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Safety and Efficacy of Probiotic Administration to Preterm Infants: Ten Common Questions

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Abstract

In spite of a large number of randomized placebo-controlled clinical trials and observational cohort studies including more than 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 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 and observational studies of probiotic use in preterm infants have been published. To summarize data presented and discussed at the 2019 NEC symposium, we have opted to address 10 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

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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 pre-clinical studies(5, 6). Recent reviews of the role of dysbiosis in such widely differing disease processes as type 1 diabetes(7), HIV 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 peri-partum 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 necrotizing enterocolitis (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, and 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 receptor(TLR)s 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 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 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

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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, *B. longum* subsp *infantis* 15697 is able to consume the full range of HMOs, *B. animalis* subsp *lactis* is unable to consume HMOs, while strains of *B. 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 randomized controlled trials found probiotics decreased colonization with Candida species in preterm infants(44). An additional prospective randomized study not included in the meta-analysis found *L. 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.

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,

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administration of *L. rhamnosus* 35 at a dose of 6×10^8 organisms/day for three 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 more than 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 one 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 or more preterm infants with three of these trials demonstrating benefit and four showing no benefit(58–64). Table 4 summarizes the published cohort studies of probiotics with more than 1000 infants with eight showing benefit, two showing no benefit, and one 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 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,

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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 (IL)1β mediated expression of IL6 and IL8, and increased expression of molecules that inhibit the TLR4 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 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 prematurity, intraventricular hemorrhage, or

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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 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 randomized controlled trials of prebiotic glycans including more than 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 more than 2200 preterm infants demonstrated no benefit in reducing NEC, sepsis or death(111).

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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 more than 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 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 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 (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, *S. 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

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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 the 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 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 towards 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 α=0.05 and β=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 relative risk for NEC of the studies in Table 3 plus the new theoretical large study would still suggest benefit (RR 0.79, 95% CI 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 under-estimated 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.

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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 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 necrotizing enterocolitis 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>..

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Conflicts of Interest:

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REFERENCES

1. Athalye-Jape G, Patole S 2019 Probiotics for preterm infants - time to end all controversies. Microb Biotechnol 12:249–253. [PubMed: 30637944]
2. Pell LG, Loutet MG, Roth DE, Sherman PM 2019 Arguments against routine administration of probiotics for NEC prevention. Curr Opin Pediatr 31:195–201. [PubMed: 30624281]
3. Weiss GA, Hennet T 2017 Mechanisms and consequences of intestinal dysbiosis. Cell Mol Life Sci 74:2959–2977. [PubMed: 28352996]
4. Takiishi T, Fenero CIM, Camara NOS 2017 Intestinal barrier and gut microbiota: Shaping our immune responses throughout life. Tissue Barriers 5:e1373208. [PubMed: 28956703]
5. Vatanen T, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M, Kolde R, Vlamakis H, Arthur TD, Hamalainen AM, Peet A, Tillmann V, Uibo R, Mokurov S, Dorshakova N, Ilonen J, Virtanen SM, Szabo SJ, Porter JA, Lahdesmaki H, Huttenhower C, Gevers D, Cullen TW, Knip M, Group DS, Xavier RJ 2016 Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. Cell 165:842–853. [PubMed: 27133167]
6. Chua HH, Chou HC, Tung YL, Chiang BL, Liao CC, Liu HH, Ni YH 2018 Intestinal Dysbiosis Featuring Abundance of Ruminococcus gnavus Associates With Allergic Diseases in Infants. Gastroenterology 154:154–167. [PubMed: 28912020]
7. Abdellatif AM, Sarvetnick NE 2019 Current understanding of the role of gut dysbiosis in type 1 diabetes. J Diabetes 11:632–644. [PubMed: 30864231]
8. Lujan JA, Rugeles MT, Taborda NA 2019 Contribution of the Microbiota to Intestinal Homeostasis and its Role in the Pathogenesis of HIV-1 Infection. Curr HIV Res 17:13–25. [PubMed: 30854974]
9. Jackson DN, Theiss AL 2019 Gut bacteria signaling to mitochondria in intestinal inflammation and cancer. Gut Microbes:1–20.
10. Zhou R, Fan X, Schnabl B 2019 Role of the intestinal microbiome in liver fibrosis development and new treatment strategies. Transl Res 209:22–38. [PubMed: 30853445]
11. Jazani NH, Savoj J, Lustgarten M, Lau WL, Vaziri ND 2019 Impact of Gut Dysbiosis on Neurohormonal Pathways in Chronic Kidney Disease. Diseases 7: pii: E21. doi: 10.3390/diseases7010021. [PubMed: 30781823]
12. Lin P 2019 Importance of the intestinal microbiota in ocular inflammatory diseases: A review. Clin Exp Ophthalmol 47:418–422. [PubMed: 30834680]
13. Henrick BM, Hutton AA, Palumbo MC, Casaburi G, Mitchell RD, Underwood MA, Smilowitz JT, Frese SA 2018 Elevated Fecal pH Indicates a Profound Change in the Breastfed Infant Gut Microbiome Due to Reduction of Bifidobacterium over the Past Century. mSphere 3: pii: e00041–18. doi: 10.1128/mSphere.00041-18. [PubMed: 29564397]
14. Rook GA, Lowry CA, Raison CL 2013 Microbial 'Old Friends', immunoregulation and stress resilience. Evol Med Public Health 2013:46–64. [PubMed: 24481186]
15. Heida FH, Beyduz G, Bulthuis ML, Kooi EM, Bos AF, Timmer A, Hulscher JB 2016 Paneth cells in the developing gut: when do they arise and when are they immune competent? Pediatr Res 80:306–310. [PubMed: 27049291]
16. Brandtzaeg P 2013 Gate-keeper function of the intestinal epithelium. Benef Microbes 4:67–82. [PubMed: 23257015]
17. Mihi B, Good M 2019 Impact of Toll-Like Receptor 4 Signaling in Necrotizing Enterocolitis: The State of the Science. Clin Perinatol 46:145–157. [PubMed: 30771815]
18. Taft DH, Salinero LK, Vongbhavit K, Kalanetra KM, Masarweh C, Yu A, Underwood MA, Mills DA 2019 Bacterial colonization and antimicrobial resistance genes in neonatal enteral feeding tubes. FEMS Microbiol Ecol 95: pii: fiz039. doi: 10.1093/femsec/fiz039. [PubMed: 30915455]
19. Gasparrini AJ, Crofts TS, Gibson MK, Tarr PI, Warner BB, Dantas G 2016 Antibiotic perturbation of the preterm infant gut microbiome and resistome. Gut Microbes 7:443–449. [PubMed: 27472377]
20. Zwittink RD, Renes IB, van Lingen RA, van Zoeren-Grobben D, Konstanti P, Norbruis OF, Martin R, Groot Jebbink LJM, Knol J, Belzer C 2018 Association between duration of intravenous antibiotic administration and early-life microbiota development in late-preterm infants. Eur J Clin Microbiol Infect Dis 37:475–483. [PubMed: 29368074]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

