Arguments for routine administration of probiotics for NEC prevention

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Purpose of review
Probiotic administration to premature infants for the purpose of prevention of necrotizing enterocolitis is common in many parts of the world but uncommon in the United States. The present review will emphasize recent findings in support of routine administration of probiotics to this highly vulnerable population.

Recent findings
Additional evidence from animal models describing mechanisms of protection of probiotics in the immature gut and updated meta-analyses of randomized placebo-controlled trials and observational cohorts are presented (now including more than 40 000 premature infants from countries across the globe).

Summary
The preponderance of evidence suggests that probiotic administration to premature infants is well tolerated and decreases the risk of death, necrotizing enterocolitis, and sepsis. Further comparisons of probiotic administration to placebo are not likely to alter these conclusions. Rather, future work should focus on assurance of high-quality products with demonstrated purity and viability of probiotic microbes, and future clinical trials should focus on comparisons between high-quality products and doses.

Keywords
Bifidobacterium, Lactobacillus, necrotizing enterocolitis, probiotic

INTRODUCTION

Necrotizing enterocolitis (NEC) is a common and devastating complication of extreme prematurity and the most common cause of death after 2 weeks of age in babies with gestational age less than 29 weeks at birth [1]. Key risk factors include prematurity (including immaturity of all aspects of the intestinal innate immune system) and intestinal dysbiosis. It is not likely coincidental that the peak time of onset for NEC in very preterm infants is 27–33 weeks corrected gestational age [2], a 'perfect storm' time period when the microbiota is dominated by pro-inflammatory Gram negative γ-Proteobacteria [3] and key components of both adaptive and innate immunity play both protective and injurious roles [4▪,5].

Probiotics are dietary supplements containing live organisms which are intended to benefit health when consumed. The potential benefits of treating premature infants with probiotics to decrease the risk of NEC first garnered attention in 1999 with the publication of two seminal papers: the first cohort study from a neonatal intensive care unit (NICU) in Colombia demonstrating a dramatic reduction in NEC and NEC-related deaths with the introduction of universal administration of a probiotic product to all admitted infants [6] and the first demonstration of NEC prevention in a rodent model [7]. Since that time a large number of animal studies, randomized controlled trials and observational cohort studies, including many studies from developing countries have been published. We now have a very large body of evidence demonstrating mechanisms of protection, safety, and efficacy of probiotic products for the prevention of NEC. Probiotics are not approved by the United States Food and Drug Administration for the prevention, mitigation or treatment of disease. The purpose of this article is to summarize the evidence in favor of routine administration of probiotics to very preterm infants with emphasis on the most recent data and to compare the strength of this evidence to that of other common practices in neonatology.

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Curr Opin Pediatr 2019, 31:188–194

DOI:10.1097/MOP.0000000000000732

KEY POINTS

- Animal studies have demonstrated mechanisms by which probiotic microbes impact the developing intestinal tract and immune system and decrease risk of NEC.
- Observational cohort studies including more than 30 000 premature infants have demonstrated decreased risk of death, NEC, and sepsis during periods of probiotic administration compared to no probiotic administration.
- Randomized placebo-controlled trials including more than 10 000 premature infants have demonstrated decreased risk of death and NEC (with a strong trend towards decreased sepsis) in infants randomized to probiotic administration.
- Parents are interested in participating in decisions regarding probiotics and human milk feeding.

MECHANISMS AND THE IMPORTANCE OF ANIMAL MODELS

The value of animal models in discovering the pathogenesis of NEC and in identifying biomarkers and preventive strategies has recently been emphasized [8▪]. Rodent and piglet models have been particularly useful in discovering mechanisms by which probiotic microbes interact with and influence the developing intestine. Key mechanisms include suppression of pro-inflammatory cytokines interleukin (IL)1β, IL6, IL8, and tumor necrosis factor (TNF)α; production of antimicrobial bacteriocins; attenuation of stress-induced apoptosis and increased intestinal permeability; and lowering of intestinal pH through production of organic acids, all of which show variability by probiotic species and/or strain [9,10]. A recent summary of probiotic studies in animal models reviewed 29 studies (rat, mouse, piglet, quail, and rabbit) of which 16 were able to be pooled in a meta-analysis with the following conclusion: 'Probiotics significantly reduced NEC via beneficial effects on immunity, inflammation, tissue injury, gut barrier, and intestinal dysbiosis' [11▪]. Since that analysis, an additional three publications have demonstrated benefit of probiotics in NEC prevention and yielded new mechanistic insights including the role of single immunoglobulin IL-1-related receptor (SIGIRR)/Toll interacting protein (TOLLIP) in NEC prevention by *B. adolescentis* [12▪], the role of toll-like receptor (TLR)2 in NEC prevention by *L. reuteri* [13], and the protective effects of a single dose of *L. reuteri* grown as a biofilm on the surface of dextranomer microspheres [14▪]. An unadjusted summary of the impact of probiotics on NEC in published animal models is included in Table 1.

