## SPECIAL ARTICLE

# Safety and efficacy of probiotic administration to preterm infants: ten common questions

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In spite of a large number of randomized placebo-controlled clinical trials and observational cohort studies including >50,000 preterm infants from 29 countries that have demonstrated a decrease in the risk of necrotizing enterocolitis, death, and sepsis, routine prophylactic probiotic administration to preterm infants remains uncommon in much of the world. This manuscript reflects talks given at the NEC Society Symposium in 2019 and is not intended to be a state-of-the-art review or systematic review, but a summary of the probiotic-specific aspects of the symposium with limited additions including a recent strain-specific network analysis and position statement from the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN). We address ten common questions related to the intestinal microbiome and probiotic administration to the preterm infant.

## INTRODUCTION

Probiotic use in neonatal units varies widely across the world. While some clinicians recommend routine prophylactic supplementation to premature infants,[1] others suggest awaiting more definitive evidence and/or better probiotic products.[2] A large number of meta-analyses of randomized controlled trials (RCTs) and observational studies of probiotic use in preterm infants have been published. To summarize data presented and discussed at the 2019 Necrotizing Enterocolitis (NEC) Symposium, we have opted to address ten common questions.

## WHAT IS INTESTINAL DYSBIOSIS?

Dysbiosis is an alteration in the composition or function of the microbes in a given anatomic location that is associated with disease. Common well-accepted examples include antibiotic-associated diarrhea and *Clostridium difficile* colitis. Novel tools that identify microbes that are difficult or impossible to grow in culture and the functional capacity of these microbes have increased understanding of how the microbes that colonize the host impact health. This is particularly important in the intestinal tract, which houses trillions of bacteria, viruses, fungi, and archaea. Indeed, the intestinal microbiome is viewed by many as an integral part of the host immune system.[3,4]

Intestinal dysbiosis has been associated with many chronic diseases with potential causal mechanisms demonstrated in preclinical studies.[5,6] Recent reviews of the role of dysbiosis in such widely differing disease processes as type 1 diabetes,[7] human immunodeficiency virus infection,[8] colorectal cancer,[9] liver fibrosis,[10] chronic kidney disease,[11] and inflammatory eye diseases[12] underscore the profound local and systemic effects associated with alterations in the gut microbiome. Modern hygiene practices and antibiotic administration have altered horizontal transmission of intestinal microbes among communities while the introduction of cesarean sections and formula feeding have altered vertical transmission from mother to baby. As a result, it is highly likely that the intestinal microbiota that evolved with our ancestors has been substantially (and perhaps irreversibly) modified. In many developed countries today, even a mother who delivers vaginally receives no peripartum antibiotics and exclusively breast-feeds may still not be able to provide certain beneficial commensal bacteria to her infant.[13,14]

## WHY DOES INTESTINAL DYSBIOSIS MATTER IN THE PRETERM INFANT?

Preterm infants are at a uniquely elevated risk for intestinal dysbiosis, with potential consequences including NEC and sepsis. The gut of the preterm infant becomes colonized at a time when gastrointestinal function and innate and adaptive immune systems are immature. For instance, intestinal motility in the very preterm infant is often reduced, when compared to more mature infants, Paneth cells are not yet functional,[15] intestinal mucus, immunoglobulins, and antimicrobial peptides are all less abundant,[16] apoptosis is poorly regulated, and signaling molecules such as Toll-like receptors (TLRs) are highly expressed. For instance, in the fetus TLR4 is highly expressed in enterocytes and is important in stimulating maturation of the gut, whereas in the term infant TLR4 expression is low and serves predominantly as a pattern recognition sensor for intestinal bacteria and viruses. In the very preterm infant, high expression of TLR4 leads to an exuberant and poorly controlled inflammatory response and impairment of the intestinal barrier, and in animal models, suppression of TLR4 reduces NEC severity.[17]

Preterm infants are exposed for long time periods to hospital surface microbes, indwelling tubes,[18] antibiotics,[19,20] and other medications,[21] all of which influence the intestinal microbiome. As

