Multiple strains probiotics appear to be the most effective probiotics in the prevention of necrotizing enterocolitis and mortality: An updated meta-analysis

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Abstract

Background

Some oral probiotics have been shown to prevent necrotizing enterocolitis (NEC) and decrease mortality effectively in preterm very low birth weight (PVLBW) infants. However, it is unclear whether a single probiotic or a mixture of probiotics is most effective for the prevention of NEC.

Objective

A meta-analysis was conducted by reviewing the most up to date literature to investigate whether multiple strains probiotics are more effective than a single strain in reducing NEC and death in PVLBW infants.

Data sources

Relevant studies were identified by searches of the MEDLINE, EMBASE, and Cochrane CENTRAL databases, from 2001 to 2016.

Data extraction and synthesis

The inclusion criteria were randomized controlled trials of any enteral probiotic supplementation that was initiated within the first 7 days and continued for at least 14 days in preterm infants (≤ 34 weeks’ gestation) and/or those of a birth weight ≤ 1500 g.

Results

A total of 25 trials (n = 7345 infants) were eligible for inclusion in the meta-analysis using a fixed-effects model. Multiple strains probiotics were associated with a marked reduction in...
the incidence of NEC, with a pooled OR of 0.36 (95% CI, 0.24–0.53; \( P < .00001 \)). Single strain probiotic using Lactobacillus species had a borderline effect in reducing NEC (OR of 0.60; 95% CI 0.36–1.0; \( P = .05 \)), but not mortality. Multiple strains probiotics had a greater effectiveness in reducing mortality and were associated with a pooled OR of 0.58 (95% CI, 0.43–0.79; \( P = .0006 \)). Trials using single strain of Bifidobacterium species and Saccharomyces boulardii did not reveal any beneficial effects in terms of reducing NEC or mortality.

**Conclusion**

This updated report found that multiple strains probiotics appear to be the most feasible and effective strategy for the prevention of NEC and reduction of mortality in PVLBW neonates. Further clinical trials should focus on which probiotic combinations are most effective.

**Introduction**

Necrotizing enterocolitis (NEC) remains the most common acquired gastrointestinal and surgical emergency in preterm very low birth weight (PVLBW) infants. The incidence of NEC \( \geq \) stage 2 varies from 2.6% to 28.0% of PVLBW infants, with associated mortality ranging between 16% and 42% \[1,2\]. Preterm infants with NEC are at risk of long-term complications, including neurodevelopmental impairment, short bowel syndrome, and growth retardation \[3,4\]. Though there are significant morbidities associated with NEC, few safe and effective therapies are available to prevent this disastrous condition \[5\]. Available strategies for primary prevention of NEC include antenatal glucocorticoids, breast milk feeding, fluid restriction, and the use of probiotics \[4,5\]. However, during the past decade, only probiotics have been studied extensively in terms of the prevention of neonatal NEC in prospective randomized control trials (RCTs). Many meta-analyses of RCTs confirmed that oral probiotics effectively prevent NEC and death \[6–14\]. Although systemic reviews of single strain has been conducted \[12,14\], clinicians are facing challenges in assessing which probiotics are most effective in PVLBW infants. Unfortunately, published studies have used a variety of different single or multiple strains probiotic with different target populations. Relatively little is known about whether single strain probiotic alone or multiple strains are most effective in the prevention of NEC and death in PVLBW infants.

Recent articles have shown a link between NEC and a lack of microbiota diversity \[15–17\]. One review article suggested that mixtures of probiotics were more beneficial than single strains for gut and immune function \[18\]. Our animal model also revealed that multiple strains probiotics were more effective in the prevention of NEC \[19\]. Furthermore, NEC does not usually occur after a gestation age of 34 weeks. We thus hypothesize that multiple strains probiotics are more effective in the prevention of NEC and death for preterm infants below a gestation age of 34 weeks. We conducted a meta-analysis by systematically reviewing the most up to date evidence available in the literature to investigate whether multiple strains probiotics are more effective than single-strain probiotics for reducing NEC and death in preterm infants.

**Materials and methods**

**Search strategy and study selection**

This meta-analysis was conducted according to PRISMA guidelines (http://www.prisma-statement.org/) (S1 PRISMA Checklist). Any trials following prespecified criteria were
enrolled in the analysis: (a) RCTs involving PVLBW infants (≤ 34 weeks' gestation or birth weight ≤ 1500 g by mean or median) and reporting on NEC ≥ stage 2 by the Modified Bell staging criteria [20] and/or death, and (b) enteral administration of any probiotic commenced within the first 7 days of life and continued for at least 28 days. We searched the PubMed, Embase, and CBM databases for studies published from January 2001 to June 2016, with the terms “extremely low birth weight infant” or “very low birth weight infant” or “premature infant” or “preterm infant” and “Lactobacillus” or “probiotics” or “Saccharomyces” or “Bifidobacterium”. There was no language restriction. Similar studies and review articles reference lists in the references were also searched. The primary outcome was the efficacy of probiotic supplementation in preventing NEC ≥ stage 2. The secondary outcome was mortality before the infant was discharged.

Data extraction
Two authors (H.Y.C. and H.C.L.) independently conducted the literature search. Information regarding study inclusion, study design, key characteristics, and outcomes was extracted independently by the 2 reviewers using a standardized data collection form. Inconsistencies were resolved by involving a third author (J.H.C.) or by discussion between all authors.

