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The Microbiota of the Extremely Preterm Infant

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KEYWORDS

• Microbiota • Dysbiosis • Intestinal tract • Skin • Oral cavity
• Necrotizing enterocolitis • Late-onset sepsis

KEY POINTS

- The intestinal microbiota of the extremely preterm infant differs dramatically from that of term infants, children, and adults with decreased diversity and high numbers of γ-proteobacteria and Firmicutes and low numbers of common commensal microbes.
- Alterations in the intestinal microbiota of the preterm infant precede the onset of necrotizing enterocolitis and sepsis.
- Altering the intestinal microbiota with diet, antibiotics, and prebiotic and probiotic supplements may be less effective in extremely preterm infants, prompting the need for novel approaches to dysbiosis in this population.

INTRODUCTION

Colonization of the fetal skin and intestinal tract begins in utero and is influenced by maternal microbial communities (particularly those that inhabit the distal intestinal tract, the mouth, the vagina, and the skin), timing of rupture of membranes, maternal genetic factors, medications and supplements. Colonization is further influenced by mode of delivery and postpartum environmental exposures and medical procedures, infant genetic factors, medications and supplements, enteral feeding, and maturity of the infant innate and adaptive immune systems. Breakthroughs in recent decades in the analysis of complex communities of bacteria and viruses and studies in germ-free and gnotobiotic animals have vastly expanded our understanding of the importance of interactions between host and microbe. The composition of the microbial community of the intestinal tract and skin impacts inflammatory pathways and is thus important in the pathogenesis of a wide variety of disease processes (**Box 1**).

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Box 1
Diseases and conditions in which the microbiota plays a role in pathogenesis

Antibiotic-associated diarrhea	Traveler's diarrhea
Necrotizing enterocolitis	Infectious diarrheas
Preterm birth	Sepsis
Infant colic	*Clostridium difficile* colitis
Inflammatory bowel disease	Food and environmental allergies
Irritable bowel syndrome	Celiac disease
Obesity	Diabetes mellitus (types 1 and 2)
Atherosclerosis	Cancer
Atopic eczema	Psoriasis
Seborrhea	Rheumatoid arthritis
Alzheimer and other neurodegenerative diseases	Mood disorders, schizophrenia, and autism

Novel mechanisms by which the microbiota influences host immunity and inflammation have recently been described.[1–3]

The importance of the intestinal microbiota in extremely preterm infants is most clearly evident in considering the risks of developing necrotizing enterocolitis (NEC) and sepsis. The roles of the skin microbiota in sepsis risk and the oral microbiota in pneumonia risk are less clear. Perhaps most compelling is the role of colonizing microbes in shaping and influencing the developing innate and adaptive immune responses in extremely preterm infants and the long-term impact of these host-microbe interactions. An additional layer of complexity is emerging with the realization that nutrients (eg, human milk, infant formulas and fortifiers, vitamins and minerals) are consumed by both host and bacterial cells, often with keen competition and overlapping effects. Host-microbe-nutrient interactions are likely to be particularly important in such processes as growth, brain development, immune development, and disease risk for the most preterm infants. In this article, we use the terms microbiota to refer to the composition of bacteria in a given anatomic niche and dysbiosis to mean an alteration in the microbiota associated with disease. There is evidence of significant colonization of the extremely preterm infant with yeasts, bacteriophages, and other viruses,[4] but discussion of these microbes is beyond the scope of this article.

DEVELOPMENT OF THE INFANT MICROBIOTA

In Utero

The development of tools to characterize the microbiota based on identification of bacterial DNA rather than relying on cultures has expanded understanding of the initial colonization of the neonate tremendously. **Table 1** summarizes the primary bacterial taxa that colonize the preterm infant. It has long been believed that the fetus grows in a sterile environment and that colonization begins at the time of rupture of the fetal membranes. More recent careful studies have shown that the amniotic fluid is not sterile, suggesting that colonization of the fetal skin and gut begins in utero.[5] The role of microbes in triggering preterm labor is perhaps the most clinically relevant observation related to this observation. Chorioamnionitis has long been recognized as a trigger of preterm labor and neonatal infection (particularly in preterm infants). The preponderance of evidence suggests a causal relationship between maternal periodontal disease and preterm labor.[6] For instance, the presence of specific bacteria (eg, *Peptostreptococcus micros* or *Campylobacter rectus*) in maternal gingival plaque was associated with increased risk of preterm delivery.[7] Treatment of periodontal