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a result of these developmental and environmental factors, the fecal microbiome of the preterm infant is often dominated by pro-inflammatory γ-Proteobacteria that contain TLR4 ligands, particularly between 28 and 33 weeks of corrected gestational age.[22] Expansion of this bacterial phylum (which includes the family Enterobacteriaceae and genera Escherichia and Klebsiella) has been described as the "signature" of intestinal dysbiosis.[23] A recent meta-analysis including almost 3000 fecal samples from nearly 400 preterm infants demonstrated that this "signature" of dysbiosis precedes the onset of NEC.[24] The peak postmenstrual age for the onset of NEC[25] coincides with the period of predominance of γ-Proteobacteria[22] and with poorly controlled pro-inflammatory responses in the immature developing gut.[26,27] Furthermore, inducers of dysbiosis such as prolonged antibiotic administration and medications that suppress gastric acid have been associated with increased risk of NEC in preterm infants.[28,29]

Late-onset sepsis (LOS) is frequent among preterm infants. Recent analyses of the intestinal microbiome and metabolome have demonstrated significant differences between infants with LOS and carefully matched controls suggesting that translocation of pathogens from the intestinal tract (including coagulase-negative staphylococci) is a common source of infection in the preterm infant.[30,31] This is likely related to the combination of intestinal dysbiosis and increased permeability of the intestinal barrier in this population. As with NEC, early exposure to prolonged antibiotics is associated with an increased risk of LOS.[28]

**DO PROBIOTICS ALTER THE INTESTINAL MICROBIOME?**
A recent cohort study demonstrated alleviation of antibiotic-associated dysbiosis in very preterm infants with probiotic administration.[32] If increased γ-Proteobacteria is the signature of dysbiosis in premature infants, a probiotic that decreases the numbers of γ-Proteobacteria and reduces inflammatory responses to these bacteria while also increasing the numbers of probiotic organisms in the intestinal lumen would seem desirable. Several studies in preterm babies have demonstrated effective colonization of ingested probiotic microbes.[33,34] Studies reporting changes in colonization with both the administered probiotic microbe and Enterobacteriaceae in preterm infants are summarized in Table 1.[35–39] Note that only two probiotics demonstrated both an increase in the administered microbe and a decrease in Enterobacteriaceae. One mechanism by which probiotic microbes displace Enterobacteriaceae in the intestinal lumen is competition for sources of nutrition. Human milk oligosaccharides (HMOs) are abundant in human milk but not digestible by the human intestinal tract. Only a few bacteria are able to consume HMOs, predominantly Bifidobacterium and Bacteroides species. For example, Bifidobacterium longum subsp. infantis 15697 is able to consume the full range of HMOs, Bifidobacterium animalis subsp. lactis is unable to consume HMOs, while strains of Bifidobacterium breve are able to consume a limited number of HMOs.[37,40–42] The capacity of probiotic B. infantis to outcompete all other gut microbes in the breast-fed term infant has recently been demonstrated.[43]

In addition to bacteria, probiotics may influence fungal colonization in the gut. A recent meta-analysis of RCTs found that probiotics decreased colonization with Candida species in preterm infants.[44] An additional prospective randomized study not included in the meta-analysis found Lactobacillus reuteri 17938 as effective as nystatin in preventing colonization with Candida in preterm very low birth weight infants; among the 300 infants enrolled, fecal colonization rates were 19% in the L. reuteri group and 16% in the nystatin group ($p = 0.54$).[45] It is noteworthy that this study was not blinded and that the infants who received L. reuteri in this clinical trial had a lower incidence of culture-positive sepsis and a shorter length of hospital stay than the infants who received nystatin.