Statistical analysis
To assess the between-study heterogeneity more precisely, both the $\chi^2$-based Q statistic test (Cochran Q statistic) to test for heterogeneity and the $I^2$ statistic to quantify the proportion of the total variation attributable to heterogeneity were used. For each meta-analysis, the Cochran Q statistic was first calculated to assess the heterogeneity of the included trials. For $P$ values less than .10, the assumption of homogeneity was deemed invalid. The outcome for our topic was binary in each trial; for example, whether NEC (or death) or not. The effects of probiotics for NEC (or death) were measured as odds ratios (ORs). For each trial, the OR is shown with a 95% confidence interval (95% CI). For NEC or mortality, a forest plot was used. For the meta-analysis, both the fixed-effects model and the random-effects model were considered. Heterogeneity existed between almost all trials in our pooled study. The $I^2$ of all trials or subgrouped trials was approximately zero. The fixed-effects model was considered to pool the estimators. Publication bias was investigated by funnel plot, and an asymmetric funnel plot suggested possible publication bias. Another way to explore the publication bias was the use of Egger’s regression test, which evaluated whether the intercept was significant. The significance level in the association test and the publication bias of our research were 0.05. Statistical analyses were performed using version 2 of the Comprehensive Meta-Analysis program (USA, 2006) and Review Manager (Cochrane Collaboration, Nordic Cochrane Centre) 5.1.

Results
Description of studies
In total, there were 123 studies identified through electronic searches; 39 trials met the inclusion criteria and were selected to be read in full text (Fig 1). Eighteen studies were excluded for the following reasons: two studies had a lack of relevant data [21,22], one study used both lactoferrin and probiotics [23], two study used both prebiotics and probiotics [24,25], five studies did not assess the NEC outcomes [26–30], the full text could not be extracted in four studies [31–34], one study was a report of additional data from a previous paper [35], and three trials were conducted before 2000 [36–38]. After eliminating these trials, this review included data from 25 RCTs [39–63]. Two studies only enrolled infants with a birth weight under 1500 g
A total of 7345 infants were included, 3679 in the probiotics group and 3666 in the control group. Each study was evaluated by Jadad score (Table 1). Of the 25 studies included in the analyses, fourteen studies used a single strain of probiotic (5 used a Lactobacillus strain [39,43,49,51,58], 6 used a Bifidobacterium strain [44,48,59,61–63], 3 used Saccharomyces

Fig 1. Flowchart showing the selection of studies for inclusion in the meta-analysis.

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[51,57]. A total of 7345 infants were included, 3679 in the probiotics group and 3666 in the control group. Each study was evaluated by Jadad score (Table 1). Of the 25 studies included in the analyses, fourteen studies used a single strain of probiotic (5 used a Lactobacillus strain [39,43,49,51,58], 6 used a Bifidobacterium strain [44,48,59,61–63], 3 used Saccharomyces
Table 1. Characteristics of the trials included in the analysis.

| Study             | Participants | Gestation or birth weight | Probiotic agents                                                                 | Outcomes            | Jadad score |
|-------------------|--------------|---------------------------|----------------------------------------------------------------------------------|---------------------|-------------|
|                     | Probiotics   | Placebo                   |                                                                                  |                     |             |
| Dani, 2002 [39]    | 295          | 290                       | < 33 wk, < 1500 g                                                              | L. rhamnosus GG     | NEC         | 4           |
| Costalos, 2003 [40]| 51           | 36                        | 28–32 wk                                                                       | S. bouardi          | NEC         | 5           |
| Bin-Nun, 2005 [41]| 72           | 73                        | < 1500 g                                                                        | B. infantis, B. bifidus, Strepto. thermophiles | NEC, mortality | 3           |
| Lin, 2005 [42]     | 180          | 187                       | < 1500 g                                                                        | L. acidophilus, B. bifidum | NEC, mortality | 5           |
| Manzoni, 2006 [43] | 39           | 41                        | < 1500 g                                                                        | L. rhamnosus GG     | NEC, mortality | 4           |
| Stratiki, 2007 [44]| 41           | 36                        | 27–37wk                                                                         | B. lactis           | NEC         | 3           |
| Lin, 2008 [45]     | 217          | 217                       | < 34 wk, < 1500 g                                                              | L. acidophilus, B. bifidum | NEC, mortality | 5           |
| Rouge, 2009 [46]   | 45           | 49                        | < 32 wk, < 1500 g                                                              | B. longum, L. rhamnosus GG | NEC, mortality | 5           |
| Samanta, 2009 [47] | 91           | 95                        | < 32 wk, < 1500 g                                                              | B. infantis, B. bifidus, B. longum, L. acidophilus | NEC, mortality | 3           |
| Mihatsch, 2010 [48]| 91           | 89                        | < 30 wk, < 1500 g                                                              | B. lactis           | NEC         | 4           |
| Sari, 2011 [49]    | 110          | 111                       | < 33 wk, < 1500 g                                                              | L. sporogenes       | NEC, mortality | 4           |
| Braga, 2011 [50]   | 119          | 112                       | 750–1499 g                                                                     | L. casei, B. breve  | NEC         | 5           |
| Rojas, 2012 [51]   | 176          | 184                       | < 1500 g*                                                                       | L. reuteri          | NEC         | 5           |
| Al-Hosni, 2012 [52]| 50           | 51                        | 501–1000g                                                                       | L. rhamnosus GG, B. infantis | NEC, mortality | 3           |
| Fernandez-Carrocera, 2013 [53]| 75          | 75                        | < 1500 g                                                                        | L. acidophilus, L. rhamnosus, L. casei, L. plantarum, B. infantis, Strepto. thermophillus | NEC, mortality | 5           |
| Demirel, 2013 [54] | 135          | 136                       | ≤ 32 wk, ≤ 1500 g                                                              | S. bouardi          | NEC, mortality | 4           |
| Jacobs, 2013 [55]  | 548          | 551                       | < 32 wk, < 1500 g                                                              | B. infantis, Strepto. thermophillus, B. lactis | NEC, mortality | 4           |
| Serce, 2013 [56]   | 104          | 104                       | ≤ 32 wk, ≤ 1500g                                                                | S. bouardi          | NEC, mortality | 4           |
| Roy, 2014 [57]     | 11           | 11                        | < 1000 g*                                                                       | B. infantis, Lactobacillus acidophilus, B. lactis | NEC         | 4           |
| Oncel, 2014 [58]   | 200          | 200                       | ≤ 32 wk, ≤ 1500 g                                                              | L. reuteri          | NEC         | 4           |
| Totsu, 2014 [59]   | 153          | 130                       | < 1500 g                                                                        | B. bifidus          | NEC, mortality | 4           |
| Saengtawesin 2014 [60]| 31          | 29                        | < 34 wk, < 1500 g                                                              | L. acidophilus, B. bifidum | NEC, mortality | 3           |
| Patole 2014 [61]    | 74           | 76                        | < 33 wk                                                                         | B. breve M-16V      | NEC         | 5           |
| Tewari 2015 [62]    | 121          | 123                       | < 34 wk                                                                         | B. clausii          | NEC         | 4           |
| Costeloe 2016 [63]  | 650          | 660                       | < 30 wk                                                                         | B. breve BBG-001    | NEC         | 5           |

