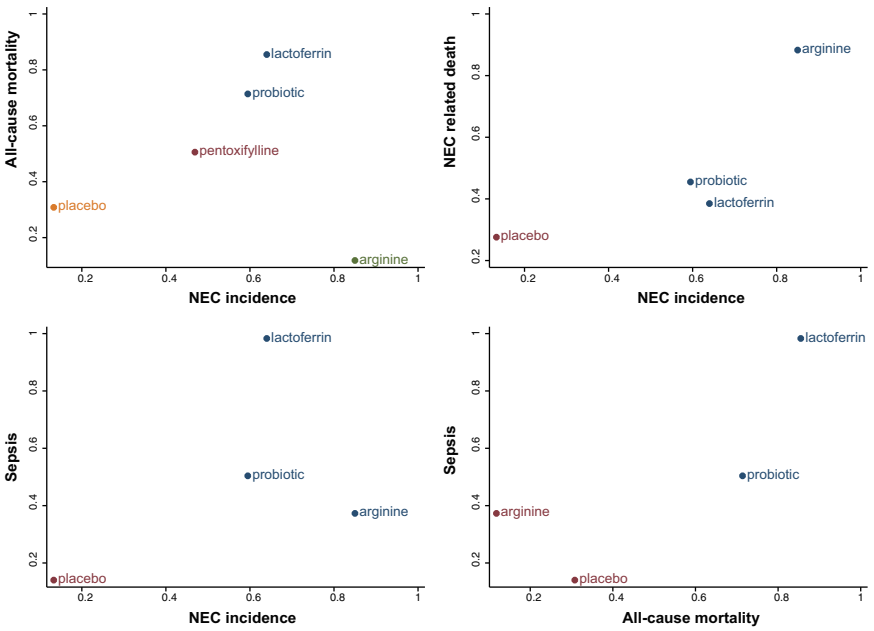

Figure 4. Two-dimensional cluster analysis for the combination of 5 endpoints. The same color of a group of treatments represents that they display a similar performance under specific 2 dimensional aspects.

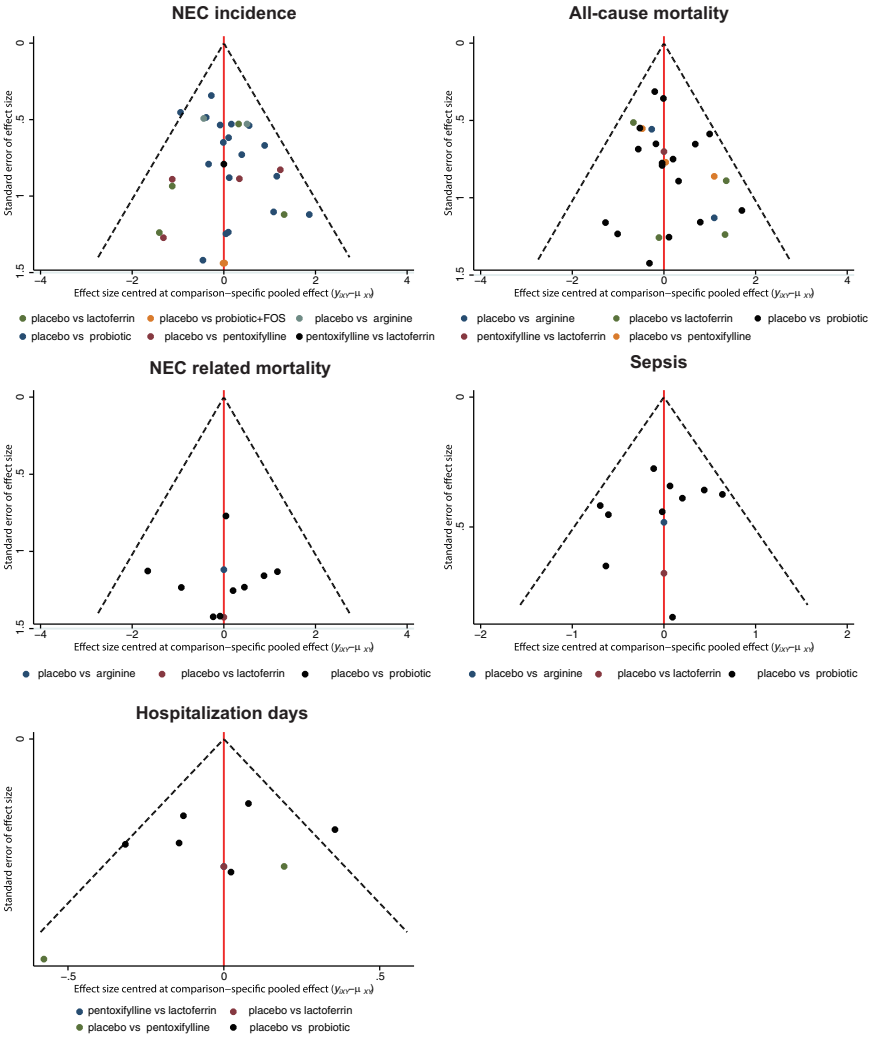

Figure 5. Comparison adjusted funnel plots for publication bias analysis.

pasteurization affects the composition of bioactive compounds. We did consider about this factor before, but the percentage of different breast milk source was only reported in 1 study,[35] some studies didn't even specify the source type. It was plausible that the above confounding factors had influence on the summary effects in a way. Second, some direct comparisons could not be achieved due to the lack of evidence and significant inconsistency may exist within the network structure. Therefore, approaches that were able to assess the risk of heterogeneity and inconsistency should be included in order to ensure that the statistical assumption of our NMA was valid. Third, the corresponding evidence in the network appeared to be substantially unbalanced, resulting in some unexpectedly unreliable estimates. Specifically speaking, the group at greatest risk of NEC, that is, those with a birth weight of less than 1000 g, though was involved in some studies,[17,18,26,33,34,38] was not considered in a separate way in our study. Moreover, the number of probiotic-related studies was obviously more than others. It's possible that the premature infants involved was underrepresented, as a result, it's a pity that we were not able to have adequate evidence of either efficacy or safety to recommend probiotics as universal prophylactic administration to all premature infants. In summary, our study indicated that probiotics had the potential to be the most preferable additive, since it exhibited a relatively balanced performance in the efficacy and safety. The use of arginine in preterm infants should be further justified, considering its high risk of resulting in mortality. More advanced research methodologies should be included in future studies to determine the most appropriate additive in preterm infants who are at great risk of NEC.

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