**Table 1.** Impact of probiotic administration to preterm infants on fecal microbes.

| Probiotic microbe (study ref.) | Lactobacillus | Bifidobacterium | Enterobacteriaceae |
|---|---|---|---|
| L. rhamnosus GG[35] | ↑ | | ↑ |
| B. breve M16V[36] | | ↑ | ↑ |
| B. longum subsp. infantis ATCC 15697[37] | | ↑ | ↓ |
| B. animalis subsp. lactis UCD316[37] | | No change | No change |
| B. lactis Bb12[38] | | ↑ | ↓ |
| L. rhamnosus GG[39] | ↑ | | ↑ |
| L. acidophilus, B. longum, B. bifidum, B. infantis[39] | ↑ | ↑ | No change |

The impact of probiotic administration on viral colonization of the intestinal tract has not been explored in preterm infants. In adults, administration of B. animalis subsp. lactis Bl-04 decreased nasal shedding following administration of rhinovirus.[46] In children, administration of Lactobacillus rhamnosus 35 at a dose of $6 \times 10^8$ organisms/day for 3 days decreased fecal shedding of rotavirus.[47]

The studies of colonization described above rely on the assumptions that the fecal microbiome is equivalent to that of the distal small bowel or proximal colon and that the organisms identified by current non-culture methods are living microbes. Clearly, both of these assumptions have limitations. In spite of these limitations, the evidence supports favorable changes in the intestinal microbiome associated with administration of several probiotic strains.

**DO PROBIOTICS PREVENT DISEASE IN PRETERM INFANTS?**
A large number of RCTs and observational cohort studies have been performed to examine the impact of probiotic administration on the outcomes of death, NEC, and LOS in preterm infants. These studies from 29 countries now include >50,000 preterm infants for whom NEC is reported as an outcome. The meta-analyses of these studies have used varying statistical approaches and included differing studies, but all have concluded that probiotics significantly decrease the risk of both NEC and death in infants weighing <1500 g at birth.[48–56] Some meta-analyses also suggest a modest reduction in the risk of LOS in this population. A critical appraisal of the quality of these systematic reviews and/or the individual studies is beyond the scope of this paper; however, a recent review of 98 meta-analyses of RCTs of interventions to reduce the risk of NEC in preterm infants included 1 of the meta-analyses of probiotic administration[49] and found the quality of the review to be high based on the AMSTAR criteria (see Table 2).[57]

One of the concerns expressed regarding the large number of probiotic clinical trials in preterm infants is that many of them are small single-center trials. Table 3 summarizes the impact of probiotics on NEC including only the RCTs with ≥400 preterm infants with 3 of these trials demonstrating benefit and 4 showing no benefit.[58–64] Table 4 summarizes the published cohort studies of probiotics with >1000 infants with 8 showing benefit, 2 showing no benefit, and 1 showing a modest benefit with multivariate regression.[65–75]

There have also been concerns about the relatively small numbers of extremely low birth weight infants in the meta-analyses published to date. In some of the early randomized clinical trials, there appeared to be no benefit of probiotic administration in prevention of NEC in the most premature infants

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**Table 2.** Impact of probiotic administration to preterm infants on NEC (ref. [49] as assessed in ref. [57]).

| Genus | Number of trials | Number of infants | RR (95% CI) |
|---|---|---|---|
| Combination of strains | 18 | 4650 | 0.41 (0.29, 0.56) |
| Any probiotic | 38 | 10,520 | 0.53 (0.42, 0.66) |
| Lactobacillus | 8 | 2596 | 0.61 (0.4, 0.95) |
| Bifidobacterium | 6 | 2056 | 0.37 (0.14, 0.97) |
| Saccharomyces | 2 | 357 | 0.72 (0.33, 1.54) |

The AMSTAR score for this meta-analysis was 9/11 (high quality).
*RR* relative risk, *CI* confidence interval.

**Table 3.** NEC in RCTs trials including at least 400 preterm infants.

| RCT (ref.) | Probiotic (NEC/total) | Placebo (NEC/total) | RR (95% CI) |
|---|---|---|---|
| Costeloe et al.[58] | 61/650 | 66/660 | 0.95 (0.69, 1.3) |
| Oncel et al.[59] | 8/200 | 10/200 | 0.80 (0.32, 2.0) |
| Manzoni et al.[60] | 0/238 | 14/258 | NA |
| Jacobs et al.[61] | 11/548 | 24/551 | 0.46 (0.23, 0.93) |
| Rojas et al.[62] | 9/372 | 15/378 | 0.61 (0.27, 1.4) |
| Lin et al.[63] | 4/217 | 14/217 | 0.29 (0.10, 0.85) |
| Dani et al.[64] | 4/295 | 8/290 | 0.49 (0.15, 1.6) |

*RCT* randomized controlled trial, *RR* relative risk, *CI* confidence interval, *NA* not applicable (relative risk cannot be estimated based on zero NEC events).