L: Lactobacillus; B: Bifidobacterium; S: Saccharomyces; Strepto: Streptococcus; NEC, necrotizing enterocolitis.
*only patients with a birth weight < 1500 g were included in these studies.

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boulardii [40,54,56]). Of the 5 Lactobacillus trials, 2 used Lactobacillus rhamnosus strain [39,43], 2 used Lactobacillus reuteri strain [51,58], and 1 used Lactobacillus sporogenes strain [49]. Of the 6 Bifidobacterium trials, 2 used Bifidobacterium lactis [44,48], 2 used Bifidobacterium breve [61,63], 1 used Bifidobacterium bifidus [59], and 1 used Bifidobacterium clausii [62]. Eleven studies used multiple strains probiotics, including 8 studies that utilized a combination of Bifidobacterium strain and Lactobacillus strain [42,45–47,50,52,57,60], and 3 studies that used a mixture of multiple probiotics strains [41,53,55].

**Efficacy of all probiotics on NEC**

We combined the trials with all probiotics, such as multiple strains probiotics, Lactobacillus, Bifidobacterium, and Saccharomyces. The incidence of NEC stage ≥ 2 was 3.9% in the probiotics group, whereas it was 6.3% in the placebo group. The $P$-value of heterogeneity was 0.54, and $I^2$ was 0%. The pooled OR was 0.60 with the fixed-effect model and the 95% CI was 0.48–0.74, $P < .00001$. The probiotics group had a lower risk of developing NEC than the placebo group (Fig 2).

**Subgroup analysis: Efficacy of multiple strains probiotics versus single-strain probiotics on NEC.** In the multiple strains probiotics trials, the incidence of NEC stage ≥ 2 was 2.4% in the probiotics group, whereas in the placebo group it was 6.5%. In the Lactobacillus trials, the developed definite NEC stage ≥ 2 was 3.0% in the Lactobacillus group and 5.0% in the placebo group. In the Bifidobacterium trials, the developed definite NEC stage ≥ 2 was 5.8% in the Bifidobacterium group and 6.8% in the placebo group. In the Saccharomyces trials, the developed definite NEC stage ≥ 2 was 6.2% in the Saccharomyces group and 7.2% in the placebo group.

The meta-analysis showed that the placebo group had a higher risk of developing NEC than the multiple strains probiotics group (pooled OR: 0.36, 95% CI: 0.24–0.53, $P < .00001$, $I^2 = 0$%). The odds of NEC occurring in the placebo group were higher than those in the single-strain probiotics groups, but these differences did not reach statistical significance (Fig 2). Single strain probiotic using Lactobacillus species had a borderline effect in reducing NEC (OR of 0.60; 95% CI 0.36–1.0; $P = .05$). Trials using single strain of Bifidobacterium species and Saccharomyces boulardii did not reveal any beneficial effects in terms of reducing NEC.

**Efficacy of probiotics on mortality**

There were 21 trials that discussed the association between mortality and probiotics. The mortality rate in the probiotics group with all the tested probiotics, including multiple strains probiotics, Lactobacillus, Bifidobacterium, and Saccharomyces, was 5.3%, whereas in the placebo group it was 6.9%. Probiotics reduced the risk of death by 25% relative to the placebo group (pooled OR: 0.75, 95% CI: 0.60–0.92, $P = .006$, $I^2 = 9$%) (Fig 3).

**Subgroup: Effect of combined probiotics and single-strain probiotics on mortality.**

We reported the effects of multiple strains probiotics, Lactobacillus, Bifidobacterium, and Saccharomyces on mortality separately. In the multiple strains probiotics cases, there were 5.3% and 8.4% mortality rates within the treatment and placebo groups, respectively.

Within the multiple strains probiotics group, the $P$-value of the heterogeneity test was 0.13, and $I^2$ was 36%. The degree of heterogeneity was moderate. The pooled OR between multiple strains probiotics and death was 0.58, and the 95% CI was 0.43 to 0.79 ($P$-value = .0006). In the single-strain probiotics group, the pooled results in the probiotics group showed no statistical significance in relation to mortality as compared with the placebo group (Fig 3).
### 1.2.1 Multiple strains

| Study or Subgroup       | Probiotics | Placebo | Odds Ratio M-H, Fixed   | 95% CI |
|-------------------------|------------|---------|-------------------------|--------|
| Events                  | Total      | Events  | Total                   | Weight |        |
| Al-Hosni 2012           | 2 50       | 2 51    | 0.9%                    | 1.02   | [0.14, 7.54] |
| Bin-Nun 2005            | 1 72       | 1 73    | 4.4%                    | 0.09   | [0.01, 0.71] |
| Braga 2011              | 0 119      | 4 112   | 2.1%                    | 0.10   | [0.01, 1.90] |
| Fernandez-Carrocera 2013| 6 75       | 12 75   | 5.0%                    | 0.46   | [0.16, 1.29] |
| Jacobs 2013             | 11 548     | 24 551  | 10.7%                   | 0.45   | [0.22, 0.93] |
| Lin 2005                | 2 180      | 10 187  | 4.4%                    | 0.20   | [0.04, 0.92] |
| Lin 2008                | 4 217      | 14 217  | 6.2%                    | 0.27   | [0.09, 0.84] |
| Rouge 2009              | 2 45       | 1 49    | 0.4%                    | 2.23   | [0.20, 25.50] |
| Roy 2014                | 1 11       | 1 11    | 0.4%                    | 1.00   | [0.05, 18.30] |
| Saengtavesin 2014       | 1 31       | 1 29    | 0.5%                    | 0.93   | [0.08, 15.65] |
| Samanta 2009            | 5 91       | 15 95   | 6.3%                    | 0.31   | [0.11, 0.89] |
| **Subtotal (95% CI)**   | 1439       | 1450    | 41.3%                   | 0.36   | [0.24, 0.53] |