Table 1
Key bacterial taxa in the preterm infant

Phylum	Class	Order	Family	Genus
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*
		Lactobacillales	Streptococcaceae	*Streptococcus*
			Enterococcaceae	*Enterococcus*
			Lactobacillaceae	*Lactobacillus*
	Clostridia	Clostridiales	Clostridiaceae	*Clostridium*
	Negativicutes	Selenomonadales	Veillonellaceae	*Veillonella*
	Mollicutes	Mycoplasmatales	Mycoplasmataceae	*Ureaplasma*
Proteobacteria	γ-Proteobacteria	Enterobacteriales	Enterobacteriaceae	*Klebsiella*
				Escherichia
				Proteus
				Serratia
				Enterobacter
				Cronobacter
		Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
			Moraxellaceae	*Acinetobacter*
Bacteroidetes	Bacteroidetes	Bacteroidales	Bacteroidaceae	*Bacteroides*
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium*
		Propionibacteriales	Propionibacteriaceae	*Propionibacterium*

disease during pregnancy is associated with decreased risk of preterm labor.[8] The demonstration of the same microbes in the amniotic fluid and periodontal plaques in women delivering preterm,[9] the observation that the most common bacterium identified in amniotic fluid from women delivering preterm is *Fusobacterium nucleatum* (a common oral microbe in adults),[10] and the observation that dental infection with *Porphyromonas gingivalis* (a common bacterium in periodontal disease) causes preterm birth, low birth weight, and colonization of the placenta in mice[11] suggests actual colonization of the placenta and fetus.

Detailed studies of the microbiota of the placenta have shed some light on early colonization of the fetus. The placenta has a low bacterial load and is easily contaminated during vaginal delivery. Analysis of placentas obtained at term cesarean delivery without rupture of the fetal membranes showed similarities among the microbiota of the placenta, the amniotic fluid, and meconium, suggesting in utero gut colonization with changes in the infant fecal samples in the first 3 to 4 days after birth reflecting the acquisition of microbes in colostrum.[12] Colonization of the placenta with *Ureaplasma* species increases the risks of preterm labor and intraventricular hemorrhage in extremely preterm infants[13] and of chorioamnionitis in moderate and late preterm infants.[14]

Shortly after birth, the neonatal microbiota in the *term* infant is heavily influenced by mode of delivery with vaginally delivered infants colonized with organisms from the maternal vagina and infants delivered by cesarean colonized with organisms from the maternal skin and no real differences in neonatal microbial communities among the mouth, nasopharynx, skin, and meconium.[15] In *preterm* infants, the microbiota of the skin diverges from that of the stool and saliva by day 8 of life, and the microbiota of the saliva and stool diverge by day 15.[16] In a study of the microbiota of meconium in preterm infants, *Staphylococcus* was the dominant genus and *Staphylococcus epidermidis* the most common species. Among those infants with gestational age less than 28 weeks, *S epidermidis* was present in meconium of 3 of the 4 infants delivered by cesarean and 1 of the 3 delivered vaginally.[17]

Fecal Microbiota

Changes in the fecal microbiota of the extremely preterm infant over the first weeks of life have been characterized.[4,17–22] The following patterns are consistent across multiple studies: (1) bacterial diversity is low in meconium and increases over time; (2) an early dominance of Firmicutes (predominantly staphylococci, enterococci, and in some studies streptococci) changes to a dominance of Proteobacteria (predominantly Enterobacteriaceae); (3) *Clostridium* and *Veillonella* species appear late compared with term infants, with *Veillonella* least common in infants born at <27 weeks; (4) diet and antibiotic exposure have a lesser impact on the fecal microbiota in extremely preterm infants than is seen in term infants (eg, the human milk oligosaccharide [HMO]-consuming organisms, bifidobacteria and *Bacteroides*, are uncommon even in exclusively human milk–fed preterm infants); and (5) postmenstrual age significantly influences the fecal microbiota. These observations suggest that environmental factors and maturation of the host immune response are the primary shapers of the developing gut microbiota in preterm infants. It is worth noting how strikingly the fecal microbiota of the preterm infant differs from that of the healthy term infant with the former often containing 1 to 2 orders of magnitude higher levels of γ-Proteobacteria and the latter commonly dominated by bifidobacteria and *Bacteroides*.

