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Probiotics and Necrotizing Enterocolitis

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Abstract

In this review, we summarize existing knowledge regarding the effects of probiotics on necrotizing enterocolitis (NEC). We review the role of the microbiome in NEC and pre-clinical data on mechanisms of probiotic action. Next, we summarize existing randomized controlled trials and observational studies of probiotics to prevent NEC. We also summarize findings from several recent meta-analyses and report a new cumulative meta-analysis of probiotic trials. Finally, we review data from cohorts routinely using commercially available probiotics. Our goal is to inform clinicians about the risks and benefits of probiotics, which may be helpful for those considering use in preterm infants to prevent NEC, death or sepsis.

Keywords

Preterm; neonate; bacteria; microbiome; gut; meta-analysis

Introduction

Probiotics are live microorganisms that confer a health benefit to the host when ingested. In preterm infants, probiotics have been widely studied and used to improve health outcomes and reduce morbidity and mortality. In particular, probiotics have been studied as a therapy to decrease the risk of necrotizing enterocolitis (NEC), a serious intestinal disorder that primary affects preterm infants (1). NEC is a multifactorial disease with a pathogenesis that is incompletely understood (2), although type of feeding (with own mother's milk associated with decreased risk and bovine-origin products associated with increased risk) (3) and an abnormal gut microbiome (4, 5) are two important determinants. In this review, we discuss mechanisms of probiotic action in the immature gut, review clinical trials investigating the use of probiotics, report a new cumulative meta-analysis of the effect of probiotics on NEC

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and discuss commercially available preparations and results from observational studies reporting on routine probiotic supplementation.

Overview of the epidemiology and pathophysiology of NEC

NEC is the most common serious gastrointestinal disease in preterm infants and the most common single cause of death in extremely preterm infants from 2 weeks to 2 months of age (6). The disease primarily affects infants <32 weeks' gestation and the incidence is inversely proportional to gestational age (3). Beyond gestational age, clinical risk factors include, but are not limited to, small for gestational age, premature rupture of membranes, assisted ventilation, sepsis, and hypotension (7). NEC does not occur in utero and is rare prior to the onset of feeding. In addition, potentially modifiable risk factors include formula feeding (8, 9), and exposure to acid suppression medications (10) and prolonged empiric antibiotics (11, 12).

The role of the microbiome in NEC

The associations between NEC and antibiotic use, acid suppression use, enteral dilute hydrochloric acid (13) and enteral antibiotics (14), all of which alter the infant's intestinal microbiome, support the role of abnormal gut bacteria (dysbiosis) as a major determinant of NEC.

Several non-culture based case-control studies have shown that early dysbiosis, with a bloom of intestinal *Gammaproteobacteria*, precedes NEC in many preterm infants (4, 5). However, the underlying causes of this bloom and mechanisms by which this results in NEC in some infants and not others remain to be elucidated. In addition, experimental models of NEC have used the administration of exogenous Gram-negative bacteria, along with hypoxia and ischemia, to cause NEC-like intestinal injury, (15) suggesting that abnormal microbiota are an important component of the causal pathway of NEC. Beneficial commensal bacteria, such as bifidobacteria, are abundant in breastfed term infants, likely due to human milk oligosaccharides which are selectively consumed by many *Bifidobacterium* species (16, 17). By contrast, these bacteria are less common in premature infants and even less abundant in preterm infants who go on to develop NEC compared to controls (16). Beyond feeding, antibiotic use may also decrease the abundance of bifidobacteria (18), which may explain some of the epidemiological associations previously noted between prolonged antibiotic exposure and a higher risk of NEC.

The immature preterm gut, which is being exposed to newly colonizing commensals and pathogens, has an innate immune system that is constantly interacting with microbial ligands such as peptidoglycan and lipopolysaccharide (19). Importantly, the immature gut has a propensity towards inflammation. A major driver of the inflammation seen in NEC is the activation of the Toll-like receptor 4, which is thought to play a central role in the pathogenesis of NEC (20, 21). Probiotics have been shown to influence the innate and adaptive immune pathways involved in the pathogenesis of NEC (19, 22).

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Mechanisms of Probiotic Action

In vitro and animal studies have demonstrated a number of mechanisms by which probiotics and commensal bacteria protect the immature gut against inflammation and injury (Figure 1). Although these mechanisms may be specific to individual commensal or probiotic strains (23), they provide insight into how probiotics prevent NEC. There are a number of mechanisms of probiotic action in the gut, which include: 1) upregulation of cytoprotective genes (24); 2) downregulation of pro-inflammatory gene expression (25–28); 3) production of butyrate and other short chain fatty acids that nourish colonocytes and lower the pH and oxygen tension within the intestinal lumen thereby suppressing growth of pathogenic Enterobacteriaceae (phylum Proteobacteria)(29, 30); 4) support of barrier maturation and function (31, 32); 5) competition with other microbes (33); 6) regulation of cellular immunity and Th1:Th2 balance (2, 34).