One promising mechanism by which probiotics decrease the risk of NEC and sepsis is the alteration of the composition of the intestinal microbiota. Carefully collected data from premature infants demonstrate that intestinal dysbiosis (defined as an alteration in the microbiota of a given anatomic niche associated with disease) precedes the onset of NEC and sepsis. The most common pattern seen is an increase in pro-inflammatory proteobacteria in the feces prior to the onset of NEC [15▪▪], whereas an increase in fecal bacilli (mostly coagulase negative staphylococci) precedes the onset of sepsis [16▪▪]. We have previously demonstrated that the probiotic *Bifidobacterium longum* subsp. *infantis* is uniquely adapted to consume human milk oligosaccharides [17] and that the combination of *B. infantis* and human milk dramatically alters the intestinal microbiota in term [18▪] and preterm infants [19] with significant decreases in proteobacteria in both groups. Administration of this probiotic strain to breast-fed term infants not only altered the composition of the fecal microbiota but decreased the abundance of fecal bacterial virulence genes [20] and reduced colonic mucin degradation [21]. This clinical trial in term breast-fed infants is unique among probiotic trials in that the composition of the fecal microbiota was dramatically changed by a three week course of probiotic (from day of life 7 to day of life 28) and that the resultant dominance of bifidobacteria persisted for several weeks after cessation of probiotic administration demonstrating the value of providing a substrate (human milk oligosaccharides) that is preferentially consumed by the administered probiotic microbe [18▪].

Table 1. Summary of studies of probiotics in the prevention of NEC

	Probiotic	No probiotic	Ratio (95% CI)
Animal models [11▪,12▪,13,14▪]	240/817 (29%)	421/696 (60%)	OR 0.27 (0.22, 0.34)
Randomized placebo-controlled trials [28]	170/5304 (3.2%)	311/5216 (6.0%)	RR 0.53 (0.42, 0.66)
Observational cohort studies [10]	419/14967 (2.8%)	806/16443 (4.9%)	OR 0.56 (0.50, 0.63)

The numerator is the number with confirmed NEC and the denominator is the total number of animals or infants studied.

SAFETY OF PROBIOTIC ADMINISTRATION

The vast majority of decisions in the care of very preterm infants are based upon weighing the potential risks and benefits of a given treatment. One of the criticisms of studies of probiotic trials in premature infants is the lack of safety data. Table 2 presents data on key safety outcomes from meta-analyses of randomized controlled trials of probiotic treatment in premature infants. Benefits of probiotic administration in these trials include a significantly lower risk of death, a shorter time to full enteral feeding and a shorter length of hospital stay with a strong trend towards decreased risk of culture positive sepsis. There were no significant differences between groups in the incidence of bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), intraventricular hemorrhage (IVH), or weight gain during hospitalization. While episodes of harm from probiotic sepsis and contamination of probiotic products have been reported [22–24], the evidence demonstrates that probiotic administration to this immunocompromised population decreases the risk of death and sepsis suggesting that sepsis and death related to probiotic administration must be very uncommon.

EFFICACY OF PROBIOTIC ADMINISTRATION

Systematic reviews and meta-analyses are generally viewed as either the pinnacle of the strength of evidence pyramid or the lens through which evidence is best viewed [25]. The most recent Cochrane review of this topic concluded: 'Enteral supplementation of probiotics prevents severe NEC and all-cause mortality in preterm infants. Our updated review of available evidence strongly supports a change in practice. Head to head comparative studies are required to assess the most effective preparations, timing, and length of therapy to be utilized [26]'. Since that Cochrane review, several additional clinical trials of probiotics in premature infants for the prevention of NEC have been published (including the largest study to date which found no benefit in NEC prevention with administration of *B. breve* BBG-001) [27] and several meta-analyses have been performed using different models and including different trials. Each meta-analysis has reached the same conclusion: probiotics decrease the risk of NEC and death in preterm infants. Table 1 includes the results of the largest of these meta-analyses (including more than 10 000 premature infants) [28].