21. Gupta RW, Tran L, Norori J, Ferris MJ, Eren AM, Taylor CM, Dowd SE, Penn D 2013 Histamine-2 receptor blockers alter the fecal microbiota in premature infants. J Pediatr Gastroenterol Nutr 56:397–400. [PubMed: 23254444]
22. La Rosa PS, Warner BB, Zhou Y, Weinstock GM, Sodergren E, Hall-Moore CM, Stevens HJ, Bennett WE Jr., Shaikh N, Linneman LA, Hoffmann JA, Hamvas A, Deych E, Shands BA, Shannon WD, Tarr PI 2014 Patterned progression of bacterial populations in the premature infant gut. Proc Natl Acad Sci U S A 111:12522–12527. [PubMed: 25114261]
23. Litvak Y, Byndloss MX, Tsolis RM, Baumler AJ 2017 Dysbiotic Proteobacteria expansion: a microbial signature of epithelial dysfunction. Curr Opin Microbiol 39:1–6. [PubMed: 28783509]
24. Pammi M, Cope J, Tarr PI, Warner BB, Morrow AL, Mai V, Gregory KE, Kroll JS, McMurtry V, Ferris MJ, Engstrand L, Lilja HE, Hollister EB, Versalovic J, Neu J 2017 Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis. Microbiome 5:31. [PubMed: 28274256]
25. Yee WH, Soraisham AS, Shah VS, Aziz K, Yoon W, Lee SK, Canadian Neonatal N 2012 Incidence and timing of presentation of necrotizing enterocolitis in preterm infants. Pediatrics 129:e298–304. [PubMed: 22271701]
26. Hackam DJ, Sodhi CP 2018 Toll-Like Receptor-Mediated Intestinal Inflammatory Imbalance in the Pathogenesis of Necrotizing Enterocolitis. Cell Mol Gastroenterol Hepatol 6:229–238.e221. [PubMed: 30105286]
27. Lueschow SR, Stumphy J, Gong H, Kern SL, Elgin TG, Underwood MA, Kalanetra KM, Mills DA, Wong MH, Meyerholz DK, Good M, McElroy SJ 2018 Loss of murine Paneth cell function alters the immature intestinal microbiome and mimics changes seen in neonatal necrotizing enterocolitis. PLoS One 13:e0204967. [PubMed: 30273395]
28. Cantey JB, Pyle AK, Wozniak PS, Hynan LS, Sanchez PJ 2018 Early Antibiotic Exposure and Adverse Outcomes in Preterm, Very Low Birth Weight Infants. J Pediatr 203:62–67. [PubMed: 30172430]
29. More K, Athalye-Jape G, Rao S, Patole S 2013 Association of inhibitors of gastric acid secretion and higher incidence of necrotizing enterocolitis in preterm very low-birth-weight infants. Am J Perinatol 30:849–856. [PubMed: 23359235]
30. Stewart CJ, Embleton ND, Marrs ECL, Smith DP, Fofanova T, Nelson A, Skeath T, Perry JD, Petrosino JF, Berrington JE, Cummings SP 2017 Longitudinal development of the gut microbiome and metabolome in preterm neonates with late onset sepsis and healthy controls. Microbiome 5:75. [PubMed: 28701177]
31. Taft DH, Ambalavanan N, Schibler KR, Yu Z, Newburg DS, Deshmukh H, Ward DV, Morrow AL 2015 Center Variation in Intestinal Microbiota Prior to Late-Onset Sepsis in Preterm Infants. PLoS One 10:e0130604. [PubMed: 26110908]
32. Esaiassen E, Hjerde E, Cavanagh JP, Pedersen T, Andresen JH, Rettedal SI, Stoen R, Nakstad B, Willassen NP, Klingenberg C 2018 Effects of Probiotic Supplementation on the Gut Microbiota and Antibiotic Resistome Development in Preterm Infants. Front Pediatr 6:347. [PubMed: 30505830]
33. Dutta S, Ray P, Narang A 2015 Comparison of stool colonization in premature infants by three dose regimes of a probiotic combination: a randomized controlled trial. Am J Perinatol 32:733–740. [PubMed: 25519197]
34. Tobin JM, Garland SM, Jacobs SE, Pirotta M, Tabrizi SN 2013 Rapid assay to assess colonization patterns following in-vivo probiotic ingestion. BMC Res Notes 6:252. [PubMed: 23830222]
35. Chrzanowska-Liszewska D, Seliga-Siwecka J, Kornacka MK 2012 The effect of Lactobacillus rhamnosus GG supplemented enteral feeding on the microbiotic flora of preterm infants-double blinded randomized control trial. Early Hum Dev 88:57–60. [PubMed: 22055271]
36. Underwood MA, Davis JCC, Kalanetra KM, Gehlot S, Patole S, Tancredi DJ, Mills DA, Lebrilla CB, Simmer K 2017 Digestion of Human Milk Oligosaccharides by Bifidobacterium breve in the Premature Infant. J Pediatr Gastroenterol Nutr 65:449–455. [PubMed: 28945208]
37. Underwood MA, Kalanetra KM, Bokulich NA, Lewis ZT, Mirmiran M, Tancredi DJ, Mills DA 2013 A comparison of two probiotic strains of bifidobacteria in premature infants. J Pediatr 163:1585–1591 e1589. [PubMed: 23993139]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

