

A meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates

Y. Yang, Y. Guo, Q. Kan, X.G. Zhou, X.Y. Zhou and Y. Li

Department of Neonates, Nanjing Children's Hospital, Nanjing Medical University, Nanjing, China

Abstract

Necrotizing enterocolitis (NEC) is one of the most common acquired diseases of the gastrointestinal tract in preterm infants. Some randomized, controlled trials (RCTs) have indicated that probiotics may potentially lower the incidence of NEC and mortality. However, debate still remains about the safety of probiotics and their influence on normal infant growth. We performed this meta-analysis to assess the safety and benefits of probiotic supplementation in preterm infants. We searched in PubMed, Embase, and Cochrane databases for English references, and in Wanfang, VIP, and CNKI databases for Chinese references. Ultimately, 27 RCTs (including 9 Chinese articles) were incorporated into this meta-analysis. Relative risk (RR) and weighted mean difference (WMD) were calculated using a random-effects or fixed-effects model, depending on the data type and heterogeneity. A total of 6655 preterm infants, including the probiotic group ($n = 3298$) and the placebo group ($n = 3357$), were eligible for inclusion in this meta-analysis. For Bell stage $\geq I$ and gestational age < 37 weeks, risk of NEC incidence was significantly lower in the probiotic group [RR = 0.35, 95% confidence interval (CI) = 0.27-0.44, $P < 0.00001$]. For Bell stage $\geq II$ or gestational age < 34 weeks, there were likewise significant differences between the probiotic and placebo groups concerning NEC incidence (RR = 0.34, 95%CI = 0.25-0.48, $P < 0.00001$; and RR = 0.39, 95%CI = 0.27-0.56, $P < 0.00001$). Risk of death was significantly reduced in the probiotic group (RR = 0.58, 95%CI = 0.46-0.75, $P < 0.0001$). In contrast, there was no significant difference concerning the risk of sepsis (RR = 0.94, 95%CI = 0.83-1.06, $P = 0.31$). With respect to weight gain and the age at which infants reached full feeds, no significant differences were found between the probiotic and placebo groups (WMD = 1.07, 95%CI = -0.21-2.34, $P = 0.10$; and WMD = -1.66, 95%CI = -3.6-0.27, $P = 0.09$). This meta-analysis has shown that, regardless of gestational age and NEC stage, probiotic supplementation could significantly reduce the risk of NEC in preterm infants. Analysis also indicated that such supplementation did not increase the incidence risk of sepsis or of mortality. Finally, the study showed that probiotic supplementation may have no adverse effect on normal feeding and growth.

Key words: Necrotizing enterocolitis; Probiotics; Meta-analysis; Preterm

Introduction

Acute inflammatory necrosis of the intestinal tract, necrotizing enterocolitis (NEC) is the most common acquired gastrointestinal disease for preterm infants in neonatal intensive care units (NICU). It has also been a leading cause of morbidity and mortality in preterm infants. According to some annual statistics for the United States, among very low birth weight (VLBW) infants, approximately 20-30% of diagnosed NEC patients will die as a result of this disease and its complications. A similarly high annual mortality, approximately 10-50%, occurs in China for preterm infants with NEC.

While remarkable advances have been made in the fields of perinatology and neonatology over the past two decades, the understanding of NEC pathogenesis remains incomplete. Among the numerous theories for pathogenesis, there is wide agreement that NEC is a

complicated syndrome characterized by intestinal injury, inflammation, and necrosis. It is characterized by a diversity of alterations in mucosal defenses, gastrointestinal microbiota, and imbalances of inflammatory responses, thus implicating a multifactorial pathophysiology – including host factors, enteral feeding, abnormal bacterial colonization, and inflammatory propensity of the immature gut (1). In the past several years, studies with limited success have focused on supplementation with immunologically relevant factors (such as IgA, glutamine, and oral lactoferrin), intravenous dexamethasone, and other approaches. However, due to lack of support for evidence-based medicine and potential side effects of such treatments, these so-called approaches are not in routine use at present, and effective measures for the prevention and treatment of NEC remain limited.

Correspondence: X.Y. Zhou and/or Y. Li, Department of Neonates, Nanjing Children's Hospital, Nanjing Medical University, 72 Guangzhou Road, Nanjing, Jiangsu 210008, China. E-mail: xyzhou161@163.com

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Currently, the most common strategies associated with a reduced risk of NEC are still conservative feeding practices and antibiotics. According to the American Society for Parenteral and Enteral Nutrition (2), breast milk feeding is also a recommended practice for reducing NEC risk. However, it is still unclear whether the amount and/or timing of mother's milk administration have an effect on the incidence of NEC, and, therefore, it has not yet been widely applied in China. Despite these approaches, the incidence and mortality of this disease have not been significantly diminished (3).

