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Impact of Probiotics on Necrotizing Enterocolitis

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Abstract

A large number of randomized placebo-controlled clinical trials and cohort studies have demonstrated a decrease in the incidence of necrotizing enterocolitis with administration of probiotic microbes. These studies have prompted many neonatologists to adopt routine prophylactic administration of probiotics while others await more definitive studies and/or probiotic products with demonstrated purity and stable numbers of live organisms. Cross-contamination and inadequate sample size limit the value of further traditional placebo-controlled randomized controlled trials. Key areas for future research include mechanisms of protection, optimum probiotic species or strains (or combinations thereof) and duration of treatment, interactions between diet and the administered probiotic, and the influence of genetic polymorphisms in the mother and infant on probiotic response. Next generation probiotics selected based on bacterial genetics rather than ease of production and large cluster-randomized clinical trials hold great promise for NEC prevention.

It has been more than 20 years since the first premature infants were enrolled in the first published cohort study of probiotic microbes for the prevention of necrotizing enterocolitis (NEC). In that landmark study, Dr. Angela Hoyos and her colleagues gave every baby admitted to the neonatal intensive care unit (NICU) in Hospital Simon Bolivar, Bogota, Colombia for a one year period, a probiotic formulation containing *Lactobacillus acidophilus* and *Bifidobacterium infantis* (Infloran, Swiss Serum and Vaccine Institute, Bern, Switzerland) at a dose of 2.5×10^8 of each organism once daily for the entire hospitalization. They then compared the incidence of NEC during the probiotic year and the previous year and found a dramatic decrease with administration of probiotics.[1]

Since that time, 35 randomized placebo controlled clinical trials with NEC, death and/or sepsis as a reported outcome (the first 33 of these trials have recently been summarized[2] with two additional trials published subsequently[3,4]) and an additional 10 cohort studies comparing periods of time with universal treatment with probiotics to control periods[2,5] have been published in English language journals. Tables 1[6-12] and 2[1,5,13-17] present the data for the largest of these trials and cohort studies (those with at least 200 infants per arm). When all 35 randomized controlled trials are combined a total of 5559 premature infants received

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probiotics and 5513 premature infants received either a placebo or a blinded non-treatment. Among those studies reporting stage 2 or greater NEC as an outcome, unweighted percentages were 3.3% in infants receiving probiotics and 6.1% in control infants. Among those studies reporting culture positive sepsis the unweighted percentages were 12% and 14%, and among those studies reporting death 5.1% and 7.2% in the probiotic and control groups respectively. When the 11 cohort studies are combined, the unweighted percentages are strikingly similar to the randomized trials: 7742 infants received probiotics (NEC 1.4%, sepsis 12%, and death 7.6%) and 7592 did not (NEC 4.4%, sepsis 14% and death 9.2%). Multiple English language meta-analyses of the randomized controlled trials have been performed (the most recent are summarized in Table 3),[18-23] all with similar conclusions: treatment of premature infants with probiotics decreases the risk of NEC and decreases mortality. Many of the published studies also included data on feeding tolerance and length of hospitalization, both of which were improved in the probiotic groups. It is possible that routine administration of probiotics to premature infants is the most studied, safe, and effective preventive intervention ever to be rejected by U.S. neonatology. In this article we will review the mechanisms by which probiotics exert their protective effects, the risks and obstacles to probiotic administration, the data favoring specific probiotic species and strains, and promising future directions.

Mechanisms of action

Probiotics are variously categorized as either dietary supplements or medications and are generally defined as containing live organisms that improve health. At the most basic level, probiotics are administered in an attempt to alter the composition of the intestinal microbiota. Most probiotic products contain one or more of the following bacterial or fungal genera: *Lactobacillus, Bifidobacterium, Streptococcus, Escherichia, Enterococcus, Bacillus*, or *Saccharomyces*. The capacity for an ingested probiotic microbe to reach the small intestine and impact the microbial community in the small and large bowel is determined by its resistance to oral and gastric enzymes and acids and to bile acids and its ability to effectively compete with other gut microbes without triggering an untoward host response. This review will focus on the probiotic genera that have been most studied in premature infants and NEC animal models: bifidobacteria and lactobacilli.