38. Mohan R, Koebnick C, Schildt J, Mueller M, Radke M, Blaut M 2008 Effects of Bifidobacterium lactis Bb12 supplementation on body weight, fecal pH, acetate, lactate, calprotectin, and IgA in preterm infants. Pediatr Res 64:418–422. [PubMed: 18552710]
39. Underwood MA, Salzman NH, Bennett SH, Barman M, Mills DA, Marcobal A, Tancredi DJ, Bevins CL, Sherman MP 2009 A randomized placebo-controlled comparison of 2 prebiotic/probiotic combinations in preterm infants: impact on weight gain, intestinal microbiota, and fecal short-chain fatty acids. J Pediatr Gastroenterol Nutr 48:216–225. [PubMed: 19179885]
40. Smilowitz JT, Lebrilla CB, Mills DA, German JB, Freeman SL 2014 Breast milk oligosaccharides: structure-function relationships in the neonate. Annu Rev Nutr 34:143–169. [PubMed: 24850388]
41. Underwood MA, German JB, Lebrilla CB, Mills DA 2015 Bifidobacterium longum subspecies infantis: champion colonizer of the infant gut. Pediatr Res 77:229–235. [PubMed: 25303277]
42. Garrido D, Ruiz-Moyano S, Jimenez-Espinoza R, Eom HJ, Block DE, Mills DA 2013 Utilization of galactooligosaccharides by Bifidobacterium longum subsp. infantis isolates. Food Microbiol 33:262–270. [PubMed: 23200660]
43. Frese SA, Hutton AA, Contreras LN, Shaw CA, Palumbo MC, Casaburi G, Xu G, Davis JCC, Lebrilla CB, Henrick BM, Freeman SL, Barile D, German JB, Mills DA, Smilowitz JT, Underwood MA 2017 Persistence of Supplemented Bifidobacterium longum subsp. infantis EVC001 in Breastfed Infants. mSphere 2: pii: e00501–17. doi: 10.1128/mSphere.00501-17.
44. Hu HJ, Zhang GQ, Zhang Q, Shakya S, Li ZY 2017 Probiotics Prevent Candida Colonization and Invasive Fungal Sepsis in Preterm Neonates: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Pediatr Neonatol 58:103–110. [PubMed: 27793494]
45. Oncel MY, Arayici S, Sari FN, Simsek GK, Yurttutan S, Erdeve O, Saygan S, Uras N, Oguz SS, Dilmen U 2015 Comparison of Lactobacillus reuteri and nystatin prophylaxis on Candida colonization and infection in very low birth weight infants. J Matern Fetal Neonatal Med 28:1790–1794. [PubMed: 25245226]
46. Turner RB, Woodfolk JA, Borish L, Steinke JW, Patrie JT, Muehling LM, Lahtinen S, Lehtinen MJ 2017 Effect of probiotic on innate inflammatory response and viral shedding in experimental rhinovirus infection - a randomised controlled trial. Benef Microbes 8:207–215. [PubMed: 28343401]
47. Fang SB, Lee HC, Hu JJ, Hou SY, Liu HL, Fang HW 2009 Dose-dependent effect of Lactobacillus rhamnosus on quantitative reduction of faecal rotavirus shedding in children. J Trop Pediatr 55:297–301. [PubMed: 19203988]
48. Alfaleh K, Anabrees J 2014 Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 4:CD005496.
49. Sawh SC, Deshpande S, Jansen S, Reynaert CJ, Jones PM 2016 Prevention of necrotizing enterocolitis with probiotics: a systematic review and meta-analysis. PeerJ 4:e2429. [PubMed: 27761306]
50. Sun J, Marwah G, Westgarth M, Buys N, Ellwood D, Gray PH 2017 Effects of Probiotics on Necrotizing Enterocolitis, Sepsis, Intraventricular Hemorrhage, Mortality, Length of Hospital Stay, and Weight Gain in Very Preterm Infants: A Meta-Analysis. Adv Nutr 8:749–763. [PubMed: 28916575]
51. Dermyshi E, Wang Y, Yan C, Hong W, Qiu G, Gong X, Zhang T 2017 The “Golden Age” of Probiotics: A Systematic Review and Meta-Analysis of Randomized and Observational Studies in Preterm Infants. Neonatology 112:9–23. [PubMed: 28196365]
52. Deshpande G, Jape G, Rao S, Patole S 2017 Benefits of probiotics in preterm neonates in low-income and medium-income countries: a systematic review of randomised controlled trials. BMJ Open 7:e017638.
53. Zhu XL, Tang XG, Qu F, Zheng Y, Zhang WH, Diao YQ 2019 Bifidobacterium may benefit the prevention of necrotizing enterocolitis in preterm infants: A systematic review and meta-analysis. Int J Surg 61:17–25. [PubMed: 30500473]
54. Jiang T, Zhang H, Xu X, Li H, Yang J 2020 Mixed probiotics decrease the incidence of stage II-III necrotizing enterocolitis and death: A systematic review and meta-analysis. Microb Pathog 138:103794. [PubMed: 31672528]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