**Total events:** 35 94

**Heterogeneity:** $\chi^2 = 6.03$, $df = 10$ ($P = 0.63$); $I^2 = 0$

**Test for overall effect:** $Z = 5.07$ ($P < 0.00001$)

### 1.2.2 Lactobacillus species

| Study or Subgroup       | Probiotics | Placebo | Odds Ratio M-H, Fixed   | 95% CI |
|-------------------------|------------|---------|-------------------------|--------|
| Events                  | Total      | Events  | Total                   | Weight |        |
| Dani 2002               | 4 285      | 8 290   | 3.6%                    | 0.48   | [0.14, 1.63] |
| Manzoni 2006            | 1 39       | 3 41    | 1.3%                    | 0.33   | [0.03, 3.35] |
| Oncel 2014              | 8 200      | 10 200  | 4.4%                    | 0.79   | [0.31, 2.05] |
| Rojas 2012              | 6 176      | 10 184  | 4.3%                    | 0.61   | [0.22, 1.73] |
| San 2011                | 6 110      | 10 111  | 4.3%                    | 0.58   | [0.20, 1.66] |
| **Subtotal (95% CI)**   | 820        | 826     | 17.8%                   | 0.60   | [0.36, 1.00] |

**Total events:** 25 41

**Heterogeneity:** $\chi^2 = 0.70$, $df = 4$ ($P = 0.95$); $I^2 = 0$

**Test for overall effect:** $Z = 1.95$ ($P = 0.05$)

### 1.2.3 Bifidobacterium species

| Study or Subgroup       | Probiotics | Placebo | Odds Ratio M-H, Fixed   | 95% CI |
|-------------------------|------------|---------|-------------------------|--------|
| Events                  | Total      | Events  | Total                   | Weight |        |
| Costelow 2016           | 61 650     | 66 660  | 27.0%                   | 0.93   | [0.65, 1.34] |
| Minatsu 2010            | 2 91       | 4 89    | 1.8%                    | 0.48   | [0.09, 2.68] |
| Patole 2014             | 0 74       | 1 76    | 0.7%                    | 0.34   | [0.01, 8.43] |
| Straki 2007             | 0 41       | 3 36    | 1.7%                    | 0.12   | [0.01, 2.31] |
| Tewari 2015             | 2 121      | 2 123   | 0.9%                    | 1.02   | [0.14, 7.34] |
| Totsu 2014              | 0 153      | 0 130   | Not estimable           |        |
| **Subtotal (95% CI)**   | 1130       | 1114    | 32.0%                   | 0.86   | [0.61, 1.20] |

**Total events:** 65 76

**Heterogeneity:** $\chi^2 = 2.72$, $df = 4$ ($P = 0.61$); $I^2 = 0$

**Test for overall effect:** $Z = 0.90$ ($P = 0.37$)

### 1.2.4 Saccharomyces species

| Study or Subgroup       | Probiotics | Placebo | Odds Ratio M-H, Fixed   | 95% CI |
|-------------------------|------------|---------|-------------------------|--------|
| Events                  | Total      | Events  | Total                   | Weight |        |
| Costales 2003           | 5 51       | 6 36    | 2.9%                    | 0.54   | [0.15, 1.94] |
| Demir 2013              | 6 135      | 7 136   | 3.0%                    | 0.86   | [0.28, 2.62] |
| Serce 2013              | 7 104      | 7 104   | 3.0%                    | 1.00   | [0.34, 2.96] |
| **Subtotal (95% CI)**   | 290        | 276     | 8.9%                    | 0.80   | [0.41, 1.56] |

**Total events:** 18 20

**Heterogeneity:** $\chi^2 = 0.53$, $df = 2$ ($P = 0.77$); $I^2 = 0$

**Test for overall effect:** $Z = 0.65$ ($P = 0.52$)

**Total (95% CI):**

| Probiotics | Placebo | 95% CI |
|------------|---------|--------|
| 3679       | 3666    | 100.0% |

**Total events:** 143 231

**Heterogeneity:** $\chi^2 = 21.65$, $df = 23$ ($P = 0.54$); $I^2 = 0$

**Test for overall effect:** $Z = 4.65$ ($P < 0.00001$)

**Test for subgroup differences:** $\chi^2 = 11.18$, $df = 3$ ($P = 0.01$), $I^2 = 73.2$

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**Fig 2. Meta-analysis of the efficacy of probiotics associated with the risk of NEC in preterm infants.**

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A funnel plot was used to show the relationship between effect size and the standard error of estimator for each trial. A symmetric funnel plot would indicate that publication bias did not
exist. Egger’s regression test, a weighted linear ordinary least squares regression, was used to explore the publication bias. The funnel plots of NEC and mortality looked generally symmetric separately. Egger’s tests for the intercepts were not statistically significant for NEC (P-value of intercept = .54) or mortality (P-value of intercept = .48). The Egger’s test corresponded with the symmetrical funnel plots. The funnel plots and Egger’s regression indicated no publication bias within our selected trials.