**Table 4.** NEC in cohort studies including at least 1000 preterm infants.

| Cohort | Probiotic (NEC/total) | No probiotic (NEC/total) | RR (95% CI) |
|---|---|---|---|
| Guthmann et al.[65] | 8/591 | 33/633 | 0.26 (0.12, 0.56) |
| Hartel et al.[66] | 116/3789 | 76/1562 | 0.63 (0.47, 0.84) |
| Bonsante et al.[67] | 4/347 | 41/783 | 0.22 (0.080, 0.61) |
| Hoyos[68 a] | 34/1237 | 85/1282 | 0.41 (0.28, 0.61) |
| Luoto et al.[69] | 19/418 | 61/1900 | 1.4 (0.86, 2.3) |
| Denkel et al.[70] | 100/5818 | 174/5072 | 0.50 (0.39, 0.64) |
| Patole et al.[71] | 12/920 | 25/835 | 0.44 (0.22, 0.86) |
| Samuels et al.[72] | 34/673 | 101/1288 | 0.64 (0.44, 0.94) |
| Sharpe et al.[73] | 7/457 | 37/1334 | 0.55 (0.25, 1.2) |
| Singh et al.[74 b] | 50/652 | 190/2436 | 0.98 (0.73, 1.3) |
| Meyer et al.[75] | 35/1973 | 70/2556 | 0.62 (0.41, 0.94) |

[a]Included infants of all gestational ages.
[b]Probiotics decreased NEC with multiple logistic regression models adjusted for gestational age, SNAPII scores > 20, outborn status, and any breast milk intake (aOR 0.64, 95% CI 0.41, 0.996).

pathway.[81,82] Probiotic microbes also decrease intestinal permeability, which may be particularly relevant in the preterm infant as a leaky gut appears to be an important aspect of increased risk for both NEC and LOS.[83] Mechanisms by which probiotic microbes alter intestinal permeability include the expression of surface molecules that interact with host immune receptors (e.g., flagella, pili, and capsular polysaccharides) and secreted molecules in addition to the previously noted organic acids and bacteriocins (e.g., secreted proteins, indoles, and microvesicles).[84,85]

## ARE PROBIOTICS SAFE FOR PRETERM INFANTS?
Clinical trials of probiotics have been criticized for not reporting adverse events and side effects.[86] In preterm infants, many trials have reported no significant difference in bloating, diarrhea, vomiting, or feeding tolerance with probiotic administration. Meta-analyses of clinical trials in preterm infants have shown shorter time to full enteral feeding and shorter length of hospitalization with probiotic administration.[50,87] The risks of probiotic administration appear to be limited to sepsis caused by the probiotic organism and to infection associated with contamination of the probiotic product. Probiotic sepsis has been reported for the common probiotic microbes administered to premature infants and may be underestimated due to the challenges of isolating and identifying *Bifidobacterium* and *Lactobacillus* species.[88] Large studies that have specifically sought evidence of probiotic sepsis have not found such cases.[89,90] The decrease in mortality and in episodes of sepsis with administration of probiotic microbes suggests that probiotic sepsis is uncommon and that there is a net beneficial effect in reducing infection.