Colonization of the fetal intestinal tract begins *in utero*. Until recently, amniotic fluid was believed to be sterile until the time of rupture of the fetal membranes, however careful studies have demonstrated that the placenta and the amniotic fluid become colonized with microbes, predominantly from the maternal vagina or by hematogenous spread of maternal oral microbes even prior to obvious membrane rupture.[24,25] Since vaginal dysbiosis and maternal periodontal disease are both associated with preterm labor,[26,27] it is likely that many preterm infants are colonized *in utero* through swallowed amniotic fluid with both maternal commensals and pathobionts. At the time of rupture of membranes and delivery the fetus/neonate is exposed to large numbers of bacteria with this initial wave of colonists influenced by delivery type and antibiotic exposure. Healthy term infants who are born vaginally and breast-fed undergo a change in the fecal microbiota in the first weeks of life from a preponderance of vaginal microbes to a preponderance of organisms that are capable of consuming human milk oligosaccharides (HMOs). Of dozens of bacterial genera tested,

only bacteroides and bifidobacteria are capable of consuming HMOs.[28] In a cohort of predominantly breast-fed infants in Bangladesh, we found the dominant fecal bacteria to be Actinobacterium at the phylum level and *Bifidobacterium infantis* at the subspecies level. Furthermore we found that the infants with the highest numbers of *B. infantis* had better growth, larger thymus size by ultrasound, and higher T cell and IgG responses to several vaccines.[29] Premature infants often have prolonged hospitalizations, periods of time without any enteral feedings, exposure to antibiotics and other agents with the potential to alter the gut microbiota (e.g. acid blocking agents) and therefore become colonized with a community of microbes that is dominated by Gram negative Proteobacteria and Gram positive Firmicutes at the phylum level and is heavily influenced by corrected gestational age.[30] Most of the pathobionts that cause sepsis and NEC in premature infants are either Proteobacteria (e.g. *E. coli*, *Klebsiella, Proteus, Serratia, Pseudomonas*) or Firmicutes (enterococci, streptococci, staphylococci and clostridia). A bloom of Proteobacteria just prior to the onset of NEC has been demonstrated.[31,32] Bifidobacteria are conspicuously absent in most very premature infants even those fed exclusively human milk.

Bifidobacteria

The primary mechanism of colonization of the intestinal tract by bifidobacteria in term infants is the species-specific capacity to consume HMOs as a food source. The bacterial genomes of many strains of bifidobacteria have been sequenced revealing a large variety of bacterial glycosidases with those species most commonly found in infant feces (infantis, bifidum, and breve) expressing the glycosidases necessary to digest HMOs and those species more commonly found in adult feces (animalis, longum, adolescentis, dentium, catenulatum, and pseudocatenulatum) expressing glycosidases necessary to digest plant oligosaccharides.[33,34] Among the bifidobacterial species, *B. infantis* is unique in the number of fucosidases, sialidases, and other glycosyl hydrolases expressed in its genome. Thus *B. infantis* is able to utilize the full range of HMOs as a nutrient source, whereas *B. bifidum* is unable to utilize fucosylated and sialylated HMOs and *B. breve* predominantly utilizes neutral HMOs with capacity to utilize fucosylated and sialylated HMOs varying by strain.[35-37] In premature infants, administration of probiotic *B. infantis* led to colonization while administration of probiotic *B. animalis* ssp *lactis* did not.[38]

In addition to an advantage in colonization in the presence of human milk, bifidobacterial species demonstrate other properties that may contribute to prevention of NEC. *B. infantis* binds to Caco-2 cells,[39] produces a single bacteriocin with activity against other lactic acid bacilli as well as staphylococci, streptococci, *Salmonella* and *E. coli*,[40] and secretes molecules with anti-inflammatory properties.[39,41] In the rat model of NEC, *B. infantis* decreases the incidence and severity of NEC and decreases expression of IL6, IL8, TNFα, IL23, and iNOS.[42] Culture medium from *B. infantis* decreases the severity of infection with *C. sakazaki* in the mouse with attenuation of three mechanisms of pathogenesis: induction of IL1β and TNFα, decrease in mucin production, and increase in apoptosis.[43]