Randomized trials of probiotics to prevent NEC

Probiotics have been extensively studied in preterm infants, with trials to date enrolling over 10,000 infants (Table 1) (35). However, studies have utilized a wide variety of bacterial strains, most commonly *Bifidobacterium*, *Lactobacillus* or a combination of the two. In addition, studies have used different total doses, ages at initiation, and durations of treatment (35, 36). Despite this clinical heterogeneity, the cumulative meta-analysis of studies show a strong treatment effect of probiotics in the reduction of NEC (pooled relative risk, random-effects: 0.53; 95% CI 0.42–0.66; Figure 2).

Following initial small studies in the latter part of the 20th century, probiotics have now been studied in over 35 randomized trials in preterm infants in both developed and developing countries. After reaching the strongest pooled cumulative treatment effect on NEC in 2009 (RR 0.32; 95% CI 0.20–0.49), which followed the publication of a multicenter study from Taiwan (37), the treatment effects of probiotics on NEC have remained significant but slightly diminished over time. Although there is a substantial amount of clinical heterogeneity in studies evaluating probiotic use in preterm infants owing to the different preparations used, there is relatively low statistical heterogeneity among studies in the pooled meta-analysis (I^2 11%) with a number of individual studies showing statistically significant effects in the reduction of NEC (Table 2) and no studies showing an increase in the risk of NEC.

Multiple meta-analyses have shown pooled estimates of treatment effect of probiotics in reducing NEC that support a clinically meaningful effect (Table 1). However, individual consideration of each study is necessary given the clinical heterogeneity of the studies of probiotics included in meta-analyses. This is highlighted by the recent Probiotics in Very Preterm Infants (PiPS) trial, which is the largest trial of probiotics use in preterm infants to date (38). The study treated 1,315 infants with *Bifidobacterium breve* or placebo and found no difference in the risk of NEC between probiotics vs. placebo treatment arms (adjusted risk ratio 0.93; 95% CI 0.68–1.27). Of note, there was no harm reported with the use of probiotics in this trial. The addition of this study to the cumulative meta-analysis led to a modest increase in heterogeneity from 0% to 11% and diminishing of treatment effect of

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probiotics on NEC from a pooled relative risk of 0.47 to 0.53 (Figure 2). Of note, the study did highlight the potential for crossover of the effect of probiotics as 20% and 49% of the infants in the placebo group were colonized with the probiotic organism by 2 weeks of life and 36 weeks post-menstrual age, respectively, with cross-contamination noted at every study site (24 hospitals). This may have diminished the results of the trial towards the null, although the incidence of NEC was not significantly different among infants colonized with the probiotic compared to those not colonized (7% vs 13%, adjusted risk ratio 0.68; 99% CI 0.43–1.09).

There have been several recent systematic-reviews and meta-analyses evaluating the use of probiotics to reduce NEC, death or sepsis (Table 1). Although systematic reviews have had different inclusion of studies, all have reported similar estimates of the treatment effects of probiotics on NEC, death and sepsis. While all recent meta-analysis have concluded that probiotics effectively decrease NEC and all-cause mortality, the analyses differ in conclusions of the effects of probiotics on sepsis, with some pooled estimates suggesting a significant benefit (39, 40) and others no significant benefit (upper 95% CI of relative risk ending at 1.0) (35). Given the number of studies to date and the strength of the treatment effect on NEC and death (Table 1), it is unlikely that additional studies will change the conclusion that probiotics decrease NEC and death, when studies are pooled together (Figure 2). However, additional trials to guide the optimal choice of preparation, including the availability of preparations that have been approved through regulatory frameworks as medications, may increase confidence in the reproducibility of the effects of probiotics observed in studies to date. The considerations related to the quality and consistency of probiotic products are discussed later in this paper.

Observational studies of probiotics to prevent NEC

As with all trials, it is important to acknowledge that the *efficacy* of a treatment in a controlled-trial may differ from the *effectiveness* of a treatment in routine practice. For the use of probiotics, multiple implementation cohort studies allow for a comparison of the treatment effects on NEC between clinical trials and observational studies (Figure 3). Reassuringly, the pooled treatment effects of probiotics on NEC, death and late-onset sepsis in clinical trials have been similar to those in observational studies, although the statistical heterogeneity is larger in the observational studies. The largest implementation cohort study to date has involved the use of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Infloran) in Germany (41). The study included over 5,000 infants and found infants supplemented with Infloran, compared to those not supplemented, had a lower risk of surgical NEC (adjusted OR 0.58, 95% CI 0.37–0.91) (Table 2). In addition to this study from Germany, other implementation cohort studies from Canada, US, France, Australia, and Switzerland have reported significant decreases in the incidence of NEC after routine use of probiotics (Table 2). These findings support the external validity of the pooled estimates of probiotic treatment effects from randomized trials (Figure 3). Table 3 presents a comparison of probiotic administration to other studied interventions intended to prevent NEC.