In the light of this situation, there has recently been an increase in the number of studies reporting the use of probiotics to prevent NEC – in both experimental animal models and clinical applications. Given their gastrointestinal introduction of mutualistic or commensal symbiotic bacteria, probiotics have the potential to confer many beneficial effects to the host at the cellular level. For example, such symbionts can promote maturation of the intestinal barrier function, reduce growth of potentially pathogenic organisms, enhance the production of anti-inflammatory cytokines, increase antioxidant activities, and regulate apoptosis (4,5). From a macroscopic perspective, many clinical studies included in this meta-analysis also showed a positive impact of probiotics in the prevention of NEC and its complications.

With these considerations in mind, our meta-analysis was designed to attempt to clarify uncertainties in three areas of probiotic supplementation. First, given the different pathological stages of NEC and the different phases of gestational age, are there significant differences between probiotic and placebo groups concerning NEC incidence? Second, with respect to safety and possible side effects of probiotics, are the incidences of sepsis and mortality higher in the probiotic group? Third, based on the potential probiotic influence on normal feeding and growth of preterm infants, are there significant differences between probiotic and placebo groups with respect to weight gain and the age at which infants reach full feeds (120-150 mL/kg per day enteral feeding)?

Material and Methods

Study selection

Guidelines from the Consolidated Standards of Reporting Trials (CONSORT) group and the CONSORT statement were followed for this systematic review and meta-analysis (6). In order to screen for eligible studies published since each database was established, a search was conducted by two investigators involved in this research in the PubMed, Embase, and Cochrane databases for studies in English, and in the Wanfang, VIP, and CNKI databases for Chinese studies (databases were last launched on March 18, 2013). The following search terms were employed: “necrotizing enterocolitis,” “preterm infants,” “probiotics,” “lactobacillus,” “saccharomyces,”

and “bifid bacterium.” The inclusion criteria of this meta-analysis were as follows: 1) randomized, controlled trials (RCTs) involving preterm infants (without consideration of birth weight) and reporting on Stage I or higher NEC (according to the modified Bell staging criteria), and 2) enteral administration of any probiotic started within the first 10 days of life and continued for at least 7 days. Hence, reviews, meta-analyses, animal experiments, and studies without sufficient clinically relevant data were excluded. Any discrepancies were independently resolved by a third investigator involved in this research.

Data abstraction

The CONSORT statement contains 22 items including participants, intervention, objectives, outcomes, randomization, blinding, statistical method, participant description, recruitment, baseline data, and others. The quality of all included RCTs was assessed according to the CONSORT items and Jadad score. Finally, from the full text and corresponding supplementary information, the following eligibility items were collected and shown in tables for each study: author, year of publication, title, abstract, birth weight, gestation, participant description, baseline data, feeding patterns, number of participants (probiotic/placebo), probiotic agents, dosage, duration, adverse events, outcomes, follow-up, randomization, blinding, Jadad score, and CONSORT items. Subsequently, the outcomes were divided into three parts. First, the primary outcome of interest was the efficacy of probiotic supplementation in the prevention of NEC in preterm infants. In addition, the assessment was further explored according to different NEC stages (Bell stages \geq II) and gestation ages (gestation age $<$ 34 weeks), respectively. Second, with respect to the possible complications of probiotics, the proportions of culture-positive sepsis and mortality were compared between probiotic and placebo groups. Third, in the light of the potential influence on normal feeding and growth in preterm infants, the potential effect of probiotic supplementation on weight gain (g/week) and age of reaching full feeds (120-150 mL/kg per day enteral feeding) were explored.

Statistical analysis

For each outcome (incidence of NEC, mortality, sepsis, weight gain, and age of reaching full feeds), either relative risk (RR) or weighted mean difference (WMD) with the 95% confidence interval (95%CI) was calculated, depending on the data type. Both a fixed-effects model and a random-effects model were considered. For each meta-analysis, the χ^2 -based Q statistic test (Cochran Q statistic) was applied to test for heterogeneity, and the I^2 statistic was also used to quantify the proportion of the total variation attributable to heterogeneity. For $P < 0.10$ or $I^2 > 50$, the assumption of homogeneity was assumed to be invalid, and the random-effects model was used; for

$P \geq 0.10$ and $I^2 \leq 50$, data were assessed using the fixed-effects model. Publication bias was investigated by funnel plot, and an asymmetric plot suggested possible publication bias. Statistical analyses were performed using Review Manager 4.2 (Cochrane Collaboration, Nordic Cochrane Centre, Denmark). A two-tailed P value of less than 0.05 was considered to be statistically significant.