B. bifidum produces two known bacteriocins[40] and is a more abundant producer of short chain fatty acids than other bifidobacteria tested (the short chain fatty acids butyrate, propionate, and acetate are produced by commensal bacteria and serve as a primary nutrient

source for host colonocytes).[44] In the rat model of NEC, *B. bifidum* decreases the incidence and severity of NEC, attenuates induction of IL6, attenuates induction of trefoil factor 3 and several antimicrobial peptides, improves barrier function, activates TLR2, and decreases apoptosis.[45-47]

B. breve has also been tested in the rat with decreased incidence and severity of NEC, attenuated TLR4 signaling, increased TLR2 signaling and suppressed inflammation.[48] In the rat weanling colitis model, *B. breve* ameliorated colitis with attenuations in IL1α, IL1β, IL10, TNFα, and TGFβ in the colon.[49]

Lactobacilli

While *Lactobacillus* species are found in abundance in the vagina, they are not typical colonizers of the newborn intestinal tract. Several species of *Lactobacillus* are utilized as food additives and sold as commercial probiotics and have been extensively characterized. Lactobacilli are not generally able to consume intact HMOs, however a species of *L casei* has recently been shown to contain a gene cluster capable of metabolizing lacto-N-biose, a key component of HMOs and a second cluster capable of metabolizing the HMO lacto-N-triose utilizing metabolic pathways that differ from those of bifidobacteria.[50,51] In premature prolonged rupture of membranes, if the maternal cervical microbiota is dominated by lactobacilli, the incidences of microbial invasion of the amniotic cavity and histologic chorioamnionitis are decreased.[52] *In vitro* studies demonstrate marked heterogeneity among species of *Lactobacillus* in the capacity to induce activation and maturation of dendritic cells with some species predominantly pro-inflammatory (e.g. *L salivarius*).[53]

L. acidophilus is a common food additive, is stable across a range of pH and temperature, and decreased disease severity in a rat NEC model.[54] *L acidophilus* strains produce at least 27 bacteriocins with antibacterial activity against a wide range of gut microbes including other lactobacilli, *Enterobacteriaceae, Bacillus* sp,, *Corynebacterium* sp, *Vibrio* sp, staphylococci, and *C. sakazaki*[55-57] *L acidophilus* secretes anti-inflammatory factors that inhibit induction of NFκB and IL-8 by platelet activating factor (PAF) an inflammatory pathway important in NEC.[58] *L acidophilus* produces short chain fatty acids and also secretes factors that facilitate uptake of butyrate by the host colonocyte.[59] A recent study demonstrated that *L. acidophilus* altered expression in human fetal intestinal cells of a variety of genes important in immune response, apoptosis and cell survival, cell adhesion, the cell cycle, development and angiogenesis.[60]

L. reuteri has direct antimicrobial activity through secretion of at least one bacteriocin (reuterin) and an antibiotic (reutericycline).[61,62] *In vitro* studies have demonstrated strain specific anti-inflammatory effects via suppression of tumor necrosis factor (TNF) in activated human macrophages.[63] In a rat model of NEC, *L reuteri* strains DSM 17938 and ATCC PTA 4659 decreased the incidence and severity of NEC, decreased mRNA expression of IL-6, TNF-α, TLR4, and NF-κB and protein expression of TLR4, TNF-α and IL-1β and increased numbers of regulatory T cells in the ileum.[64,65] *L reuteri* also impacts intestinal motility which may be of importance in the premature infant given immaturity of peristaltic activity.[66,67]

A single strain of *L. rhamnosus* (GG ATCC 53103) is perhaps the most studied probiotic organism. This strain has antimicrobial activity that appears to be predominately related to lowering the luminal pH through production of lactic acid and other bacterial products.[68] *L rhamnosus* GG has anti-inflammatory effects; the primary mechanism appears to be upregulation of the IL10R2 receptor subunit which decreases expression of TNFα and MIP2 and increases expression of the suppressors of cytokine signaling (SOCS) family.[69] *L rhamnosus* GG also improves intestinal barrier function.[70-72] In both the piglet and mouse models of NEC, a different strain of *L. rhamnosus* (HN001) decreased the incidence and severity of NEC by altering TLR9 signaling.[73]