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Commercial probiotic preparations

One of the important decisions involved in the use of probiotics to prevent NEC is the choice of product. Clinicians and researchers must choose from a large number of commercially available preparations, some of which are summarized in Tables 2 and 4. The variability in preparations used in the US was evaluated in a phone survey of neonatal intensive care units (NICUs) (42). The survey reported that 16 different commercial products were used in 44 US NICUs or 9% of those surveyed. The most common probiotics, accounting for over 50% of use in NICUs, were single strain preparations of *Lactobacillus rhamnosus* GG (LGG) or *Lactobacillus reuteri* (Table 4). LGG, in the form of Culturelle, was the most common probiotic used in the US. Manzoni et al. evaluated LGG, as Diclofor, alone and with Lactoferrin in several studies (43, 44). In addition, a recent cohort study in France reported a decrease in the risk of NEC from 5.3% to 1.2% with the use of LGG (45). Of note, nasogastric tube clogging has been reported with the use of LGG (46).

The second and third most common probiotic products used in the US included *Lactobacillus reuteri* (42). This strain has been used in several trials and observational studies (Table 4). A strain-specific meta-analysis reported that *Lactobacillus reuteri* decreases the risk of late-onset sepsis but not NEC (47). Of note, a randomized trial of *Lactobacillus reuteri* in Turkey did not show a beneficial effect on NEC (48), which contrasts a single-center observational study in which the incidence of NEC decreased from 15.1% to 2.5% following the adoption of routine supplementation (46). *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Infloran) is one of the most widely studied probiotic preparations, having been used in 5 studies involving over 7,000 infants (Table 4), including a large implementation study in Germany (41). However, the *Bifidobacterium* species and strain in this probiotic has been changed over the years and this product has not been commonly used in the US and has been associated with several case reports of probiotic-associated sepsis (49).

Given the lack of a regulator-approved probiotic preparation that is widely available, we recommend that quality improvement principles be used to assess the beneficial (or harmful) effects of routine clinical use of probiotics should centers decided to use currently available commercial supplements. Such approaches should measure the adherence to probiotic supplementation as a process measure, NEC as an outcome measure and episodes of probiotic-associated sepsis as a balancing measure. Such initiatives could then inform the decision to continue with a given probiotic or change to another preparation based on the observed changes over time in the incidence of NEC. Based on the experience in Germany and other centers, multicenter quality improvement may accelerate efforts to decrease the incidence of NEC. Currently, there are insufficient data to recommend any particular probiotic product, although we have summarized common products used in both randomized trials and observational studies in Tables 2 and 4.

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Remaining questions about probiotic use

Quality of preparations

Concerns regarding the quality of probiotic products have been raised by scientific societies (50), with specific concerns regarding the quality control process and differences between the label and actual content. The lack of adequate quality control for some products was illustrated in a recent study in which 16 products were evaluated to determine if the bacterial species noted on the label matched the contents identified by both DNA and culture-based methods (51). In this study, only 1 of 16 products containing bifidobacteria exactly matched the label. In addition, there was substantial variability in the composition of probiotic products by differing lots and pills. As many probiotic products are considered dietary supplements and do not fall under regulatory frameworks for pharmaceutical products in most countries, the balance between improving oversight of probiotic quality and discouraging additional study due to regulatory burdens has been highlighted by the proceedings from an international workshop on probiotics (52). Several phase 2 randomized, placebo-controlled, multicenter trials are ongoing that will evaluate the use of probiotics to prevent NEC (ClinicalTrials.gov NCT02472769, NCT01954017). These trials, if they progress and are successful, may yield products approved by regulatory agencies such as the Food and Drug Administration that could address some of the quality concerns noted and increase the use of probiotics in preterm infants in the US; however, it is likely to be a number of years before such products could potentially be available.

Safety

Although several meta-analyses reported an overall decrease in the incidence of late-onset sepsis with probiotic use (Table 1), there are concerns about the risk of probiotic-associated sepsis when administering live microorganisms to immature infants. There have been several case reports of probiotic-associated sepsis, mostly from *Bifidobacterium longum* associated with the use of Infloran (49), from *Lactobacillus rhamnosus* (53) and from the fungal probiotic *Saccharomyces* (54, 55). However, given the large number of infants studied in randomized trials to date and the overall favorable effect of probiotic supplementation on the risk of late-onset sepsis and death (Table 1), the absolute risk of sepsis from probiotic supplementation is likely to be low. Of note, the incidence of probiotic-sepsis is difficult to characterize due to the infrequent occurrence and potential ascertainment bias due to different blood culture media used to grow bacteria. In addition, issues related to the quality control of probiotic products remain as evidenced by the single case report of death in a premature infant from contamination of a commercial probiotic product with a pathogen (56, 57).