Studies of commercially available probiotics have demonstrated that many products do not contain the advertised strain or contain additional strains that are not noted on the label.[91] The report of a death in a premature infant who received a contaminated probiotic[92] underscores the importance of increased oversight of probiotic production in the USA. Probiotics produced in other countries that provide a higher level of oversight than that provided in the USA for dietary supplements represent a reasonable option for neonatologists who prefer not to continue to await a product produced in the USA.[74]

Recent meta-analyses have found no differences in common outcomes such as bronchopulmonary dysplasia, retinopathy of

with a possible increase in sepsis.[76] However, subgroup analyses should be viewed cautiously, particularly due to issues with interpretation.[77] As more studies have been published, it has become clear that the incidences of NEC, death, and sepsis are not higher in extremely premature infants receiving probiotics. In addition, two large cohort studies have shown a lower incidence of NEC in extremely preterm infants receiving probiotics.[74,78]

## HOW DO PROBIOTICS WORK?
In addition to competition for nutrients within the gut lumen, the metabolic products of the probiotic microbe likely shape the intestinal microbiota. For instance, *Lactobacillus* and *Bifidobacterium* species produce lactate and short-chain fatty acids, such as acetate, propionate, and butyrate. These microbial products lower the pH in the colon inhibiting the growth of microbes that are less tolerant of an acidic environment.[13,38,43] In addition, several probiotic microbes produce bacteriocins, which have antibacterial properties inhibiting the growth of competing bacteria. Bacteriocins produced by *Lactobacillus* species and other lactic-acid bacteria have been the most studied to date with emphasis on their capacity to inhibit growth of bacteria associated with food spoilage.[79] How effective probiotic-associated bacteriocins are at preventing or treating infections remains uncertain.[80]

Some probiotic microbes also have an anti-inflammatory effect, particularly in the preterm infant. The mechanisms underlying this effect include decreased expression of TLR4 and TLR2, activation of TLR9 leading to inhibition of TLR4 activity, decreased interleukin-1β (IL-1β)-mediated expression of IL-6 and IL-8, and increased expression of molecules that inhibit the TLR4

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**Table 5.** Common interventions and impact on mortality in preterm infants.

| Intervention | Number of trials | Number of infants | RR of mortality |
|---|---|---|---|
| Antenatal corticosteroids[a] [98] | 22 | 7188 | 0.69 (0.59, 0.81) |
| Kangaroo mother care[b] [99] | 8 | 1736 | 0.60 (0.39, 0.92) |
| Prophylactic indomethacin[100] | 18 | 2769 | No difference in mortality |
| Prophylactic indomethacin[c] [100] | 2 observational studies | 11,289 | 0.81 (0.66, 0.98) |
| Surfactant vs no treatment[101] | 7 | 540 | 0.67 (0.50, 0.90) |
| Surfactant type[d] [102] | 9 | 901 | 1.44 (1.04, 2.00) |
| Low vs high oxygen saturation target[e] [103] | 5 | 4873 | 1.16 (1.03, 1.31) |
| Formula vs donor human milk[f] [104] | 11 | 1809 | No difference in mortality |
| Medical treatment of PDA vs placebo or no treatment[105] | 68 | 4802 | No difference in mortality |
| High vs low amino acid intake[g] [106] | 14 | 1407 | No difference in mortality |
| Interventions to prevent hypothermia[107] | 25 | 1986 | No difference in mortality |
| Inhaled nitric oxide for preterm infants[108] | 17 | 4065 | No difference in mortality |

[a]Most studies in developed countries. The quality of this meta-analysis for the prevention of NEC (10 studies, 4702 infants) received an AMSTAR score 11/11 (highest) and certainty of evidence GRADE of moderate and RR for NEC 0.50 (0.32, 0.78). See ref. [57].
[b]Most studies in developing countries.
[c]Outcome is risk-adjusted odds of mortality.
[d]Bovine minced lung vs porcine minced lung (no differences between bovine lavage and bovine minced or between bovine lavage and porcine minced).
[e]Outcome was death at 18–24 months of corrected age.
[f]The quality of this meta-analysis for the prevention of NEC (8 studies, 1605 infants) received an AMSTAR score 9/11 (high) and certainty of evidence GRADE of low and RR for NEC with formula 1.87 (1.23, 2.85). See ref. [57].
[g]The quality of this meta-analysis for the prevention of NEC (14 studies, 1301 infants) received an AMSTAR score 10/11 (high) and certainty of evidence GRADE of low and RR for NEC 1.0 (0.68, 1.47)). See ref. [57].