Risks of probiotic administration

The risks and drawbacks of administration of probiotics fall into four categories. First, case reports clearly document occurrences of sepsis caused by the administered probiotic microbe. This risk is particularly relevant to premature infants given the immaturity of their intestinal barrier and increased risk of translocation of intestinal microbes into the lymphatic and/or systemic circulation. Among the probiotic products commonly administered to premature infants, there are reports of sepsis from *Saccharomyces boulardii* in one preterm infant and three term infants (two of these infants were in a bed adjacent to an infant receiving the probiotic),[74-76] *L. rhamnosus* GG in several premature infants and term infants many of whom had either congenital heart disease, gastroschisis, or short gut syndrome,[77-82] and in a few infants receiving probiotic bifidobacteria.[83-85] It has been argued that the actual incidence of probiotic-induced sepsis is likely under-reported as anaerobic cultures may not be routinely performed in infants receiving probiotics, however among the clinical trials of premature infants reporting mortality and/or culture-negative clinical sepsis, the incidences of both are either decreased or unchanged suggesting that probiotic-induced sepsis is likely very rare.

In adults, bacteremia caused by *Lactobacillus* species occurs most commonly in the elderly, the immunocompromised, and those with central venous catheters with *L. salivarius* the most common species in Taiwan[86] and *L. rhamnosus* the most common in Europe.[87,88] In Finland, where a database of all positive blood cultures for the entire country is maintained, there was no increase in *Lactobacillus* bacteremia over a period of years in which use of probiotic lactobacilli increased dramatically.[89] In Stockholm, the incidence of *Lactobacillus* bacteremia remained <1% of all positive blood cultures in spite of marked increases in probiotic consumption and none of the organisms isolated were identical to the probiotic strains commonly available there.[90] In a Quebec hospital, administration of a probiotic containing three species of *Lactobacillus* to every adult patient receiving antibiotics (a total of 44,835 inpatients over a decade) resulted in a dramatic decrease in *Clostridium difficile* infection with no cases of *Lactobacillus* bacteremia.[91] Infections with *L. acidophilus* appear to be less common than with *L. rhamnosus* (including occasional cases of endocarditis).[92] Most lactobacilli are sensitive to erythromycin, penicillins, clindamycin and vancomycin and resistant to metronidazole and aminoglycosides, with the most notable exception that many *L. rhamnosus* strains are resistant to vancomycin.[93,94]

Bacteremia caused by *Bifidobacterium* species in adults is much less commonly reported, predominantly affects the immunocompromised, and often presents with fever and abdominal pain.[95] It is possible that the incidence of *Bifidobacterium* bacteremia is underreported due to the challenges of growing this organism in standard culture media, though increased utilization of matrix-assisted laser desorption-ionization–time of flight mass spectrometry may clarify this. The importance of molecular diagnosis to confirm that an ingested probiotic is identical to the organism isolated from the infection has been stressed.[96] Bifidobacteria are generally sensitive to ampicillin and vancomycin and resistant to aminoglycosides.[97,98]

Second, contamination of commercially available products with pathogenic organisms is a possibility that likely varies with the quality control methods employed by the manufacturer. The recent report of a premature infant who received the probiotic ABC Dophilus (containing *B. lactis*, *Streptococcus thermophilus*, and *L rhamnosus*) and developed NEC with evidence of intestinal ischemia from the esophagus to the rectum is particularly disturbing. Histopathologic analysis of resected necrotic bowel revealed invasive fungal infection and fungal DNA from the tissue block demonstrated *Rhizopus oryzae*. Unopened containers of ABC Dophilus of the same lot as that administered to the infant were found to be contaminated with the same species of fungus.[99] The Health Advisory released by the Centers for Disease Control following the death of this infant emphasized that probiotic products available in the U.S. are viewed as dietary supplements and therefore are not evaluated for safety or efficacy by the Food and Drug Administration (FDA) (http://emergency.cdc.gov/HAN/han00373.asp accessed 5 May 2016). It is noteworthy that ABC Dophilus decreased the incidence of NEC in a large multi-center trial with no increase in the incidence of sepsis and no reports of probiotic-related sepsis or mucormycosis.[11]