55. Balasubramanian H, Ananthan A, Rao S, Patole S 2020 Probiotics for Preterm Infants in India - Systematic Review and Meta-Analysis of Randomized Controlled Trials. Indian J Pediatr doi: 10.1007/s12098-020-03223-0.
56. Olsen R, Greisen G, Schroder M, Brok J 2016 Prophylactic Probiotics for Preterm Infants: A Systematic Review and Meta-Analysis of Observational Studies. Neonatology 109:105–112. [PubMed: 26624488]
57. Xiong T, Maheshwari A, Neu J, Ei-Saie A, Pammi M 2020 An Overview of Systematic Reviews of Randomized-Controlled Trials for Preventing Necrotizing Enterocolitis in Preterm Infants. Neonatology 117:46–56. [PubMed: 31838477]
58. Costeloe K, Hardy P, Juszczak E, Wilks M, Millar MR, Probiotics in Preterm Infants Study Collaborative G 2016 Bifidobacterium breve BBG-001 in very preterm infants: a randomised controlled phase 3 trial. Lancet 387:649–660. [PubMed: 26628328]
59. Oncel MY, Sari FN, Arayici S, Guzoglu N, Erdeve O, Uras N, Oguz SS, Dilmen U 2014 Lactobacillus Reuteri for the prevention of necrotising enterocolitis in very low birthweight infants: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed 99:F110–115. [PubMed: 24309022]
60. Manzoni P, Mostert M, Leonessa ML, Priolo C, Farina D, Monetti C, Latino MA, Gomirato G 2006 Oral supplementation with Lactobacillus casei subspecies rhamnosus prevents enteric colonization by Candida species in preterm neonates: a randomized study. Clin Infect Dis 42:1735–1742. [PubMed: 16705580]
61. Jacobs SE, Tobin JM, Opie GF, Donath S, Tabrizi SN, Pirotta M, Morley CJ, Garland SM, ProPrems Study G 2013 Probiotic Effects on Late-onset Sepsis in Very Preterm Infants: A Randomized Controlled Trial. Pediatrics 132:1055–1062. [PubMed: 24249817]
62. Rojas MA, Lozano JM, Rojas MX, Rodriguez VA, Rondon MA, Bastidas JA, Perez LA, Rojas C, Ovalle O, Garcia-Harker JE, Tamayo ME, Ruiz GC, Ballesteros A, Archila MM, Arevalo M 2012 Prophylactic probiotics to prevent death and nosocomial infection in preterm infants. Pediatrics 130:e1113–1120. [PubMed: 23071204]
63. Lin HC, Hsu CH, Chen HL, Chung MY, Hsu JF, Lien RI, Tsao LY, Chen CH, Su BH 2008 Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. Pediatrics 122:693–700. [PubMed: 18829790]
64. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF 2002 Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biol Neonate 82:103–108. [PubMed: 12169832]
65. Guthmann F, Arlettaz Mieth RP, Bucher HU, Buhrer C 2016 Short courses of dual-strain probiotics appear to be effective in reducing necrotising enterocolitis. Acta Paediatr 105:255–259. [PubMed: 26600335]
66. Hartel C, Pagel J, Rupp J, Bendiks M, Guthmann F, Rieger-Fackeldey E, Heckmann M, Franz A, Schiffmann JH, Zimmermann B, Hepping N, von der Wense A, Wieg C, Herting E, Gopel W, German Neonatal N 2014 Prophylactic use of Lactobacillus acidophilus/Bifidobacterium infantis probiotics and outcome in very low birth weight infants. J Pediatr 165:285–289 e281. [PubMed: 24880888]
67. Bonsante F, Iacobelli S, Gouyon JB 2013 Routine probiotic use in very preterm infants: retrospective comparison of two cohorts. Am J Perinatol 30:41–46. [PubMed: 22773285]
68. Hoyos AB 1999 Reduced incidence of necrotizing enterocolitis associated with enteral administration of Lactobacillus acidophilus and Bifidobacterium infantis to neonates in an intensive care unit. Int J Infect Dis 3:197–202. [PubMed: 10575148]
69. Luoto R, Matomaki J, Isolauri E, Lehtonen L 2010 Incidence of necrotizing enterocolitis in very-low-birth-weight infants related to the use of Lactobacillus GG. Acta Paediatr 99:1135–1138. [PubMed: 20219023]
70. Denkel LA, Schwab F, Garten L, Geffers C, Gastmeier P, Piening B 2016 Protective Effect of Dual-Strain Probiotics in Preterm Infants: A Multi-Center Time Series Analysis. PLoS One 11:e0158136. [PubMed: 27332554]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