prematurity, intraventricular hemorrhage, or neurodevelopmental delay between infants receiving probiotics and those receiving a placebo.[50,93,94] As noted above, early analyses suggested that the most premature infants may not benefit from probiotic administration[95]; however, the largest observational study to date, including 4683 infants with birth weight <1000 g, demonstrated a decrease in the incidence of NEC and death with probiotic administration in this more immature group of infants.[70,74,78]

Several studies have demonstrated cross-contamination with probiotics within a given neonatal intensive care unit (NICU) wherein the probiotic strain has been isolated from the feces of infants who did not receive the probiotic. This suggests that probiotic microbes are passed from infant to infant or from care provider to infant in the NICU. Whether this is a problem or not remains unclear, as the intestinal tract is continually being exposed to environmental bacteria. One case report of a probiotic fungus Saccharomyces boulardii causing infection in an infant adjacent to an infant who was receiving the probiotic suggests that cross-contamination may involve risk.[96] This appears to be a very rare phenomenon.

In summary, probiotic administration has potential risks as well as benefits. As with essentially all interventions in very preterm infants, clinicians and parents must weigh the risks and benefits in deciding to provide probiotic administration. The risks of probiotic administration to very preterm infants appear to be low and perhaps comparable to, or even lower than, the risk of symptomatic cytomegalovirus infection from feeding unpasteurized mother's milk to preterm infants.[97] It is our impression that the benefits of mother's milk and probiotics both outweigh the risks. We believe it is important to engage parents in this decision.

## HOW DO THE SAFETY AND EFFICACY OF PROBIOTICS COMPARE TO OTHER COMMON INTERVENTIONS IN PRETERM INFANTS?

The practice of evidence-based medicine is particularly challenging in neonatology given the relative paucity of high-quality studies in support of common interventions. Table 5 summarizes several meta-analyses in very preterm infants of common interventions that included mortality as an outcome.[98–108] The beneficial effect of probiotic administration on mortality in preterm infants is similar to that seen with studies of antenatal corticosteroids and with the early studies of a single dose of surfactant vs no treatment; there are stronger data to support probiotic administration than many of the other listed common interventions in very preterm infants.

Other interventions, besides human milk and probiotics, to alter the intestinal microbiota or augment the innate immune system have been studied in preterm infants. For instance, a recent meta-analysis of 18 RCTs of prebiotic glycans including >1300 preterm infants showed a decrease in LOS and death but no significant impact on the incidence of NEC. All of the included trials were individually underpowered to detect clinically important differences in these three outcomes.[109] Lactoferrin, a component of human milk with antibacterial properties, showed low-quality evidence of benefit in reducing sepsis and NEC in a recent meta-analysis of small clinical trials in preterm infants[110]; however, a subsequent much larger clinical trial including >2200 preterm infants demonstrated no benefit in reducing NEC, sepsis, or death.[111]

## WHICH PROBIOTIC PRODUCT IS MOST EFFECTIVE FOR PRETERM INFANTS?

Most of the RCTs and cohort studies published to date have compared a single probiotic product (containing one or more strains) to either a placebo or to no probiotic treatment. The largest probiotic RCT to date in preterm infants compared a single strain probiotic, B. breve BBG-001 in >1300 infants and found no difference between the probiotic and placebo groups for NEC, sepsis, or death.[58] This strain was chosen based on previous demonstration of improved weight gain[112]; however, there were no mechanistic data from human or preclinical studies to suggest alterations in inflammation or intestinal permeability with this strain. The observation that more studies of combination products (containing more than one probiotic strain) have shown

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benefit in reduction of NEC than studies of single microbe probiotic products has been interpreted as evidence that multi-strain products are more effective; however, in the absence of comparisons between actual products or consistent findings of subgroup heterogeneity in meta-analyses, such conclusions are not justified.[113]