Results

Demographic characteristics of the studies

After searching the above databases, 115 potentially relevant studies on probiotic supplementation for preterm infants were obtained. Details of the search process are shown in Figure 1. After carefully reviewing and extracting data from the publications, 3 RCTs were further excluded because of inconsistent research content pertaining to our topic or an absence of relevant clinical data. A search of other aforementioned databases did not identify any additional eligible studies. Ultimately, we identified 27 original RCTs (18 in English, 9 in Chinese), including the probiotics group ($n = 3298$) and placebo group ($n = 3357$; Table S1). The quality of all RCTs included in this meta-analysis was assessed by the Jadad score and CONSORT items (Table S2).

Effect of probiotics on NEC

First, with respect to Stage I or higher NEC (Bell stage $\geq I$; gestational age < 37 weeks), data on definite NEC were reported for all 27 trials (probiotic group/placebo group = 3298/3357; Figure S1). There was no significant heterogeneity among these trials ($\chi^2 = 16.96$, $P = 0.88$; $I^2 = 0\%$). Meta-analysis of data using a fixed-effects model estimated a reduced risk of NEC in the probiotic supplement group. Probiotic supplementation was associated with a

significantly decreased risk of Stage I or higher NEC in preterm infants with an RR of 0.35 (95%CI = 0.27-0.44; $P < 0.00001$) compared with the placebo group.

Second, regarding the effect of probiotics on Stage II or higher NEC in preterm infants (Bell Stage $\geq II$; gestational age < 37 weeks), there were 17 eligible studies included (probiotic group/placebo group = 2042/2156), and no significant heterogeneity was detected among these trials ($\chi^2 = 9.96$, $P = 0.82$; $I^2 = 0\%$). A significantly decreased risk of NEC (Bell Stage $\geq II$) was found in the probiotic group compared with the placebo group (RR = 0.34; 95%CI = 0.25-0.48; $P < 0.00001$; Figure S2).

Third, with respect to the influence on NEC in early preterm infants (Bell Stage $\geq I$; gestational age < 34 weeks), 12 studies were included for this meta-analysis (probiotic group/placebo group = 1266/1229). The analysis showed that, compared to the placebo group, there was still a significantly lower risk in the probiotic group (RR = 0.39; 95%CI = 0.27-0.56; $P < 0.00001$; Figure S3), and there was no significant heterogeneity in these trials ($\chi^2 = 6.68$, $P = 0.75$; $I^2 = 0\%$).

Effect of probiotics on mortality and sepsis

In assessing the major risks of probiotic use, mortality and sepsis were compared between probiotic and placebo groups in this meta-analysis. First, data for mortality between the probiotic group and placebo group were reported in 14 trials (probiotic group/placebo group = 1789/1794). There was no significant heterogeneity among these trials ($\chi^2 = 13.61$, $P = 0.40$; $I^2 = 4.5\%$); therefore, a fixed-effects model was applied. The result showed a reduced risk for mortality from all causes in the probiotic group vs the placebo group (RR = 0.58; 95%CI = 0.46-0.75; $P < 0.00001$; Figure S4).

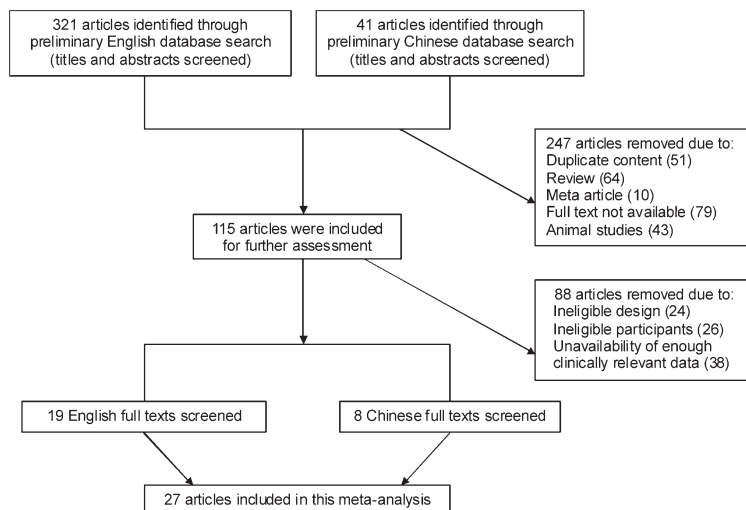


Figure 1. Flow diagram of the selection of articles for inclusion in the meta-analysis.