71. Patole SK, Rao SC, Keil AD, Nathan EA, Doherty DA, Simmer KN 2016 Benefits of Bifidobacterium breve M-16V Supplementation in Preterm Neonates - A Retrospective Cohort Study. PLoS One 11:e0150775. [PubMed: 26953798]

72. Samuels N, van de Graaf R, Been JV, de Jonge RC, Hanff LM, Wijnen RM, Kornelisse RF, Reiss IK, Vermeulen MJ 2016 Necrotising enterocolitis and mortality in preterm infants after introduction of probiotics: a quasi-experimental study. Sci Rep 6:31643. [PubMed: 27545195]

73. Sharpe J, Way M, Koorts PJ, Davies MW 2018 The availability of probiotics and donor human milk is associated with improved survival in very preterm infants. World J Pediatr 14:492–497. [PubMed: 29951973]

74. Singh B, Shah PS, Afifi J, Simpson CD, Mitra S, Dow K, El-Naggar W, Canadian Neonatal Network I 2019 Probiotics for preterm infants: A National Retrospective Cohort Study. J Perinatol 39:533–539. [PubMed: 30692619]

75. Meyer MP, Chow SSW, Alsweiler J, Bourchier D, Broadbent R, Knight D, Lynn AM, Patel H 2020 Probiotics for Prevention of Severe Necrotizing Enterocolitis: Experience of New Zealand Neonatal Intensive Care Units. Front Pediatr 8:119. [PubMed: 32318522]

76. Lin HC, Wu SF, Underwood M 2011 Necrotizing enterocolitis. N Engl J Med 364:1878–1879; author reply 1879. [PubMed: 21568003]

77. Wallach JD, Sullivan PG, Trepanowski JF, Sainani KL, Steyerberg EW, Ioannidis JP 2017 Evaluation of Evidence of Statistical Support and Corroboration of Subgroup Claims in Randomized Clinical Trials. JAMA Intern Med 177:554–560. [PubMed: 28192563]

78. Denkel LA, Schwab F, Garten L, Geffers C, Gastmeier P, Piening B 2017 Dual-strain probiotics reduce NEC, mortality and neonatal bloodstream infections among extremely low birthweight infants. Arch Dis Child Fetal Neonatal Ed 102:F559–F560. [PubMed: 28923984]

79. Favaro L, Todorov SD 2017 Bacteriocinogenic LAB Strains for Fermented Meat Preservation: Perspectives, Challenges, and Limitations. Probiotics Antimicrob Proteins 9:444–458. [PubMed: 28921417]

80. Mokoena MP 2017 Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens: A Mini-Review. Molecules 22: pii: E1255. doi: 10.3390/molecules22081255. [PubMed: 28933759]

81. Arciero J, Bard Ermentrout G, Siggers R, Afrazi A, Hackam D, Vodovotz Y, Rubin J 2013 Modeling the interactions of bacteria and Toll-like receptor-mediated inflammation in necrotizing enterocolitis. J Theor Biol 321:83–99. [PubMed: 23238281]

82. Ganguli K, Meng D, Rautava S, Lu L, Walker WA, Nanthakumar N 2013 Probiotics prevent necrotizing enterocolitis by modulating enterocyte genes that regulate innate immune-mediated inflammation. Am J Physiol Gastrointest Liver Physiol 304:G132–141. [PubMed: 23139215]

83. Halloran K, Underwood MA 2019 Probiotic mechanisms of action. Early Hum Dev 135:58–65. [PubMed: 31174927]

84. Meng D, Sommella E, Salviati E, Campiglia P, Ganguli K, Djebali K, Zhu W, Walker WA 2020 Indole-3-lactic acid, a metabolite of tryptophan, secreted by Bifidobacterium longum subspecies infantis is anti-inflammatory in the immature intestine. Pediatr Res doi: 10.1038/s41390-019-0740-x.