Gastric Microbiota

Gastric aspirates have recently been studied using bacterial DNA techniques. Analysis of 22 neonates with an average gestational age of 27.7 weeks ($\pm$2.8) demonstrated a relative paucity of species in the stomach, with *Bacteroides* spp predominant in the first 4 weeks of life and *Bifidobacterium* colonization significantly higher in infants receiving human milk. These results differ dramatically from studies of the fecal microbiota and raise the possibility that, although rare in the feces of preterm infants, the 2 genera of bacteria capable of consuming HMOs may be present in their small bowel.[23] In this study, *Helicobacter pylori* and *Ureaplasma* were not identified; however, a different study of 12 neonates with an average gestational age of 27 weeks ($\pm$0.5) found the predominant species in the first week of life to be *Ureaplasma*, with a predominance of *S epidermidis* in subsequent weeks. By the fourth week, Proteobacteria and Firmicutes each accounted for 50% of the total gastric organisms.[24] The reasons for this disparity are unclear, but may represent differences in technique (denaturing gradient gel electrophoresis in the first study and direct sequencing of polymerase chain reaction [PCR]-amplified clones in the second), differences in population (both studies were performed in the United States, but diversity in maternal colonization with *Ureaplasma* may have played a role), or the relatively small numbers of infants analyzed.

Oral Microbiota

Investigations of the development of the oral microbiota in extremely preterm infants are limited. The largest study to date included 110 preterm infants with birth weight less than 1000 g with weekly oral swabs for the first 6 weeks of life, but used culture techniques rather than bacterial DNA-based approaches. At birth the oral swabs did not show significant growth of culturable bacteria, but by week one, 21 infants were colonized with methicillin-resistant *Staphylococcus aureus* (MRSA), 6 infants had other pathogenic bacteria (*S aureus,* Enterobacteriaceae*, Escherichia coli*), 56 infants were colonized with "nonpathogenic bacteria" (*S epidermidis* was most common followed by *Corynebacterium*, *Lactobacillus*, and *Streptococcus*), and 22 infants still showed no significant growth of culturable bacteria. By 6 weeks, 60 of the infants

were colonized with MRSA. It is noteworthy that MRSA sepsis cases were less common in those infants with early oral colonization with the "nonpathogenic" microbes.[25] Smaller studies of preterm infants using culture-only technology have shown colonization of the mouth in the first 10 days of life with coagulase-negative staphylococci, enterococci, Enterobacteriaceae, *Pseudomonas*, and *Candida*.[26] A study using bacterial DNA-based technology included 1 infant with gestational age 24 weeks; the saliva microbiota differed from the other 4 preterm infants analyzed (gestational age 30–31 weeks) in that there were Enterobacteriaceae at days 8 and 10 and *Pseudomonas* and *Mycoplasma* at days 15, 18, and 21 that were not seen in the older preterm infants.[16] We analyzed the oral microbiota of 7 preterm infants (gestational age 25–27 weeks) with bacterial DNA techniques at 3 time points in the first 5 days of life and found a predominance of Mycoplasmataceae and Moraxellaceae in the first 36 hours of life and Staphylococcaceae and Planococcaceae by day of life 5.[27]

Skin Microbiota

The skin of the extremely preterm infant changes dramatically in the first weeks of life. The stratum corneum, which functions as the epidermal barrier, is nearly absent at 23 weeks' gestation, has a few cornified layers at 26 weeks, and is not fully mature until approximately 34 weeks' gestation.[28] When infants are born preterm, the epidermis matures fairly rapidly, and even the most immature neonate has functionally and histologically mature epidermis by approximately 2 weeks postnatal age.[29] Studies of the skin microbiota of the extremely preterm infant are limited. Several studies have demonstrated that pathogens commonly colonize the skin of preterm infants (mostly staphylococci, enterococci, Enterobacteriaceae, Pseudomonadales, and *Candida*) and that MRSA colonization is more common in the preterm infant; however, these studies were not designed to analyze the broader skin microbiota.[30–32] The previously noted study comparing changes over time in the saliva, skin, and feces included 1 infant with gestational age 24 weeks. The skin of this infant was dominated by staphylococci during the time of testing (day 8 to day 21) and did not differ from the older preterm infants.[16] Environmental factors that influence the skin microbiota include parental skin, feeding type, environmental surfaces and caregiving equipment, health care provider skin, and antibiotic use.[33]