Discussion
Our updated meta-analysis showed that multiple strains probiotics resulted in a marked reduction of the incidence of NEC and of the incidence of mortality in preterm infants ≤ 34 weeks’ gestation or of a birth weight ≤ 1500 g.

NEC is a multifactorial disease, and its pathophysiology remains unclear. Several factors appear to contribute to the pathogenesis, including immaturity of multiple intestinal functions, such as gastrointestinal dysmotility, impaired digestive capacity, altered regulation of intestinal blood flow, barrier dysfunction, altered anti-inflammatory control, and impaired host defense. Frequent use of antibiotic therapy and anti-acid medications, followed by enteral feeding, are believed to increase the risk of NEC. Establishing a core microbiota of diverse commensal species is critical of PVLBW infants. Reasons for disruption or delay of this critical process include delivery modes, gestational age, birth weight, infectious diseases, antibiotics therapy, parenteral feeding, feeding type, and hospital period and environment. The dysbiosis of microbial succession [64,65], abnormal bacterial colonization, and lower bacterial diversity [15–17] in PVLBW infants also has been linked to the occurrence of NEC, which is the rationale for the need for probiotic supplements.

Although probiotics are the most promising treatment for reducing NEC, the use of different probiotics in all studies has made the selection of an optimal probiotic regimen difficult. Furthermore, preterm infants range up to ≤ 34 weeks’ gestational age, making it difficult to determine which preterm infants would benefit the most. The 25 trials that were enrolled in our review are summarized in Table 1 [39–53]. Our updated meta-analysis confirmed the results of other systematic reviews, which reported that probiotics prevent NEC and death. However, we focused on preterm infants of ≤ 34 weeks’ gestation or of a birth weight ≤ 1500 g, who were a high-risk group for developing NEC or death, who had undergone enteral administration of probiotics commenced within the first 7 days of life and continued for at least 28 days. Our study discovered that multiple strains probiotics resulted in a marked reduction in NEC, which was comparable with results from one recent meta-analysis [12]. The potential mechanisms by which multiple strains probiotics may provide better protection from developing NEC might include increased diversity of the intestinal microbiota and offering healthy bacteria such as Lactobacillus and Bifidobacteria to balance normal microbiota in this vulnerable human population. However, it is unclear whether this is due to synergistic interactions between strains or a consequence of the higher probiotics doses.

Our updated meta-analysis further confirmed the results of other systematic reviews, which reported that probiotics supplements had a significant effect in reducing mortality in preterm infants of ≤ 34 weeks’ gestation or of a birth weight ≤ 1500 g. However, after further analysis, 3 trials using a Lactobacillus strain alone, 6 trials using Bifidobacterium alone, and 2 trials using Saccharomyces alone did not reveal a beneficial effect on mortality. On the other hand, analyses from 10 studies showed a greater effectiveness of multiple strains probiotics in reducing mortality in PVLBW infants. It is unclear from this meta-analysis whether this was due to a reduction in NEC-related or sepsis-related deaths or other etiologies. In theory and animal studies, different probiotics may function differently in the modification of the intestinal
immune system. Therefore, the effects of multiple strains probiotics probiotics on NEC or death may be acting through the synergetic effect, inhibiting the growth of pathogens, promoting up-regulation of the immune responses, or strengthening the mucosal barrier [17].

Concerns regarding safety issues and complications associated with the use of probiotics in these relatively immunocompromised preterm infants have been debated. However, the reported risk of sepsis due to translocation of the probiotics through the intestinal wall is extremely rare, and no apparent adverse effects were observed in any of the studies. Based on the currently available clinical trial results, probiotics use in preterm infants is generally considered to be safe. We did not analyze the efficacy of probiotics on sepsis, the time to full oral feeding, or the duration of hospitalization, because these items were not the primary or secondary outcomes of the enrolled studies. Only a fraction of the enrolled studies described these outcomes, which could not be analyzed because of publication bias.

There were several possible limitations that warranted careful review in this meta-analysis. First of all, the multiple strains probiotics treatment regimens varied widely. Questions regarding the optimal combination of species and dosing remain unanswered. Further studies directly comparing probiotic mixtures with single strains are warranted. Further research should also identify which multiple strains probiotics might be associated with improved health outcomes or enhance the preparation’s effectiveness. Second, publication bias may have existed for trials using single strain probiotic alone because of the limited study numbers. Probiotic effects are known to be strain specific, statistically and clinically significant benefits could relate to strain-specific differences. Although strainspecific meta-analysis data has been performed [12,14], recommendation cannot be made because of limited RCTs using the same single strain. Furthermore, there is significant heterogeneity among included studies. Some trials with small sample size and inadequate power, NEC or mortality as a secondary outcome are also affected our meta-analysis results. We should also point out that some of the probiotic products or placebos in the control group contained maltodextrin, which according to a recent animal study could increase the incidence of NEC [66]. However, our further analysis showed that probiotics containing maltodextrin had the same effect in terms of the prevention of NEC.

Conclusions

Multiple strains probiotics, a therapeutic modification of the gut microbiota and restoring a healthy complement and diversity of commensal bacteria is the most logical approach to prevent NEC and death in PVLBW infants. The current evidence provided by this meta-analysis supported that multiple strains probiotics seemed to be the most feasible method and the most effective way to prevent NEC and reduce mortality in preterm infants of \( \leq 34 \) weeks’ gestation or of a birth weight \( \leq 1500 \) g. Single strain probiotic using Lactobacillus species had a borderline effect in reducing NEC. Single strain of Bifidobacterium species and Saccharomyces boulardii did not reveal any beneficial effects in terms of reducing NEC or mortality. The optimal combination of species and dosing, long-term immune and neurodevelopment outcomes of probiotic supplementation in PVLBW infants still need to be explored in further studies.

Supporting information

S1 PRISMA Checklist.

(DOC)

Author contributions

Conceptualization: HYC J.-H. Chang HCL.
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Supervision: HCL.