Second, data for culture-positive sepsis in infants with probiotic supplementation were reported in 17 trials (probiotic group/placebo group = 1950/2093). There was no significant heterogeneity among the trials ($\chi^2=21.1$, $P=0.17$; $I^2=24.2\%$); therefore, a fixed-effects model was applied. No significant difference in the risk for sepsis was found between the two groups (RR = 0.94; 95%CI = 0.83-1.06; $P=0.31$; Figure S5).

Effect of probiotics on weight gain and age reaching full feeds

The potential effect of probiotic supplementation on normal feeding and growth, weight gain, and the age at which infants reached full feeds (120-150 mL/kg per day enteral feeding) were compared between probiotic and placebo groups in this meta-analysis. First, data for weight gain in probiotic-supplemented and placebo groups were reported in 2 trials (probiotic group/placebo group = 205/199). There was heterogeneity among these 2 trials ($\chi^2=3.47$, $P=0.06$; $I^2=71.1\%$); therefore, a random-effects model was applied. The results showed that there was no difference for weight gain between the probiotic group and the placebo group (WMD = 1.07; 95%CI = -0.21-2.34; $P=0.10$; Figure S6).

Second, data for the age at which infants reached full feeds in the probiotic-supplemented and placebo groups were reported in 9 trials (probiotic group/placebo group = 821/805). There was significant heterogeneity among these trials ($\chi^2=78.93$, $P<0.00001$; $I^2=89.9\%$); therefore, a random-effects model was applied. The results showed that there was no significant difference for the age at which infants reached full feeds in the probiotic group vs the placebo group (WMD = -1.66; 95%CI = -3.60-0.27; $P=0.09$; Figure S7).

Publication bias

All trials included in the meta-analysis had Jadad quality scores ≥ 3 . A funnel plot was performed in order to assess the potential publication bias in this meta-analysis. In analyzing the effect of probiotic supplementation on NEC risk (Bell Stage $\geq I$), we visually evaluated the symmetry of funnel plot shape and did not find obvious evidence of asymmetry.

Discussion

NEC is one of the most devastating diseases in the NICU. Data obtained from large, multicenter neonatal network databases showed a mean prevalence of 7% in infants weighing <1500 g and an estimated mortality of 15-30% in the United States and Canada (34). In light of this grim situation, an increasing number of neonatologists have turned their attention to the study of this fatal disease. Unfortunately, regarding its pathogenesis, NEC has always been viewed as a complex disease. It appears that multiple factors involving immature intestinal function

contribute to the pathogenesis: gastrointestinal dysmotility, impaired digestive capacity, altered regulation of intestinal blood flow, barrier dysfunction, altered anti-inflammatory regulation, and impaired host defenses (35). Moreover, NEC can rapidly progress from early clinical signs to extensive intestinal necrosis within hours, thereby limiting the effectiveness of therapeutic intervention.

In the past several years, studies using probiotics to prevent NEC have aroused the interest of neonatologists. Probiotic microorganisms are often referred to as commensal bacteria or protective microorganisms; they are nonpathogenic and are part of the normal intestinal flora. As previously mentioned, numerous factors are believed to contribute to the risk for NEC development in premature infants. The gastrointestinal tract of premature infants exhibits abnormal bacterial colonization, deficient barrier function, immature mesenteric circulation, and imperfect immune defenses (36). Probiotics have the potential to play a constructive role in mitigating these abnormalities. Probiotic supplementation can allow the acquisition of normal commensal flora and can support the transition to an intestinal microbiome with beneficial microbes through enhancing epithelial barrier function and producing direct anti-inflammatory effects on pathogens in epithelial signaling pathways (37).