Long-term outcomes

There are limited follow-up studies of preterm infants enrolled in probiotic trials to guide an assessment of long-term efficacy or safety. In a randomized trial of 400 VLBW infants with follow-up of 249 infants at 18–24 months' corrected age, the use of *Lactobacillus reuteri* did not increase or decrease the risk of adverse neurocognitive outcome assessed using the Bayley Scales of Infant and Toddler Development II (58). Follow-up of the ProPrems trial is ongoing and will provide additional data regarding the long-term risks and benefits of

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probiotics. Other long-term outcomes such as atopic disease have been studied in more mature populations of infants. A systematic-review and meta-analyses of these studies have found probiotics may prevent infantile eczema but do not affect other atopic diseases such as asthma or wheezing (59).

Optimal dose, age and duration of treatment initiation

There is variability in the doses, age at initiation and duration of probiotic supplementation in randomized trials, as highlighted in a previous review (36). The majority of trials have used a dose of 1 to 6 × 10^9 CFU/d with initiation of treatment within the first several days of birth. Studies using probiotics at a daily dose below 1 × 10^9 CFU per day have had mixed results. The PiPs trial showed no benefit with a preparation of *Bifidobacterium breve* at a dose of 8.3 to 8.8 × 10^8 CFU/d (38). By contrast, cohort studies using *Lactobaccilus reuteri* at a daily dose of 1 ×10^8 CFU/d (46) and LGG at 4 ×10^8 CFU/d (45) have both reported associations between probiotic supplementation and a lower risk of NEC. In the only published dose escalation trial of probiotics in premature infants to date, doses of 1.4 × 10^9 CFU twice daily of *Bifidobacterium infantis* led to maximal fecal colonization while there was no significant colonization at any of the studied doses for *Bifidobacterium lactis* (60). In terms of duration of therapy, most trials have provided probiotic supplementation for at least 28 days, with several continuing through discharge (35, 36). In a retrospective cohort study in 3 NICUs in Switzerland and Germany, a shorter duration of probiotics supplementation for 10 to 14 days was associated with a lower risk of NEC (61). Therefore, it remains unclear if treatment for a greater duration of time confers a larger benefit to preterm infants and additional studies are needed to guide the optimal dose and duration of therapy.

Conclusion

In conclusion, a large number of pre-clinical studies provide mechanistic insight into how probiotics support gut health and may decrease NEC. These results support the beneficial effect of probiotics observed in meta-analyses of both randomized trials and observational studies. The cumulative meta-analysis demonstrates a significant but diminished treatment effect of probiotics on NEC over time; however, the overall effect on NEC is unlikely to change substantially given the large number of trials and patients studied to date. Additional studies are needed to guide clinicians in the most appropriate probiotic product to decrease NEC; however, further small, traditional placebo-controlled trials that are not pursuing a drug regulatory pathway are of questionable ethical and clinical value. Cluster-randomized clinical trials (i.e. randomization of the neonatal intensive care unit rather than the individual infant) comparing available commercial probiotics to each other would require a very large sample size but would be of great value. Future studies will be of greatest value if they report independent confirmation of the purity and viability of administered probiotic strains.

If, after reviewing available data with relevant stakeholders including the parents of premature infants, clinicians opt to pursue routine supplementation of currently available products, quality improvement approaches should be utilized to measure for the desired effects of probiotics on the risk of NEC and also to assess for safety at a given center. The NEC Society website (NECSociety.org) contains information for clinicians interested in

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participating in a planned large multi-center quality improvement study of probiotic administration (see the tab marked “low-cost NEC QI”).