Observations which suggest that administration of a *Bifidobacterium* strain would be potentially beneficial include (1) antibiotic administration decreases fecal bifidobacteria[20] and is associated with increased risk of NEC; (2) preterm infants with LOS have lower numbers of bifidobacteria prior to onset of sepsis[30]; (3) as noted above, many strains of bifidobacteria consume HMOs and are historically the dominant gut microbes in breast-fed infants[13]; and (4) a meta-analysis of RCTs of *Bifidobacterium* strains demonstrated reduced risk of NEC and death[53] and a strain-specific network meta-analysis demonstrated a decrease in NEC with *B. lactis* Bb12 (relative risk (RR) 0.25 (0.10, 0.56).[114]

Observations in favor of administration of *Lactobacillus* strains to preterm infants include (1) a large body of animal studies demonstrating protective mechanisms in the preterm gut[115]; (2) a meta-analysis of clinical trials of *L. reuteri* 17938 demonstrating benefit[116]; and (3) the previously noted strain-specific network meta-analysis demonstrating benefit with *L. rhamnosus* GG and *L. reuteri* 17938.[114]

Combination probiotic products containing more than one microbial strain offer the potential advantage of synergism between strains and the potential disadvantage of negative interactions between administered strains. Several combination products have shown benefit in decreasing the risks of death and NEC.[114]

Among products available in North America, there are good data available suggesting safety and efficacy in preterm infants for several commercial products, including *L. reuteri* 17938 (Biogaia, Gerber)[117] and Ultimate Flora Baby (Renew Life, a combination of four *Bifidobacterium* and one *Lactobacillus* strains).[118] The combination of *B. infantis* Bb-02, *Streptococcus thermophilus* TH-4, and *B. lactis* Bb12 (ABC Dophilus) demonstrated a significant decrease in NEC in a large multicenter trial.[61] Following the clinical trial, this product was altered to contain *B. lactis*, *S. thermophilus*, and *L. rhamnosus* and then taken off the market following the death of a premature infant from contamination as noted above. The original combination product is now available in the U.S. (Similac Probiotic Tri-blend) and Europe (Neobiomics ProPrems). Safety and sustained colonization have been shown in term breast-fed infants for the *B. infantis* EVC001 strain.[43,119] Long-term follow-up of term infants receiving *L. rhamnosus* GG has demonstrated safety and some efficacy at decreasing allergic disease.[120,121] *L. rhamnosus* GG (Culturelle) is the most commonly administered probiotic strain in U.S. NICUs. The previously noted strain-specific network analysis demonstrated decreased risk of NEC (RR 0.24 (0.064, 0.67)) with administration of *L. rhamnosus* GG,[114] though a recent US cohort study of preterm infants did not demonstrate benefit.[90]

Dose comparison studies are few and do not address the possibility that different strains have different optimum dosage.[37,122] Similarly, duration of probiotic administration varies with most studies commencing probiotic administration with the first enteral feeding and continuing until 34–36 weeks corrected gestation.

A recent position paper from the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) Committee on Nutrition and the ESPGHAN Working Group for Probiotics and Prebiotics summarized current evidence with reliance on the strain-specific network meta-analysis.[114] Emphasis was placed on quality assurance of the probiotic product, absence of transferable antibiotic-resistance genes, and local ability to routinely detect probiotic sepsis with a conditional recommendation (with low certainty of evidence) to provide either

*L. rhamnosus* GG (e.g., Culturelle) or the combination of *B. infantis* Bb-02, *B. lactis* Bb-12, and *S. thermophilus* TH-4 (e.g., Similac Tri-blend, NeoBiomics ProPrems) in order to reduce NEC rates.[123]