85. Liu Q, Yu Z, Tian F, Zhao J, Zhang H, Zhai Q, Chen W 2020 Surface components and metabolites of probiotics for regulation of intestinal epithelial barrier. Microb Cell Fact 19:23. [PubMed: 32024520]

86. Bafeta A, Koh M, Riveros C, Ravaud P 2018 Harms Reporting in Randomized Controlled Trials of Interventions Aimed at Modifying Microbiota: A Systematic Review. Ann Intern Med 169:240–247. [PubMed: 30014150]

87. Athalye-Jape G, Deshpande G, Rao S, Patole S 2014 Benefits of probiotics on enteral nutrition in preterm neonates: a systematic review. Am J Clin Nutr 100:1508–1519. [PubMed: 25411286]

88. Esaiassen E, Cavanagh P, Hjerde E, Simonsen GS, Stoen R, Klingenberg C 2016 Bifidobacterium longum Subspecies infantis Bacteremia in 3 Extremely Preterm Infants Receiving Probiotics. Emerg Infect Dis 22:1664–1666. [PubMed: 27532215]

89. Costeloe K, Bowler U, Brocklehurst P, Hardy P, Heal P, Juszczak E, King A, Panton N, Stacey F, Whiley A, Wilks M, Millar MR 2016 A randomised controlled trial of the probiotic

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bifidobacterium breve BBG-001 in preterm babies to prevent sepsis, necrotising enterocolitis and death: the Probiotics in Preterm infantS (PiPS) trial. Health Technol Assess 20:1–194.

90. Kane AF, Bhatia AD, Denning PW, Shane AL, Patel RM 2018 Routine Supplementation of Lactobacillus rhamnosus GG and Risk of Necrotizing Enterocolitis in Very Low Birth Weight Infants. J Pediatr 195:73–79 e72. [PubMed: 29402455]
91. Jackson SA, Schoeni JL, Vegge C, Pane M, Stahl B, Bradley M, Goldman VS, Burguiere P, Atwater JB, Sanders ME 2019 Improving End-User Trust in the Quality of Commercial Probiotic Products. Front Microbiol 10:739. [PubMed: 31105649]
92. Vallabhaneni S, Walker TA, Lockhart SR, Ng D, Chiller T, Melchreit R, Brandt ME, Smith RM 2015 Notes from the Field: Fatal Gastrointestinal Mucormycosis in a Premature Infant Associated with a Contaminated Dietary Supplement — Connecticut, 2014. Morbidity and Mortality Weekly Report 64:155–156. [PubMed: 25695322]
93. Villamor-Martinez E, Pierro M, Cavallaro G, Mosca F, Kramer B, Villamor E 2017 Probiotic Supplementation in Preterm Infants Does Not Affect the Risk of Bronchopulmonary Dysplasia: A Meta-Analysis of Randomized Controlled Trials. Nutrients 9: pii: E1197. doi: 10.3390/nu9111197. [PubMed: 29088103]
94. Cavallaro G, Villamor-Martinez E, Filippi L, Mosca F, Villamor E 2017 Probiotic supplementation in preterm infants does not affect the risk of retinopathy of prematurity: a meta-analysis of randomized controlled trials. Sci Rep 7:13014. [PubMed: 29026199]
95. Berrington J, Ward Platt M 2016 Recent advances in the management of infants born <1000 g. Arch Dis Child 101:1053–1056. [PubMed: 27166220]
96. Perapoch J, Planes AM, Querol A, Lopez V, Martinez-Bendayan I, Tormo R, Fernandez F, Peguero G, Salcedo S 2000 Fungemia with Saccharomyces cerevisiae in two newborns, only one of whom had been treated with ultra-levura. Eur J Clin Microbiol Infect Dis 19:468–470. [PubMed: 10947224]
97. Hamprecht K, Goelz R 2017 Postnatal Cytomegalovirus Infection Through Human Milk in Preterm Infants: Transmission, Clinical Presentation, and Prevention. Clin Perinatol 44:121–130. [PubMed: 28159200]
98. Roberts D, Brown J, Medley N, Dalziel SR 2017 Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev 3:CD004454. [PubMed: 28321847]
99. Conde-Agudelo A, Diaz-Rossello JL 2016 Kangaroo mother care to reduce morbidity and mortality in low birthweight infants. Cochrane Database Syst Rev:Cd002771. [PubMed: 27552521]
100. Jensen EA, Foglia EE, Schmidt B 2018 Association between prophylactic indomethacin and death or bronchopulmonary dysplasia: A systematic review and meta-analysis of observational studies. Semin Perinatol 42:228–234. [PubMed: 29907397]
101. Hennes HM, Lee MB, Rimm AA, Shapiro DL 1991 Surfactant replacement therapy in respiratory distress syndrome. Meta-analysis of clinical trials of single-dose surfactant extracts. Am J Dis Child 145:102–104. [PubMed: 1824667]
102. Singh N, Halliday HL, Stevens TP, Suresh G, Soll R, Rojas-Reyes MX 2015 Comparison of animal-derived surfactants for the prevention and treatment of respiratory distress syndrome in preterm infants. Cochrane Database Syst Rev:Cd010249. [PubMed: 26690260]
103. Askie LM, Darlow BA, Davis PG, Finer N, Stenson B, Vento M, Whyte R 2017 Effects of targeting lower versus higher arterial oxygen saturations on death or disability in preterm infants. Cochrane Database Syst Rev 4:Cd011190. [PubMed: 28398697]
104. Quigley M, Embleton ND, McGuire W 2018 Formula versus donor breast milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev 6:Cd002971. [PubMed: 29926476]
105. Mitra S, Florez ID, Tamayo ME, Mbuagbaw L, Vanniyasingam T, Veroniki AA, Zea AM, Zhang Y, Sadeghirad B, Thabane L 2018 Association of Placebo, Indomethacin, Ibuprofen, and Acetaminophen With Closure of Hemodynamically Significant Patent Ductus Arteriosus in Preterm Infants: A Systematic Review and Meta-analysis. Jama 319:1221–1238. [PubMed: 29584842]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