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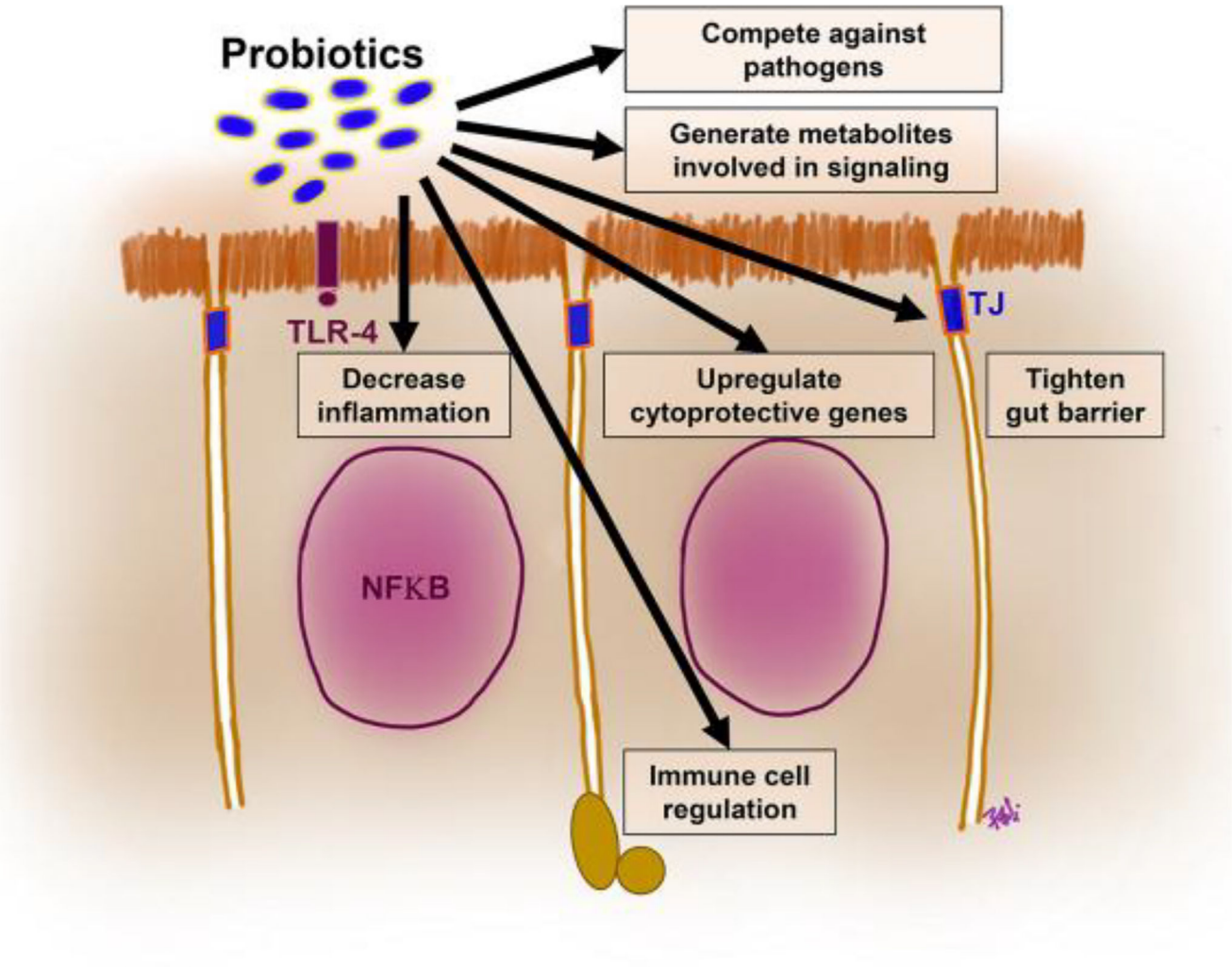

Figure 1. Mechanisms of probiotic action

Figure depicts potential mechanisms by which probiotics exert beneficial effects on the immature gut. Abbreviations: TLR-4, Tolllike receptor 4, TJ, tight junction; NFKB, nuclear factor kappa B.

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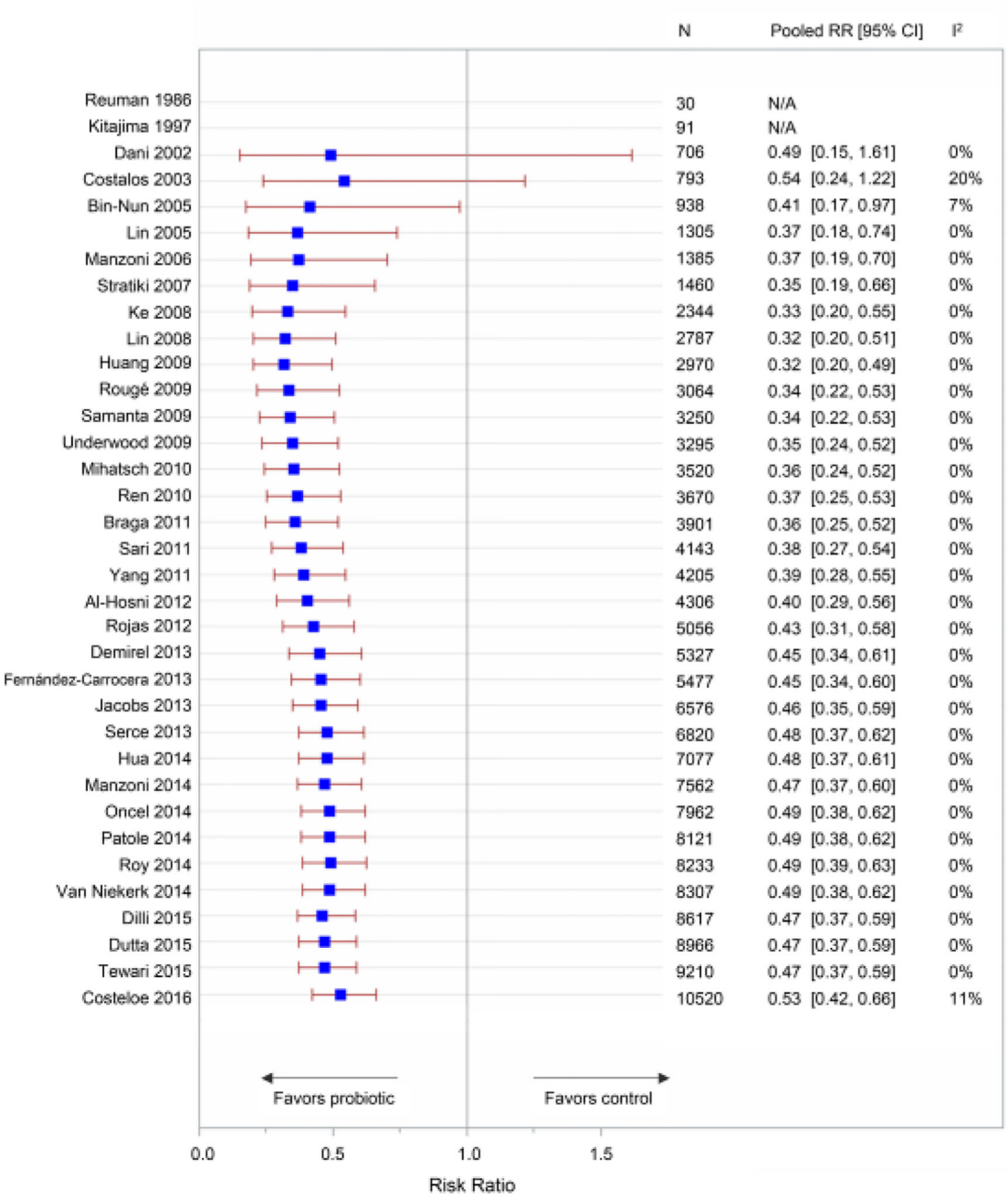