As such, probiotics are, theoretically, the most promising treatment on the horizon for this devastating disease. In our meta-analysis, 24/27 RCTs (probiotic group/placebo group = 3298/3357) showed the positive effect of probiotics in preventing NEC (Stage I or higher) with an RR of 0.35 compared with the placebo group. In addition, to avoid error and bias as much as possible, we further explored the probiotic effect on higher NEC stages (NEC Stage $\geq II$; probiotic group/placebo group = 2042/2156) and early preterm infants (gestation age <34 weeks; probiotic group/placebo group = 1266/1229). For both parameters, this analysis also showed a significant probiotic protective effect in the prevention of NEC (RR = 0.34; 95%CI = 0.25-0.48; $P<0.00001$ and RR = 0.39; 95%CI = 0.27-0.56; $P<0.00001$). Hence, probiotic supplementation has potential as an effective way to minimize or prevent NEC.

Conversely, there remain concerns that limit widespread clinical use, with many researchers concerned about probiotic safety. Probiotic supplementation may improve colonization by commensal bacteria, but there are also numerous other factors influencing colonization. Many RCTs use live probiotic organisms, which makes infectious processes such as sepsis a potentially serious problem. In our study, regarding the incidence of sepsis, we found 9/17 studies (probiotic group/placebo group = 1950/2093) that reported a lower incidence of sepsis in the probiotic group but, in general, showed no significant difference in the risk for sepsis between the two groups (RR = 0.94; 95%CI = 0.83-1.06; $P=0.31$). In addition, regarding mortality, data from 12/14 studies

(probiotic group/placebo group = 1789/1794) showed that probiotic supplementation could significantly reduce mortality (RR = 0.58; 95%CI = 0.46-0.75; $P < 0.00001$). These results are basically in agreement with a previous meta-analysis (38), which, at least to some extent, supports the safety of clinical probiotic use. However, it should be noted that probiotics contain numerous obligate anaerobes and that some studies did not report or utilize specific anaerobic culture methods. Hence, the resulting analysis of sepsis linked to probiotic supplementation should be viewed with caution.

In general, adverse events constitute a significant problem facing pediatricians. Along these lines, the Agency for Healthcare Research and Quality conducted a comprehensive systematic review to assess the safety of probiotics (39). Of their 622 reviewed studies, nearly half made only a nonspecific safety statement; interventions and adverse events were likewise poorly documented. Additionally, some reviewed case studies described fungemia, and others, bacteremia, potentially associated with administered probiotic organisms. However, in discussions of RCTs, no statistically significant increased relative risk of adverse events was found. Similarly, in our meta-analysis, many studies carefully observed and compared possible adverse events such as vomiting, apnea, jaundice, and infection (see Table S2), with the result that there was no significant difference in adverse events. It should be emphasized, however, that these outcomes were in the context of short-term probiotic use; hence the potential adverse effects of long-term probiotic administration remain unknown. Additionally, many probiotic studies do not systematically address and report adverse events.

There has been a paucity of studies that have discussed the effects of probiotic administration on normal feeding and growth of preterm infants. In order to approach these aspects in our meta-analysis, we chose to explore the possible effect of probiotic supplementation via the related parameters of weight gain and the age at which infants reach a complete feeding pattern. The results showed that there was no significant difference between probiotic and placebo groups for these parameters

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(WMD = -1.66, 95%CI = -3.60-0.27, $P = 0.09$; and WMD = 1.07, 95%CI = -0.21-2.34, $P = 0.10$). Another recent study with 2 year olds showed that oral probiotics given to premature VLBW infants after age 1 week to reduce NEC incidence did not affect either their growth or their normal neurodevelopmental progress (40), thereby further supporting probiotic safety using a different but related approach.

In addition to the aforementioned concerns, we must note additional limitations to some recent research. The available studies do not focus on one specific product or dosing regimen. In addition, methods of specific randomization and detailed feeding regimens are generally not included in published reports. Some studies include the declaration that the research to date is not adequate to draw precise conclusions. Given these limitations, perhaps the focus of future studies should not be directed simply at questioning the benefits of probiotics but, rather, should explore, in more depth, optimal dosing and duration of therapy for specific target groups. Fortunately, some large, multicenter RCTs are in progress to address these concerns. Perhaps these will become guidelines for clinicians in the use of probiotics for the prevention of NEC.

In conclusion, regardless of gestational age and NEC stage, our results indicated that probiotic supplementation could significantly lower the risk of NEC in preterm infants. In addition, it suggests that probiotic supplementation does not increase the risk of either the incidence of sepsis nor mortality and that probiotics may also have no adverse effect on the normal feeding and growth of preterm infants.

Supplementary Material

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Acknowledgments

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