Observational cohort studies are generally considered to provide a lower strength of evidence, however these studies can be very useful, particularly in the investigation of uncommon diseases and in situations wherein the population to be studied can be carefully monitored. Observational studies of premature infants have particular value for outcomes that are limited to the period of the NICU stay, such as NEC and sepsis. Two meta-analyses of observational studies comparing periods of no probiotic administration to periods of probiotic administration have been published to date [29,30▪▪]. An unadjusted odds ratio from a recent summary of these studies and an additional 11 observational studies is included in Table 1 (including more than 30 000 premature infants) [10]. Of the 23 published observational cohort studies published to date, 13 have demonstrated a benefit in reduction of NEC with administration of probiotics, eight have found no significant difference, and two have demonstrated an increase in the incidence of NEC during the probiotic administration period.

Table 2. Safety of probiotic administration to premature infants from meta-analyses of randomized controlled trials (except the last two outcomes)

	Number of trials	Number of infants	RR (95% CI)	*P* value
All-cause mortality [28]	27	9507	0.79 (0.68, 0.93)	0.003
Culture positive sepsis [28]	28	8707	0.88 (0.77, 1.0)	0.05
BPD [45]	12	4384	1.07 (0.96, 1.2)	0.20
ROP [46]	11	4250	1.05 (0.96, 1.23)	0.51
IVH [47▪▪]	10	3431	0.91 (0.73, 1.14)	0.42
Weight gain [47▪▪]	15	3751	−0.29 (−1.16, 0.58)	0.51
Time to full feeding [48]	19	4527	−1.5d (−2.8, −0.32)	<0.001
Length of NICU stay [47▪▪]	19	5443	−3.8d (−5.8, −1.9)	<0.001
All cause mortality[a] [10]	22	28 260	0.83 (0.76, 0.90)	<0.001
Culture positive sepsis[a] [10]	21	25 785	0.91 (0.85, 0.97)	0.004

[a]Observational cohort studies.

PROBIOTIC DATA IN THE CONTEXT OF OTHER COMMON NEONATAL INTENSIVE CARE UNIT PRACTICES

Given the high degree of vulnerability of premature infants, neonatologists are often early adopters of promising practices. Rapid adoption of postnatal administration of prolonged courses of corticosteroids is frequently cited as evidence of the need for caution in the NICU, however the question of which, whether, what dose, what route, and how long to provide corticosteroids to decrease risk of BPD without increasing the risk of cerebral palsy remains uncertain in spite of 47 RCTs including 6747 premature infants [31]. This degree of uncertainty has not seemed to limit administration of corticosteroids to premature infants. Table 3 summarizes the current evidence supporting several common practices in the NICU, including those intended to decrease the risk of NEC and other poor outcomes. The current level of evidence supporting routine administration of probiotics to premature infants in hopes of decreasing the risk of NEC, death, sepsis and feeding intolerance is stronger than the evidence for many common NICU practices.

INCLUDING PARENTS IN THE DECISIONS TO UTILIZE HUMAN MILK AND PROBIOTICS

Historically, parents were often excluded from the NICU based on beliefs that their presence would increase the risk of infection or other poor outcomes. However, as parents have become integral to the care of their premature infants in the NICU, outcomes have improved [32–34]. In small group sessions and focus groups, parents of premature infants have repeatedly requested more communication and resources [35]. Parents of infants who have developed NEC almost universally express frustration that they were unaware of this devastating disease and of potential preventive approaches [36▪▪]. Sharing information about NEC, sepsis, and the potential risks and benefits of human milk and probiotics with parents of preterm infants – either as part of the prenatal consultation or after delivery – can be accomplished either directly or through a handout or flyer. The NEC Society website has an example of a simple handout: https://necsociety.org/wp-content/uploads/2018/01/probiotic-information-for-parents-2018.pdf and suggestions as to promising probiotic products with evidence for efficacy and purity plus details regarding a low-cost multicenter quality improvement project. Many parents express a preference to participate as an essential part of the team caring for their premature infant as opposed to the more paternalistic model in which the physician dictates all care decisions [37].