Third, several studies have demonstrated that many commercially available probiotic products do not contain the numbers or strains of bacteria advertised on the product label, that there is significant variation between lots of a given product, and that species and strain changes in probiotics are common and often not reflected clearly on the label.[100,101] This may be due in part to lack of oversight of probiotic manufacture and to challenges related to the changing taxonomy of probiotic microbes. The result is that it is difficult for the clinician to be certain of the viability and purity of available products and for those reviewing clinical trials to be certain which probiotic was administered. In the U.S., the risks of contamination or suboptimal purity or viability of probiotic microbes could be obviated by production of probiotic products that qualify as drugs rather than dietary supplements and by testing these products under the auspices of Investigational New Drug (IND) oversight by the FDA. U.S. probiotic manufacturers have been hesitant to adopt this approach due to concerns that the IND process would preclude them from selling the probiotic product as a dietary supplement (a much larger market). An appealing alternative would be to follow the lead of the Canadian government and create a new classification for oversight of probiotic products recognizing that probiotics differ significantly from dietary supplements, drugs, and vaccines. The result of the Canadian approach is that our neighbors to the north have access to probiotics that are tested for purity and viability.[102]

Fourth, probiotic organisms administered to one infant have been identified in the feces of infants in the NICU that did not receive the probiotic.[76] The mechanisms of cross-contamination in the NICU have not been completely characterized but likely include transmission through contamination of NICU surfaces and the hands of caregivers. In a trial of *B infantis* vs *B lactis*, the former was identified in the stools of infants receiving the latter and confirmed at the strain level.[38] The challenges of cross-contamination include both the potential impact on NICU infants and the blunting of any protective effects of probiotic organisms in clinical trials. The largest randomized clinical trial of probiotics in premature infants to date demonstrated cross contamination in all study sites with 49% of the placebo infants colonized with the study probiotic.[6] These observations question the value of meta-analyses and of further randomized clinical trials of probiotics. The recent call for large cluster or cross-over cluster randomized trials in which the NICU is randomized rather than the infant is particularly applicable to probiotics and NEC.[103]

Which Probiotic to prevent NEC?

Clinical trials comparing probiotic strains, doses, and duration of administration are uncommon and have not been powered to detect differences in NEC incidence.[104,105] Animal studies and meta-analyses of available trials have been somewhat helpful in comparing differing strains of probiotics and single organism products to multiple organism cocktails. The following general principles have emerged: combination products may have advantages over single organisms;[106-108] if a single organism is administered bifidobacteria appear to be more effective than lactobacilli and both are more effective than *Saccharomyces*;[18,106] among bifidobacteria *B. infantis* has advantages in colonization over other species and subspecies;[37] among lactobacilli *L acidophilus* appears to have advantages over *L. reuteri* and *L rhamnosus* GG; and a short course of probiotics (10-14 days early in life with repeat courses following antibiotic administration) may be as useful in NEC prevention as longer courses particularly in the human milk-fed infant.[5] Unfortunately comparisons of NEC prevention between studies suffer from the well-established observation that the incidence of NEC varies dramatically over time and from NICU to NICU for unclear reasons.

It is striking that much of the world has embraced routine administration of probiotic microbes to premature infants with positive outcomes while neonatologists in the U.S. and the American Academy of Pediatrics remain hesitant to embrace this approach (somewhat reminiscent of U.S. neonatologists being the last to accept room air resuscitation at birth). While we wait for more definitive studies, based on the conservative assumptions of 3000 NEC cases/year (about 5% of infants with birth weight < 1500 grams) and 600 NEC deaths/year (20% mortality) in the U.S. and a relative risk reduction of 0.5, it becomes feasible to estimate the savings per year of increased probiotic use in lives, meters of intestines, millions of dollars and billions of neurons[109] (I come up with 300, 120, 250, and 12000 respectively).

If a neonatologist were to decide in conjunction with the parents after review of the evidence that the potential benefits of decreased risk of NEC, sepsis, death, feeding intolerance, and prolongation of hospital stay outweighed the risks of sepsis and contamination, what

products currently available would be most appealing? FloraBaby (contains *L. rhamnosus* plus four strains of Bifidobacterium: infantis, longum, breve and bifidum) decreased the incidence of NEC in a cohort study and is produced in Canada under the auspices of Health Canada which means that the manufacturers follow good manufacturing practice and the strains are known.[16] Infloran (the current version contains *L. acidophilus* and *B. infantis*) is produced in Europe and licensed in Switzerland as a drug and has perhaps the best success rate in randomized controlled trials. Natren Life Start powder contains a strain of *B. infantis* that has been shown to completely metabolize HMOs. Biogaia ProTectis contains a single strain of *L. reuteri* (the same strain described in the meta-analysis)[22], is available as a liquid drop either with or without added Vitamin D, and has been shown in several studies of term infants to decrease colic.[110] An alternative would be to encourage the motivated parents to take a probiotic product themselves in hopes of transmitting the desired microbes to their infant.[111]