Figure 2. Cumulative pooled meta-analysis of the effects of probiotics on NEC

The cumulative pooled risk ratio for NEC among trials from 1997 through 2016. Studies selected from a recent meta-analysis (DOI: 10.7717/peerj.2429/supp-1)(35) and sorted, first, by year of publication and then alphabetically by author. Cumulative pooled risk ratios (Mantel-Haenszel method with random effects model) including each study along with prior studies generated using RevMan 5.3 (Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). The N reflects the cumulative number of enrolled patients. Abbreviations: NEC, necrotizing

enterocolitis; N/A, no applicable as no events in either group; N, cumulative number of infants; RR, relative risk; CI, confidence interval.

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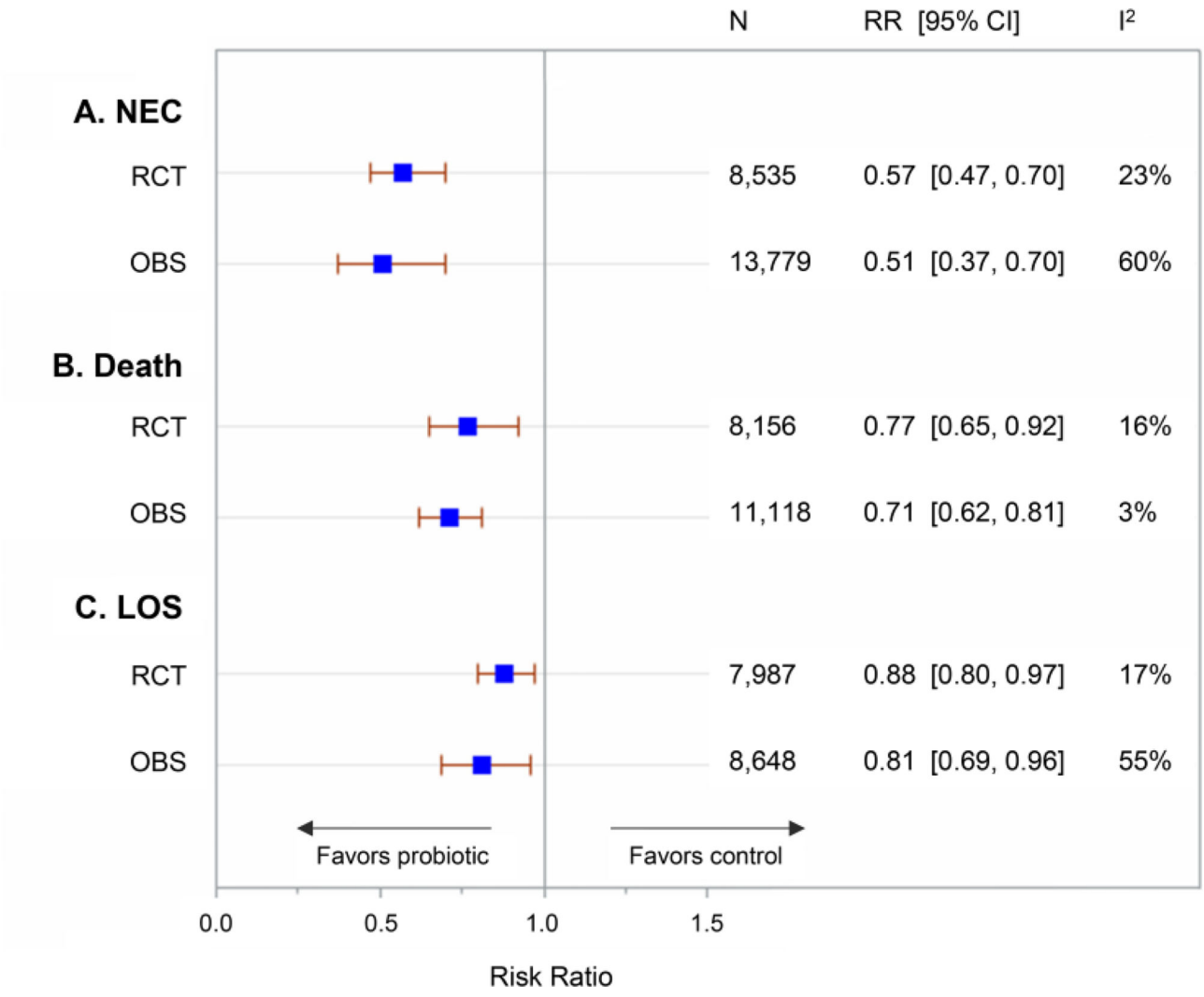