106. Osborn DA, Schindler T, Jones LJ, Sinn JK, Bolisetty S 2018 Higher versus lower amino acid intake in parenteral nutrition for newborn infants. Cochrane Database Syst Rev 3:Cd005949. [PubMed: 29505664]

107. McCall EM, Alderdice F, Halliday HL, Vohra S, Johnston L 2018 Interventions to prevent hypothermia at birth in preterm and/or low birth weight infants. Cochrane Database Syst Rev 2:Cd004210. [PubMed: 29431872]

108. Barrington KJ, Finer N, Pennaforte T 2017 Inhaled nitric oxide for respiratory failure in preterm infants. Cochrane Database Syst Rev 1:Cd000509. [PubMed: 28045472]

109. Chi C, Buys N, Li C, Sun J, Yin C 2019 Effects of prebiotics on sepsis, necrotizing enterocolitis, mortality, feeding intolerance, time to full enteral feeding, length of hospital stay, and stool frequency in preterm infants: a meta-analysis. Eur J Clin Nutr 73:657–670. [PubMed: 30568297]

110. Pammi M, Suresh G 2017 Enteral lactoferrin supplementation for prevention of sepsis and necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 6:Cd007137. [PubMed: 28658720]

111. Griffiths JJP, Vargova M, Bowler U, Juszczak E, King A, Linsell L, Murray D, Partlett C, Patel M, Berrington J, Dorling J, Embleton ND, Heath PT, Oddie S, McGuire W, Ainsworth S, Boyle E, Clarke P, Craig S, Johnson K, Mactier H, Scorrer T, Ledwidge M, Story I, Holder G, Ohadike P, Ellis S, Vaikute R, Gowda G, Yates H, Garg S, Pilling E, Roehr C, Batra D, Gibson D, Johnson M, Kumar Y, Bartle D, Peters C, Quine D, Gupta R, Matthes J, Kennea N, Reynolds P, Geethanath R, Janakiraman S, Vasu V, Manjunatha CM. 2019 Enteral lactoferrin supplementation for very preterm infants: a randomised placebo-controlled trial. Lancet 393:423–433. [PubMed: 30635141]

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

113. Chang HY, Chen JH, Chang JH, Lin HC, Lin CY, Peng CC 2017 Multiple strains probiotics appear to be the most effective probiotics in the prevention of necrotizing enterocolitis and mortality: An updated meta-analysis. PLoS One 12:e0171579. [PubMed: 28182644]

114. van den Akker CHP, van Goudoever JB, Szajewska H, Embleton ND, Hojsak I, Reid D, Shamir R, Espghan Working Group for Probiotics Prebiotics, Committee on Nutrition 2018 Probiotics for Preterm Infants: a strain specific systematic review and network meta-analysis. J Pediatr Gastroenterol Nutr 67:103–122. [PubMed: 29384838]

115. Underwood MA 2019 Probiotics and the prevention of necrotizing enterocolitis. J Pediatr Surg 54:405–412. [PubMed: 30241961]

116. Athalye-Jape G, Rao S, Patole S 2016 Lactobacillus reuteri DSM 17938 as a Probiotic for Preterm Neonates: A Strain-Specific Systematic Review. JPEN J Parenter Enteral Nutr 40:783–794. [PubMed: 26059900]