Writing – original draft: HYC J.-H. Chang HCL.

Writing – review & editing: HYC J.-H. Chang HCL CCP.

References

1. Luig M, Lui K, NSW & ACT NICUS Group. Epidemiology of necrotizing enterocolitis-Part I: Changing regional trends in extremely preterm infants over 14 years. J Paediatr Child Health 2005; 41: 169–173. doi: 10.1111/j.1440-1754.2005.00582.x PMID: 15813869

2. Fitzgibbon SC, Ching Y, Yu D, Carpenter J, Kenny M, Weldon C, et al. Mortality of necrotizing enterocolitis expressed by birth weight categories. J Pediatr Surg 2009; 44: 1072–1075. doi: 10.1016/j.jpedsurg.2009.02.013 PMID: 19524719

3. Hintz SR, Kendrick DE, Stoll BJ, Vohr BR, Fanaro FF, Donovan EF, et al. Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis. Pediatrics 2005; 115: 696–703. doi: 10.1542/peds.2004-0569 PMID: 15741374

4. Schulzke SM, Deshpande GC, Patole SK. Neurodevelopmental outcomes of very low-birth-weight infants with necrotizing enterocolitis: a systematic review of observational studies. Arch Pediatr Adolesc Med 2007; 161: 583–590. doi: 10.1001/archpedi.161.6.583 PMID: 17548764

5. Chen AC, Chung MY, Chang JH, Lin HC. Pathogenesis implication for necrotizing enterocolitis prevention in preterm very-low-birth-weight infants. J Pediatr Gastroenterol Nutr 2014; 58: 7–11. PMID: 24378520

6. Barclay AR, Stenson B, Simpson JH, Weaver LT, Wilson DC. Probiotics for necrotizing enterocolitis: a systematic review. J Pediatr Gastroenterol Nutr 2007; 45: 569–576. PMID: 18030235

7. Deshpande G, Rao S, Patole S. Probiotics for prevention of necrotizing enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. Lancet 2007; 369: 1614–1620. doi: 10.1016/S0140-6736(07)60748-X PMID: 17499603

8. Alfaleh K, Anabrees J, Bassler D. Probiotics reduce the risk of necrotizing enterocolitis in preterm infants: a meta-analysis. Neonatology 2010; 97: 93–99. doi: 10.1159/0003235684 PMID: 19707025

9. Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. Pediatrics 2010; 125: 921–930. doi: 10.1542/peds.2009-1301 PMID: 20403939

10. Wang Q, Dong J, Zhu Y. Probiotic supplement reduces risk of necrotizing enterocolitis and mortality in preterm very-low-birth-weight infants: an updated meta-analysis of 20 randomized, controlled trials. J Pediatr Surg 2012; 47: 241–248. doi: 10.1016/j.jpedsurg.2011.09.064 PMID: 22244424

11. Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2014; 4: CD005496

12. Aceti A, Gori D, Barone G, Callegari ML, Di Mauro A, Fantini MP, et al. Probiotics for prevention of necrotizing enterocolitis in preterm infants: systematic review and meta-analysis. Ital J Pediatr. 2015; 41: 89. doi: 10.1186/s13052-015-0199-2 PMID: 26567539

13. Lau CS, Chamberlain RS. Probiotic administration can prevent necrotizing enterocolitis in preterm infants: A meta-analysis. J Pediatr Surg. 2015; 50: 1405–1412. doi: 10.1016/j.jpedsurg.2015.05.008 PMID: 26216544

14. Athalye-Jape G, Rao S, Patole S. Lactobacillus reuteri DSM 17938 as a probiotic for preterm neonates: A strain-specific systematic review. J Parenter Enteral Nutr. 2016; 40: 783–794.

15. Mai V, Young CM, Ukhanaova M, Wang X, Sun Y, Casella G, et al. Fecal microbiota in premature infants prior to necrotizing enterocolitis. PLoS One. 2011; 6: e20647. doi: 10.1371/journal.pone.0020647 PMID: 21674011
16. Wang Y, Hoenig JD, Malin KJ, Qamar S, Petrof EO, Sun J, Antonopoulos DA, Chang EB, Claud EC. 16S RNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis. ISME J 2009; 3: 944–954. doi: 10.1038/ismej.2009.37 PMID: 19369970

17. Shiou SR, Yu Y, Guo Y, He SM, Mziray-Andrew CH, Hoenig J, et al. Synergistic protection of combined probiotic conditioned media against neonatal necrotizing enterocolitis-like intestinal injury. PLoS One 2013; 8: e65108 doi: 10.1371/journal.pone.0065108 PMID: 23717690

18. Chapman CM, Gibson GR, Rowland I. Health benefits of probiotics: are mixtures more effective than single strains? Eur J Nutr 2011; 50: 1–17. doi: 10.1007/s00394-010-0166-z PMID: 21229254

19. Wu SF, Chiu HY, Chen AC, Lin HY, Lin HC, Caplain M. Efficacy of different probiotic combinations on death and necrotizing enterocolitis in a premature rat model. J Pediatr Gastroenterol Nutr 2013; 57: 23–28. PMID: 23535766

20. Bell MJ, Ternberg JL, Feigen RD, Keating JP, Marshall R, Barton L, et al. Neonatal necrotizing enterocolitis: therapeutic decisions based upon clinical staging. Ann Surg 1978; 187: 1–7. PMID: 413500

21. Dilli D, Aydin B, Fettah ND, Özayazıcı E, Beken S, Zenciroğlu A, et al. The propre-save study: effects of probiotics and prebiotics alone or combined on necrotizing enterocolitis in very low birth weight infants. J Pediatr. 2015; 166: 545–551. doi: 10.1016/j.jpeds.2014.12.004 PMID: 25596096

22. Umezaki H, Shinohara K, Satoh Y, Shoji H, Satoh H, Ohtsuka Y, et al. Bifidobacteria prevents preterm infants from developing infection and sepsis. Int J Probiotics Prebiotics 2010; 5: 33–36.