Table 3. Strength of evidence for common NICU practices

	Number of trials	Number of infants	RR (95% CI)
RCTs with NEC as the outcome			
Slow vs. fast feeding advancement [49]	9	949	1.02 (0.64, 1.62)
Formula vs. donor human milk [50]	9	1070	2.77 (1.40, 5.46)
Exclusive human diet vs. bovine-based protein [51]	2	260	0.31 (0.14, 0.68)
Observational studies with NEC as the outcome			
Mother's own milk vs. no mother's own milk in first 7 days [52▪▪]	1	14 678	0.69 (0.60, 0.78)
No bovine products vs. any bovine products in first 14 days [52▪▪]	1	14 678	0.61 (0.39, 0.83)
No donor human milk available vs. donor human milk available [53]	1	42 532	1.15 (1.03, 1.28)
Exclusive human diet vs. bovine-based fortifier [54–56]	3	2494	0.70 (0.56, 0.87)

Other common NICU practices				
	Outcome	**Number of trials**	**Number of infants**	**RR (95% CI)**
Therapeutic hypothermia [57]	Death or neurodevelopmental disability at 18 months	11	1505	0.75 (0.68, 0.83)
Less invasive surfactant administration [58]	Death or BPD	6	895	0.75 (0.59, 0.94)
Newborn individualized developmental care and assessment program [59]	Death or major sensorineural disability at 18 months	3	302	0.89 (0.61, 1.29)
Prophylactic indomethacin [60]	Death or BPD	2[a]	11 289	0.93 (0.76, 1.13)

[a]Observational studies.

Table 4. Meta-analyses of probiotic administration in children and adults

Disease	Number of studies	Number of patients	Ratio (95% CI)
Hirschprung-associated enterocolitis [61]	5	198	OR 0.72 (0.37, 1.39)
Clostridium difficile associated diarrhea [62]	31	8672	RR 0.40 (0.30, 0.52)
Hepatic encephalopathy [63]	14	1152	OR 0.40 (0.26, 0.60)
Pancreatitis mortality [64]	3	403	OR 0.83 (0.14, 4.83)

PREMATURE INFANTS ARE NOT VERY LITTLE ADULTS

Table 4 summarizes recent meta-analyses of probiotic administration in children and adults; whereas some of these meta-analyses show benefit and some do not, none shows a worse outcome with probiotic administration. The limited evidence of benefit from multiple studies of probiotics in adults and children does not imply that probiotic administration is ineffective in premature infants. The intestinal microbiota of the child and adult is highly diverse, well established and difficult to modify with probiotic organisms [38]. The intestinal innate immune system of the healthy child and adult is a highly effective barrier between pathogens and food antigens in the intestinal lumen and the host [39,40]. Conversely, the intestinal microbiota of the healthy breast-fed infant has low diversity and is dominated by bifidobacteria, particularly in developing countries where antibiotic exposure, high-fat diets, formula feeding, and other practices that alter this community of microbes are less common [41,42], whereas the intestinal microbiota of the premature infant is dominated by proteobacteria, particularly from 27 to 32 weeks corrected gestational age (the window in which most cases of NEC occur) [2,3]. In addition, essentially all aspects of the innate immune system are immature and poorly regulated in the term and premature infant [43,44]. It is therefore not surprising that the impact of probiotic microbes differs in premature infants compared to term infants, children, and adults.

CONCLUSION

Probiotic microbes have been shown in animal models to prevent NEC by improving barrier function of the immature intestine, suppressing an excessive inflammatory response, and by altering the composition of the intestinal microbiota. Cohort studies including more than 30 000 infants have demonstrated decreased incidences of NEC, death, and sepsis among premature infants receiving probiotics. Randomized placebo-controlled trials including more than 10 000 premature infants have demonstrated statistically significant decreased risks of NEC and death, decreased feeding intolerance and decreased length of hospital stay with a strong trend towards decreased risk of culture positive sepsis with probiotic administration. The evidence for benefit far outweighs the evidence for risk in this highly vulnerable population (much like the evidence for benefit from mother's own milk). The evidence for routine administration of probiotics to premature infants is stronger than current evidence for many common NICU practices. Although many questions remain, including the optimum dose, strain, duration, and number of strains of probiotics, these questions are unlikely to be answered definitively in the near future (not unlike current questions about optimal feeding strategies and postnatal steroids). My opinions: withholding probiotics from premature infants has become difficult to justify, and withholding information from parents about the risks and benefits of both human milk and probiotics is unconscionable.

Acknowledgements

None.

Financial support and sponsorship

The author has received financial support from the NIH (R01 HD059127, R21 HD096247).

Conflicts of interest

The author has received honoraria from Abbott, is on the scientific advisory board for Avexegen, and has served as the chair of the Data Safety Monitoring Board for clinical trials conducted by Infant Bacterial Therapeutics (administered through Premier Research). The author was the principal investigator for a clinical trial funded by Evolve Biosystems, but has not received any salary or other financial support for this trial.

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