Future Directions

Historically, most probiotic microbes were chosen based on ease of manufacture, stability and resistance to gastric acid. Advances in understanding of the “healthy” neonatal microbiota and the impact of changes in these communities of microbes over time in preterm and term infants and mechanisms of action of various probiotic species and strains suggest that next generation probiotics with specific properties may allow targeted manipulations of the gut microbiota. For instance “activated” probiotic microbes with specific desirable genes up-regulated during manufacture may improve colonization or increase specific anti-inflammatory or barrier strengthening effects. Currently probiotic products are limited to a few species of bacteria and a single fungal species. As ability to identify differences in intestinal archaea and viruses (both phages and human viruses) between health and disease develops, it is likely that a broad range of probiotics from multiple kingdoms may become available. Precision medicine is likely to play an important role in individualizing responses to dysbiosis. This will require more rapid and interpretable analysis of the intestinal microbiota or reliable microbial biomarkers of a disordered microbial community. For instance, recent descriptions of a microbiota for age z-score to identify dysmaturation of the intestinal microbiota in undernutrition or obesity may have applicability in the identification of the high risk premature infant microbiome.[112] The recent identification of host genetic polymorphisms that influence the intestinal microbiota suggests the possibility of early identification of preterm infants at higher risk for intestinal dysbiosis and NEC (which may require differing probiotic and/or prebiotic strategies).[113,114] Furthermore the marked heterogeneity of HMOs between mothers and in a given mother over time and differential effects on the infant intestinal microbiota suggest that some HMOs may have more beneficial effects than others.[115] The capacity to measure HMOs in an individual milk sample and then select a paired intervention (augmentation of specific prebiotic HMOs in combination with a probiotic capable of consuming those HMOs) may eventually be feasible.

Conclusion

Probiotic administration has been shown to decrease mortality and NEC in several randomized clinical trials and in combination with human milk appears to represent a promising and relatively safe intervention. Many NICUs throughout the world routinely provide prophylactic probiotics to premature infants with good evidence of success. Improvement in quality control by manufacturers, oversight by governmental agencies in the U.S., and development of new strains of probiotic microbes are urgently needed. Cluster randomized clinical trials comparing commercially available probiotic strains sufficiently powered to determine differences in NEC incidence (including thousands of premature infants in each arm) are needed.

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Table 1

Probiotic RCTs with 200 or more premature infants in each arm

Author Year	Country	Probiotic Species (strain)	Brand (Company)	Population	Dose × duration	Number enrolled		NEC cases Stage 2, 3		Culture + sepsis cases		Deaths	
						Prob	Cont	Prob	Cont	Prob	Cont	Prob	Cont
No benefit													
Costeloe 2015 *[6]	U K	B breve (BBG 001)	NR (Yakult)	GA 23-30 w	1.6e8 - 1.6e9/d until 36w	650	660	62	66	73	77	54	56
Oncel 2014[7]	Turkey	L reuteri (DSM 17938)	NR (Biogaia AB)	BW < 1500 g + GA < 32 w	1e8/d until discharge	200	200	8	10	13	25	15	20
Rojas 2012[8]	Colombia	L reuteri (DSM 17938)	NR (Biogaia AB)	BW < 2000 g	1e8/d until dischrge	372	378	9	15	24	17	22	28
Dani 2002[9]	Italy	L rhamnous (GG)	Dicoflor (Dicofarm)	BW < 1500 g and GA < 33 w	6e9/d until discharge	295	290	4	8	14	12	0	2
Benefit													
Manzoni 2014[10]	Italy + NZ	L rhamnosus (GG) + Bovine lactoferrin	NR	BW < 1500 g	6e9/d until day 30	238	258	0	14	NR	NR	9	26
Jacobs 2013 **[11]	Australia + NZ	B infantis (BB-02) + S thermophiles (TH-415957) + B lactis (BB-12 15954)	ABC Dophilus (Solgar)	BW < 1500 g + GA < 32 w	1e9/d until discharge	548	551	11	24	72	89	27	28
Lin 2008[12]	Taiwan	L acidophilus (NCDO 1748) + B bifidum (NCDO 1453)	Infloran (Laboratorio Farmaceutico)	BW < 1500 g + GA < 34 w	1e9 BID until 6 w	217	217	4	14	40	24	2	9

* Subgroup analysis showed a decrease in NEC among infants with colonization with the probiotic compared to those not colonized.