23. Li Y, Shimizu T, Hosaka A, Kaneko N, Ohtsuka Y, Yamashiro Y. Effects of bifidobacterium breve supplementation on intestinal flora of low birth weight infants. Pediatr Int 2004; 46: 509–515. doi: 10.1111/j.1442-200x.2004.01953.x PMID: 15491374

24. Mohan R, Koebnick C, Schildt J, Schmidt S, Mueller M, Possner M, et al. Effects of Bifidobacterium lactis Bb12 supplementation on intestinal microbiota of preterm neonates: a double placebo controlled, randomized study. J Clin Microbiol 2006; 44: 4025–4031. doi: 10.1128/JCM.00767-06 PMID: 16971641

25. Romeo MG, Romeo DM, Trovato L, Oliveri S, Palermo F, Cota F, et al. Role of probiotics in the prevention of the enteric colonization by Candida in preterm newborns: incidence of late-onset sepsis and neurological outcome. J Perinatol 2011; 31: 63–69. doi: 10.1038/jp.2010.57 PMID: 20410904

26. Ke D, Su Z, Li L. Control study on preventing necrotizing enterocolitis in 438 premature infants by using bifico. Chin Pediatr Emerg Med 2008; 15: 69–71. [Article in Chinese].

27. Huang BZ, Yang HY, Huang XY. Prevention and cure effect of micro ecosystem praeparatum on necrotizing enterocolitis of very low birth weight infant. J Guangdong Med Coll 2009; 27: 37–39. [Article in Chinese].

28. Ren B. Preventive effect of Bifidobacterium tetravaccine tablets in premature infants with necrotizing enterocolitis. J Pediatr Pharm 2010; 16: 24–25. [Article in Chinese].

29. Mohan R, Koebnick C, Schildt J, Mueller M, Radke M, Blaut M. Effects of Bifidobacterium lactis Bb12 supplementation on body weight, fecal pH, acetate, lactate, calprotectin, and IgA in preterm infants. Pediatr Res 2008; 64: 418–422. doi: 10.1203/PDR.0b013e318181b7fa PMID: 18552710

30. Reuman PD, Duckworth DH, Smith KL, Kagan R, Bucciarelli RL, Ayoub EM. Lack of effect of Lactobacillus on gastrointestinal bacterial colonization in premature infants. Pediatr Infect Dis 1986; 5: 663–668. PMID: 3099269
Multiple strains probiotics in the prevention of necrotizing enterocolitis

37. Millar MR, Bacon C, Smith SL, Walker V, Hall MA. Enteral feeding of premature infants with Lactobacillus GG. Arch Dis Child 1993; 69: 483–487. PMID: 8285750

38. Kitajima H, Sumida Y, Tanaka R, Yuki N, Takayama H, Fujimura M. Early administration of Bifidobacterium breve to preterm infants: randomised controlled trial. Arch Dis Child Fetal Neonatal Ed 1997; 76: F101–107. PMID: 9135288

39. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biol Neonate 2002; 82: 103–108. PMID: 12169832

40. Costalos C, Skouteri V, Gounaris A, Sevastiadou S, Triandafilou A, Ekonomidou C, et al. Enteral feeding of premature infants with Saccharomyces boulardii. Early Hum Dev 2003; 74: 89–96. PMID: 14580749

41. Bin-Nun A, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. J Pediatr 2005; 147: 192–196. doi: 10.1016/j.jpeds.2005.03.054 PMID: 16126048

42. Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Pediatrics 2005; 115: 1–4. doi: 10.1542/peds.2004-1463 PMID: 15629973

43. Manzoni P, Mostert M, Leonessa ML, Priolo C, Farina D, Monetti C, et al. Oral supplementation with Lactobacillus casei subspecies rhamnosus prevents enteric colonization by Candida species in preterm neonates: a randomized study. Clin Infect Dis 2006; 42: 1735–1742. doi: 10.1086/504324 PMID: 16705585

44. Stratiki Z, Costalos C, Sevastiadou S, Kastanioudi O, Skouroliakou M, Giakoumatou A, et al. The effect of a Bifidobacterium supplemented bovine milk on intestinal permeability of preterm infants. Early Hum Dev 2007; 83: 575–579. doi: 10.1016/j.earhumdev.2006.12.002 PMID: 17229535

45. Lin HC, Hsu CH, Chen HL, Chung MY, Hsu JF, Lien RL, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. Pediatrics 2008; 122: 693–700. doi: 10.1542/peds.2007-3007 PMID: 18829790

46. Rougé C, Piloquet H, Butel MJ, Berger B, Rochat F, Ferraris L, et al. Oral supplementation with probiotics in very-low-birth-weight infants: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 2009; 89: 1828–1835. doi: 10.1093/ajcn.2008.26919 PMID: 19369375

47. Samanta M, Sarkar M, Ghosh P, Ghosh J, Sinha M, Chatterjee S. Prophylactic probiotics for prevention of necrotizing enterocolitis in very low birth weight newborns. J Trop Pediatr 2009; 55: 128–131. doi: 10.1093/tropej/fm091 PMID: 18842610

48. Mihatsch WA, Vossbeck S, Eikmanns B, Hoegel J, Pohlandt F. Effect of Bifidobacterium lactis on the incidence of nosocomial infections in very-low-birthweight infants: a randomized controlled trial. Neonatology 2010; 98: 156–163. doi: 10.1159/000280291 PMID: 20234140

49. Sari FN, Dizard EA, Oguz S, Erdeve O, Uras N, Dilmen U. Oral probiotics: Lactobacillus casei oral supplementation on necrotizing enterocolitis in very-low-birth-weight preterm infants: a multicenter study. J Perinatol 2012; 32: 539–545. doi: 10.1038/jp.2011.51 PMID: 21358064