Figure 3. Treatment effects of probiotics in randomized trials and observational studies

Pooled risk ratios with error bars to indicate 95% CI (Mantel-Haenszel method with fixed effects) are reported along with sample sizes for each pooled estimate with corresponding statistical measure of heterogeneity (I^2). Data from Dermyshi E. et al. The "Golden Age" of Probiotics: A Systematic Review and Meta-Analysis of Randomized and Observational Studies in Preterm Infants. *Neonatology*. 2017 (39). Abbreviations: RR, relative risk; CI, confidence interval; NEC, necrotizing enterocolitis; RCT, randomized controlled trials; OBS, observational studies; LOS, late-onset sepsis.

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Table 1

Summary of recent meta-analyses evaluating treatment effects of probiotics.

Outcome	Year	Trials, n	Patients, n	RR (95 % CI)	I^2	Effects
NEC (Bell Stage 2 or 3)						
Sawh et al.(35)	2016	35	10520	0.53 (0.42–0.66)	11%	Random
Dermyshi et al.(39)	2017	29	8535	0.57 (0.47–0.70)	23%	Fixed
Chang et al.(62)	2017	25	7345	0.60 (0.48–0.74)	0%	Fixed
Thomas et al.(49)	2017	23	7325	0.57 (0.43–0.74)	22%	Random
Late-onset sepsis						
Sawh et al.(35)	2016	28	8707	0.88 (0.77–1.00)	31%	Random
Rao et al.(40)	2016	37	9416	0.86 (0.78–0.94)	35%	Fixed
Dermyshi et al.(39)	2017	28	7987	0.88 (0.80–0.97)	17%	Fixed
Death						
Sawh et al.(35)	2016	27	9507	0.79 (0.68–0.93)	0%	Random
Dermyshi et al.(39)	2017	27	8156	0.77 (0.65–0.92)	16%	Fixed
Chang et al.(62)	2017	21	6291	0.75 (0.60–0.92)	9%	Fixed
Thomas et al.(49)	2017	22	6954	0.72 (0.57–0.92)	17%	Random

Abbreviations: RR, relative risk; CI, confidence interval; NEC, necrotizing enterocolitis.

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Table 2

Individual trials and observational studies of probiotics reporting a significant reduction in NEC

Study author, year; country (Reference)	Population	Preparation (Product)	Total dose in CFU/d	Risk of NEC (probiotic vs. placebo/control)[a]	RR or aOR for NEC (95% CI)[b]	NEC primary outcome
Randomized trials Bin Nun, 2005; Israel (63)	BW ≤1500g, n=145	*B. infantis, S. thermophilu B.bifidus* (ABC Dophilus)	1.05×10^9	1.4% vs 13.7%	0.10 (0.01–0.77)	Yes
Lin, 2005; Taiwan (64)	BW <1500g, n=367	*L. acidophilus* and *B. infantis* (Infloran)	Not specified	1.1% vs 5.3%	0.21 (0.05–0.94)	Yes
Lin, 2008; Taiwan (37)	BW <1500g; GA <34wk; n=434	*L. acidophilus* and *B. infantis* (Infloran)	Not specified	1.8% vs. 6.3%	0.28 (0.10–0.85)	Yes
Samanta, 2009; India (65)	BW <1500g; GA <32wk; n=186	*B.infantis, B.bifidum, B. longum & L. acidophilus*	2×10^{10}	5.5% vs. 15.8%	0.35 (0.13–0.92)	Yes (1 of several)
ProPrems, 2013; AU/NZ (66)	BW <1500g; GA <32wk; n=1099	*B. infantis, S. thermophilus , B. bifidum* (ABC Dophilus)	1×10^9	2.0% vs. 4.4%	0.46 (0.23–0.93)	No
Dilli, 2015; Turkey (67)	BW <1500g; GA <32wk; n=200	*B. lactis* (synbiotic not included)	5×10^9	2.0% vs. 18.0%	0.11 (0.03–0.47)	Yes
Observationa l studies Janvier, 2014; Canada (68)	GA<32wk; n=611	*B. breve, B bifidum, B. infantis, B. longum* & LGG (FloraBaby)	2×10^9	5.4% vs. 9.8%	aOR 0.51 (0.26–0.98)	Yes
Hartel, 2014; Germany (41)	BW <1500g; GA 22–31wk; n=5351	*L. acidophilus* and *B. infantis* (Infloran)	Not specified	2.6% vs. 4.2% (Group 1 & 3); 4.0% vs. 6.2% (Group 2)	aOR 0.58 (0.37–0.91) for all groups	Yes (surgical)
Hunter, 2012; US (46)	BW ≤ 1000g; n=311	*L. reuteri* (BioGaia)	1×10^8	2.5% vs. 15.1%	Not provided (P=0.048)	Yes
Bonsante, 2013; France (45)	GA 24–31 wk; n=1,130	*L. rhamnosus* GG (LCR restituto)	4×10^8	1.2% vs. 5.3%	aOR 0.23 (0.08–0.69)	Yes
Guthmann, 2016; Germany/S W (61)	BW 400–1500g; n=1,224	*L. acidophilus* and *B. infantis* (Infloran)	2×10^9	1.4% vs. 5.2%	RR 0.26 (0.12–0.55)	Yes
Patole, 2016; Australia (69)	<34wk, n=1755	*B. breve* (Morinaga Milk Industry Co.)	$1.5 \text{ to } 3 \times 10^9$	1.3% vs. 3.0%	aOR 0.43 (0.21–0.87)	Yes