** Subgroup analysis showed a decrease in sepsis in infants > 28 weeks but not < 28 weeks.

Table 2

Probiotic cohort studies with 200 or more premature infants in each cohort

Author Year	Country	Probiotic Species (strain)	Brand (Company)	Population	Dose × duration	Number enrolled		NEC cases Stage 2, 3		Culture + sepsis cases		Deaths	
						Prob	Cont	Prob	Cont	Prob	Cont	Prob	Cont
No benefit													
Repa 2015 *[13]	Austria	L acidophilus + B infantis (NR)	Infloran (Laboratorio Farmaceutico)	GA < 34 w	2e9 BID until discharge or 37 w	230	233	16	24	60	78	16	30
Li 2013[14]	U S	B bifidum + B infantis + S thermophiles (NR)	ABC Dophilus (NR)	BW < 1500 g + GA < 33 w	5e8-1e9/d until discharge or 36 w	291	289	7	9	NR	NR	4	3
Benefit													
Hartel 2014 **[15]	Germany	L acidophilus + B infantis (NR)	Infloran (Berna)	BW < 1500 g + GA 23-31 w	1e9/d from d2 to d14	3789	1562	116 (3.6)	76 (4.8)	428 (11)	195 (12)	292 (7.7)	160 (10)
Guthmann 2016[5]	Switzerland and Germany	L acidophilus + B infantis (NR)	Infloran (Laboratorio Farmaceutico)	BW<1 500 g + GA <32 w	2e9 BID d1-3 to d10-14	591	633	8	33	NR	NR	21	32
Janvier 2014[16]	Canada	B bifidum + B breve + B infantis + B (NR) + L rhamnosus (GG)	FloraBABY (Renew Life)	GA < 32 w	2e9/d until 34 w	294	317	16	31	54	57	20	31
Bonsante 2013[17]	France	L reuteri (LCR35)	Lcr Restituo (Probionov)	GA 24-31 w	2e8 BID until discharge or 36 w	347	783	4	41	37	130	8	38
Hoyos 1999 ***[1]	Colombia	L acidophilus + B infantis (NR)	Infloran Berna 7 (Swiss Serum and Vaccine Institute)	All NICU patie nts	5e8/d until discharge	1237	1282	34	85	69	70	137	140

* The probiotic cohort was smaller and more premature. Subgroup analysis showed a significant decrease in NEC among human milk fed infants but not formula fed infants.

** percentages included in parentheses due to difference in size of the two cohorts.

*** included both term and premature infants.

Table 3

Recent meta-analyses of probiotic trials in premature infants

Author Year	Number of studies included	RR for NEC (95% CI)	RR for death (95% CI)	Other analyses	
Aceti 2016[18]	26	0.47 (0.36-0.60), p < 0.00001		Probiotics containing Bifidobacterium: RR 0.24 (0.10-0.54), p = 0.0006	Probiotic combinations: RR 0.48 (0.37-0.62), p < 0.00001
Olsen 2016[19]	12	0.55 (0.39-0.78), p = 0.0006	0.72, (0.61-0.85), p < 0.0001		
Aggarwal S 2016[20]	23		0.69 (0.56 and 0.86), p=0.0007		
Lau 2015[21]	20	0.51 (0.39-0.67), p<0.001	0.73 (0.58-0.93), p=0.009		
Athalye-Jape 2015[22]	8 (limited to L reuteri)	0.69 (0.47-1.01), 3 RCTs	0.79 (0.57-1.09), 3 RCTs		
Al Faleh 2014[23]	24	0.43 (0.33-0.56), 20 RCTs	0.65 (0.52-0.81), 17 RCTs	Probiotics containing either Lactobacillus alone or in combination with Bifidobacterium were effective	

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