## WHAT WOULD THE DEFINITIVE CLINICAL TRIAL OF PROBIOTICS IN PRETERM INFANTS LOOK LIKE?

Given the large magnitude of treatment benefit toward reduction of NEC and death demonstrated in both RCTs and observational studies of probiotic administration in preterm infants, it is unlikely that further placebo-controlled RCTs will alter the conclusion that probiotics prevent NEC and death or meaningfully change the estimates of treatment benefit. For instance, using the larger clinical trials summarized in Table 3 (incidence of NEC 97/2520 or 3.8% for infants receiving probiotic and 151/2554 or 5.9% for infants receiving placebo) to guide a reasonable effect size estimate and assuming $\alpha = 0.05$ and $\beta = 0.9$, a sample size of 2292 babies in each group would be needed for a definitive trial to detect a similar or larger magnitude difference. Even if the incidence of NEC were equivalent in each group (and midway between the incidences in Table 3) in such a large study, the unadjusted RR for NEC of the studies in Table 3 plus the new theoretical large study would still suggest benefit (RR 0.79, 95% confidence interval 0.66, 0.95). For this reason, future studies should ideally compare promising probiotic products to each other rather than to placebo. Furthermore, as noted earlier, cross-contamination occurs frequently in the NICU,[37,89] suggesting that beneficial effects of probiotics may be underestimated with traditional placebo-controlled parallel-group randomized trial designs. For this reason, a cluster-randomized cross-over trial (in which the NICU is randomized to a given probiotic product for a time period and then crosses over to the other product) may be of particular value. Such a study would require a large sample size, possibly in the tens of thousands, and significant funding. In the absence of such a definitive study, pooling the observational data on routine probiotic use from dozens or hundreds of NICUs, as has been recently reported from Germany[70] and Canada,[74] has high value.

## HOW CAN WE BEST COMMUNICATE WITH NICU PARENTS ABOUT PROBIOTICS AND HUMAN MILK?

The role of parents in the NICU has changed dramatically over the past decades. Today, many units are moving away from their traditional clinician-driven hierarchical structure toward family-centered care, where parents are considered core members of their baby's care team. In this model, clinicians and staff value parents' concerns and actively seek their engagement. Family-centered care has the potential to improve outcomes, including reduced length of stay[124,125] and complications.[124] While the benefits of inclusion of parents as an essential part of the team caring for their preterm neonate are becoming apparent,[126,127] parental participation in NICU care and decision-making remains highly variable.[128] In focus groups and in surveys, parents of very premature infants expressed frustration at the limited information they received in the NICU.[129] Parents overwhelmingly report to the NEC Society that they wish they had more information about NEC and potential NEC prevention strategies, and sooner.[68]

The current variability in practice regarding probiotics is an additional reason to include parents in this discussion. Parents should understand why the decision has been made to give their baby probiotics or not. The fear of overwhelming families with information should not preclude such a discussion. A conversation with the parents of the risks of sepsis and NEC and the potential risks and benefits of mother's own milk and probiotics in the first days after the delivery of a very preterm infant is viewed by families as empowering rather than frightening. Ensuring that the family understands and actively supports care decisions sets the

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stage for a more positive and less stressful parent experience, regardless of the outcome.

It may well be that family-centered care in the NICU will have greater long-term benefit for both parents and extremely preterm infants than many common interventions.[95] An example of a concise summary for parents of NEC and the risks and benefits of human milk and probiotics is presented on the NEC website: https://necsociety.org/wp-content/uploads/2019/02/Probiotics_HumanMilk_Resource_Parents.pdf.

## AUTHOR CONTRIBUTIONS
M.A.U. wrote the initial and final drafts; E.U. and R.M.P. edited the initial draft and approved the final draft.

## ADDITIONAL INFORMATION
**Competing interests:** M.A.U. has received grant support from Evolve Bioscience, honoraria and travel support from Abbott, and received consulting fees from Avexegen. R.M.P. has received honoraria and travel support from Mednax, Inc., partial travel support from Danone to attend the SIGNEC UK meeting, and consults for Shipman & Goodwin, LLP. M.A.U. and R.M.P. serve on the data-monitoring committee for a probiotic study conducted by Premier Research/Infant Bacterial Therapeutics. E.U. serves as a director of the NEC Society, the sponsor of the NEC Symposium and of this supplement to *Pediatric Research*. None of these entities had any role in this manuscript. The authors are not endorsing the use of any specific probiotic product.

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