50. Al-Hosni M, Duenas M, Hawk M, Stewart LA, Borghese RA, Cahoon M, et al. Probiotics-supplemented feeding in extremely low-birth-weight infants. J Perinatol 2012; 32: 253–259. doi: 10.1038/jp.2011.51 PMID: 21546942

51. Rojas MA, Lozano JM, Rojas MX, Rodriguez VA, Rondon MA, Bastidas JA, et al. Prophylactic probiotics to prevent death and nosocomial infection in preterm infants. Pediatrics 2012; 130: e1113–1120. doi: 10.1542/peds.2011-3584 PMID: 23071204

52. Demirel G, Erdeve O, Celik IH, Dilmen U. Saccharomyces boulardii for prevention of necrotizing enterocolitis in preterm infants: A randomized, controlled study. Acta Paediatr 2013; 102: e560–e563. doi: 10.1111/apaa.12416 PMID: 24008629

53. Strati Z, Costalos C, Sevastiadou S, Kastanioudi O, Skouroliakou M, Giakoumatou A, et al. The effect of a Bifidobacterium supplemented bovine milk on intestinal permeability of preterm infants. Early Hum Dev 2007; 83: 575–579. doi: 10.1016/j.earhumdev.2006.12.002 PMID: 17229535

54. Manzoni P, Mostert M, Leonessa ML, Priolo C, Farina D, Monetti C, et al. Oral supplementation with Lactobacillus casei subspecies rhamnosus prevents enteric colonization by Candida species in preterm neonates: a randomized study. Clin Infect Dis 2006; 42: 1735–1742. doi: 10.1086/504324 PMID: 16705585

55. Fernetas-Carrocera LA, Solis-Herrera A, Cabanillas-Ayon M, Gallardo-Sarmiento RB, Garcia-Perez CS, Montaño-Rodriguez R, et al. Double-blind, randomised clinical assay to evaluate the efficacy of probiotics in preterm newborns weighing less than 1500g in the prevention of necrotising enterocolitis. Arch Dis Child Fetal Neonatal Ed 2013; 98: F5–9. doi: 10.1136/archdischild-2011-300435 PMID: 22556209

56. Jacobs SE, Tobin JM, Opeil GF, Donath S, Tabrizi SN, Pirotta M, et al. Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. Pediatrics 2013; 132: 1055–1062. doi: 10.1542/peds.2013-1338 PMID: 24249817
56. Serce O, Benzer D, Gursoy T, Karatekin G, Ovali F. Efficacy of saccharomyces boulardii on necrotizing enterocolitis or sepsis in very low birth weight infants: A randomised controlled trial. Early Hum Dev 2013; 89: 1033–1036. doi: 10.1016/j.earlhumdev.2013.08.013 PMID: 24041815

57. Roy A, Chaudhuri J, Sarkar D, Ghosh P, Chakraborty S. Role of Enteric Supplementation of Probiotics on Late-onset Sepsis by Candida species in Preterm Low Birth Weight Neonates: A Randomized, Double Blind, Placebo-controlled Trial. N Am J Med Sci 2014; 6: 50–57. doi: 10.4103/1947-2714.125870 PMID: 24678479

58. Oncel MY, Sari FN, Arayici S, Guzoglu N, Erdeve O, Uras N, et al. Lactobacillus Reuteri for the prevention of necrotising enterocolitis in very low birthweight infants: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed 2014; 99: F110–115. doi: 10.1136/archdischild-2013-304745 PMID: 24309022

59. Totsu S, Yamasaki C, Terahara M, Uchiyama A, Kusuda S; Probiotics Study Group in Japan. Bifidobacterium and enteral feeding in preterm infants: Cluster-randomized trial. Pediatr Int 2014; 56: 714–719. doi: 10.1111/ped.12330 PMID: 24617812

60. Saengtawesin V, Tangpolkaiwalsak R, Kanjanapattankul W. Effect of oral probiotics supplementation in the prevention of necrotizing enterocolitis among very low birth weight preterm infants. J Med Assoc Thai 2014; 97 Suppl 6: S20–5.

61. Patole S, Keil AD, Chang A, Nathan E, Doherty D, Simmer K, et al. Effect of Bifidobacterium breve M-16V supplementation on fecal bifidobacteria in preterm neonates -a randomised double blind placebo controlled trial. PLoS One. 2014; 9: e89511. doi: 10.1371/journal.pone.0089511 PMID: 2494833

62. Tewari VV, Dubey SK, Gupta G. Bacillus clausii for Prevention of late-onset sepsis in preterm infants: a randomized controlled trial. J Trop Pediatr. 2015; 61: 377–385. doi: 10.1093/tropej/fmv050 PMID: 26246087

63. Costeloe K, Hardy P, Juszczak E, Wilks M, Millar MR; Probiotics in Preterm Infants Study Collaborative Group. Bifidobacterium breve BBG-001 in very preterm infants: a randomised controlled phase 3 trial. Lancet. 2016; 387: 649–660. doi: 10.1016/S0140-6736(15)01027-2 PMID: 26628328

64. Cassir N, Simeoni U, La Scola B. Gut microbiota and the pathogenesis of necrotizing enterocolitis in preterm neonates. Future Microbiol. 2016; 11: 273–292. doi: 10.2217/fmb.15.136 PMID: 26855351

65. Warner BB, Deych E, Zhou Y, Hall-Moore C, Weinstock GM, Sodergren E, et al. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. Lancet. 2016; 387(10031):1928–36. doi: 10.1016/S0140-6736(16)00081-7 PMID: 26969089

66. Thymann T, Meller HK, Stoll B, Stey AC, Buddington RK, Bering SB, et al. Carbohydrate malabsorption induces necrotizing enterocolitis in preterm pigs. Am J Physiol Gastrointest Liver Physiol 2009; 297: G1115–1125. doi: 10.1152/ajpgi.00261.2009 PMID: 19808655