117. Rolnitsky A, Ng E, Asztalos E, Shama Y, Karol D, Findlater C, Garsch M, Dunn M 2019 A Quality Improvement Intervention to Reduce Necrotizing Enterocolitis in premature infants with Probiotic Supplementation. Pediatr Qual Saf 4:e201. [PubMed: 31745505]

118. Sekhon MK, Grubb PH, Newman M, Yoder BA 2019 Implementation of a probiotic protocol to reduce rates of necrotizing enterocolitis. J Perinatol 39:1315–1322. [PubMed: 31358866]

119. Smilowitz JT, Moya J, Breck MA, Cook C, Fineberg A, Angkustsiri K, Underwood MA 2017 Safety and tolerability of Bifidobacterium longum subspecies infantis EVC001 supplementation in healthy term breastfed infants: a phase I clinical trial. BMC Pediatr 17:133. [PubMed: 28558732]

120. Scalabrin D, Harris C, Johnston WH, Berseth CL 2017 Long-term safety assessment in children who received hydrolyzed protein formulas with Lactobacillus rhamnosus GG: a 5-year follow-up. Eur J Pediatr 176:217–224. [PubMed: 27975116]

121. Lundelin K, Poussa T, Salminen S, Isolauri E 2017 Long-term safety and efficacy of perinatal probiotic intervention: Evidence from a follow-up study of four randomized, double-blind, placebo-controlled trials. Pediatr Allergy Immunol 28:170–175. [PubMed: 27779809]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

122. Watkins C, Murphy K, Dempsey EM, O'Shea CA, Murphy BP, O'Toole PW, Ross RP, Stanton C, Ryan CA 2019 Dose-interval study of a dual probiotic in preterm infants. Arch Dis Child Fetal Neonatal Ed 104:F159–f164. [PubMed: 29925539]

123. van den Akker CHP, van Goudoever JB, Shamir R, Domellof M, Embleton ND, Hojsak I, Lapillonne A, Mihatsch WA, Canani RB, Bronsky J, Campoy C, Fewtrell MS, Mis NF, Guarino A, Hulst JM, Indrio F, Kolacek S, Orel R, Vandenplas Y, Weizman Z, Szajewska H 2020 Probiotics and Preterm Infants: A Position Paper by the ESPGHAN Committee on Nutrition and the ESPGHAN Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr 70(5):664–680. [PubMed: 32332478]

124. Ortenstrand A, Westrup B, Brostrom EB, Sarman I, Akerstrom S, Brune T, Lindberg L, Waldenstrom U 2010 The Stockholm Neonatal Family Centered Care Study: effects on length of stay and infant morbidity. Pediatrics 125:e278–285. [PubMed: 20100748]

125. White-Traut RC, Rankin KM, Yoder JC, Liu L, Vasa R, Geraldo V, Norr KF 2015 Influence of H-HOPE intervention for premature infants on growth, feeding progression and length of stay during initial hospitalization. J Perinatol 35:636–641. [PubMed: 25742287]

126. Raiskila S, Axelin A, Rapeli S, Vasko I, Lehtonen L 2014 Trends in care practices reflecting parental involvement in neonatal care. Early Hum Dev 90:863–867. [PubMed: 25463833]

127. Gianni ML, Sannino P, Bezze E, Comito C, Plevani L, Roggero P, Agosti M, Mosca F 2016 Does parental involvement affect the development of feeding skills in preterm infants? A prospective study. Early Hum Dev 103:123–128. [PubMed: 27591506]

128. Franck LS, McNulty A, Alderdice F 2017 The Perinatal-Neonatal Care Journey for Parents of Preterm Infants: What Is Working and What Can Be Improved. J Perinat Neonatal Nurs 31:244–255. [PubMed: 28737545]

129. Umberger E, Canvasser J, Hall SL 2018 Enhancing NICU parent engagement and empowerment. Semin Pediatr Surg 27:19–24. [PubMed: 29275811]

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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

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Table 2:

Impact of probiotic administration to preterm infants on NEC (reference 49 as assessed in reference 57). The AMSTAR score for this meta-analysis was 9/11 (high quality).

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)

Abbreviations:; RR, relative risk; CI, confidence interval

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Table 3:

NEC in RCTs trials including at least 400 preterm infants

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

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

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Table 4:

NEC in cohort studies including at least 1000 preterm infants

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

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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 (98)[a]	22	7188	0.69 (0.59, 0.81)
Kangaroo mother care (99)[b]	8	1736	0.60 (0.39, 0.92)
Prophylactic indomethacin (100)	18	2769	No difference in mortality
Prophylactic indomethacin (100)[c]	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 (102)[d]	9	901	1.44 (1.04, 2.00)
Low vs high oxygen saturation target (103)[e]	5	4873	1.16 (1.03, 1.31)
Formula vs donor human milk (104)[f]	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 (106)[g]	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, RR for NEC 0.50 (0.32, 0.78). See reference 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 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, RR for NEC with formula 1.87 (1.23, 2.85). See reference 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, RR for NEC 1.0 (0.68, 1.47)). See reference 57