Summary only includes published studies in which a full-text was available.

Abbreviations: CFU/d, colony forming unit per d; NEC, necrotizing enterocolitis; RR, relative risk, aOR, adjusted odds ratio; CI, confidence interval; BW, birth weight; GA, gestational age; *B, Bifidobacterium*; *L, Lactobacillus*; *S, Streptococcus;* LGG, *L. rhamnosus* GG, AU/NZ, Australia, New Zealand, SW, Switzerland;

[a] NEC Bell Stage 2 or 3, with percentages derived using all randomized infants in the denominator.

[b] Calculated relative risks are reported for randomized trials

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Table 3

Comparison of interventions studied for the prevention of NEC Meta-analyses of randomized controlled trials

Intervention	Number of trials	Number of infants	RR (95% CI)
Slow vs. fast feeding advancement (70)	9	949	1.02 (0.64–1.62)
Formula vs. donor human milk (8)	9	1070	2.77 (1.40–5.46)
Exclusive human diet vs. bovine-based protein (71)	2	260	0.31 (0.14–0.68)
Probiotic vs. no probiotic (35)	35	10,520	0.53 (0.42–0.66)
Observational studies			
Intervention	Number of infants		RR (95% CI)
Mother's own milk within 7 days of birth vs. all others (3)	14,678		0.69 (0.60–0.78)
No bovine products vs. any bovine products within 14 days of birth (3)	14,678		0.61 (0.39–0.83)
No donor human milk available vs. donor human milk available (72)	42,532		1.15 (1.03–1.28)[a]
Exclusive human diet vs. bovine based human milk fortifier (73–75)	2494		0.70 (0.56–0.87)
Probiotic vs no probiotic (39)	13,779		0.51 (0.37–0.70)

Abbreviations: NEC, necrotizing enterocolitis; RR, relative risk; CI, confidence interval.

[a]Adjusted odds ratio

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Table 4

Summary of probiotic strains and associated products evaluated in randomized trials and observational studies for NEC

Product Name or Supplier (Country)	Bacterial species on label	Randomized trials (infants), n	Observational studies (infants), n	Ref.
Culturelle (US), Diclofor (Italy)	*Lactobacillus rhamnosus* GG (LGG)	3 (984)	1 (3342)	(43, 44, 76, 77)
BioGaia (Sweden), Gerber Soothe (US)	*Lactobacillus reuteri*	2 (1150)	1 (311)	(46, 48, 78)
Infloran (Multiple)	*Lactobacillus acidophilus, Bifidobacterium bifidum*[a]	2 (810)	3 (7038)	(37, 41, 61, 64, 79)
ABC Dophilus (US)[b]	*Bifidobacterium infantis, Bifidobacterium bifidum, Streptococcus thermophilus*	2 (1244)	1 (580)	(63, 66, 80)
Align (along with Culturelle) (US)	*Bifidobacterium infantis* (*with* LGG)	1 (101)	1 (221)	(81, 82)
Yakult (Japan), Morinaga Milk	*Bifidobacterium breve*	4 (1584)	1 (1755)	(38, 69, 83–85)
Industry Co. (Japan)				
FloraBaby (Canada)	*Bifidobacterium breve, bifidum, infantis*, and		1 (611)	(68)

Products selected based on reporting of probiotic use in Viswanathan et al. *Journal of Perinatology*. 2016 (42) and evaluation of studies in Dermyshi et al. *Neonatology*. 2017 (39).

[a] Various Infloran products have contained *B. bifidum, longum* or *infantis*

[b] Recalled from the US market (56, 57)