THE MICROBIOTA AND DISEASE RISK IN EXTREMELY PRETERM INFANTS

The Fecal Microbiota and Necrotizing Enterocolitis and Sepsis

The incidences of NEC and sepsis are highest in the most preterm infants, likely due to immaturity of intestinal and skin barriers and immaturity of immune responses. Cases and outbreaks of NEC have been associated with a striking variety of organisms (**Table 2**), suggesting that there is not a single organism responsible. The evidence that the early or colonizing microbiota of the intestine influences the risk for subsequent development of NEC and/or sepsis has become quite compelling. The observation that NEC is most common in infants born at less than 28 weeks gestation and most commonly occurs at 30 to 32 weeks corrected gestational age[34] suggests that maturation of the host immune response and/or maturation of the intestinal microbiota are important in NEC pathogenesis. The Paneth cells of the small intestine produce large quantities of antimicrobial peptides that shape the intestinal microbiota.[35] It is not likely coincidental that Paneth cells increase in numbers and become immunecompetent at 29 weeks corrected gestational age.[36,37] Lower fecal bacterial diversity and/or richness is common in extremely preterm infants and has been demonstrated

Table 2
Microbes associated with cases or outbreaks of necrotizing enterocolitis

Gram-Positive Bacteria	Gram-Negative Bacteria	Fungi	Viruses
Enterococcus faecalis	*Klebsiella pneumoniae*	*Candida albicans*	Coronavirus
Clostridium perfringens	*Escherichia coli*	*Candida parapsilosis*	Coxsackie B2 virus
Clostridium butyricum	*Pseudomonas aeruginosa*	*Candida glabrata*	Rotavirus
Clostridium neonatale	*Enterobacter cloacae*	*Aspergillus*	Adenovirus
Clostridium difficile	*Cronobacter sakazakii*	*Mucoraceae*	Torovirus
Staphylococcus aureus	*Cronobacter muytjensii*		Astrovirus
Staphylococcus epidermidis	*Shigella*		Echovirus 22
	Salmonella		Norovirus
			Cytomegalovirus

in some studies of infants with NEC compared with matched controls,[22,38–40] although this is not universal.[41,42] Studies investigating the fecal microbiota before the onset of NEC compared with matched controls are summarized in **Table 3**.[40,41,43–54] These studies demonstrate the following: (1) colonization patterns differ between preterm infants who subsequently develop NEC and those who do not; (2) these differences are heavily influenced by maturation, NICU location, antibiotic exposure, and perhaps feeding type; (3) it remains unclear whether NEC risk is associated with the absence of potentially protective microbes (eg, *Propionibacterium, Bifidobacterium, Bacteroides*, or *Veillonella* species) or the dominance of potentially pathogenic microbes (eg, Enterobacteriaceae or *Clostridium* species); and (4) it remains unclear whether dysbiosis is the cause of NEC or a marker of alterations in host genetics or immune development. Two observations support the hypothesis that Enterobacteriaceae are important in the pathogenesis of NEC: (1) recognition of lipopolysaccharide in the cell wall of Gram-negative Enterobacteriaceae by Toll-like receptor 4 triggers a proinflammatory response and an influx of lymphocytes that in animal models is essential to the development of NEC,[55] and (2) Enterobacteriaceae have unique metabolic pathways by which they both trigger inflammation and use the products of the host inflammatory response as an energy source allowing them to outcompete other gut microbes.[56]

Many of the organisms responsible for late-onset sepsis (LOS), including staphylococci, in extremely preterm infants originate in the intestinal tract.[57] Several studies have demonstrated organisms in the feces before or concurrent with the onset of LOS caused by the identical organism in extremely preterm infants.[58–60] Decreased bacterial diversity and a predominance of staphylococci in early fecal specimens were associated with later sepsis in one small study of infants with gestational age 24 to 27 weeks.[61]

The Skin Microbiota and Sepsis

Efforts to decrease LOS with emphasis on the skin microbiota (eg, hand washing, protocols for line placement and care, early removal of central lines) have been partially successful, suggesting that a portion of these infections originate in the skin. Studies correlating skin colonization with LOS have relied on culture-based approaches and therefore likely give a limited view of the microbiota.[62]

The Oral and Gastric Microbiota and Pneumonia

In critically ill adults and children, attention to oral care has been shown to decrease the risk of ventilator-associated pneumonia, suggesting that aspirated oral microbes

may play a role in pathogenesis. No studies to date have demonstrated similar decreases in ventilated preterm infants. Tracheal pepsin has been proposed as a marker of aspiration of gastric contents and appears to be common in preterm infants.[63] Bacterial DNA techniques have demonstrated an association between the gastric microbiota and chronic lung disease, with *Ureaplasma* the most common genus.[64]

The Tracheal Microbiota and Chronic Lung Disease

The lower airway is not sterile in the preterm infant. Tracheal aspirates from very preterm intubated infants have predominantly been studied with culture-based techniques.[65] A study of 25 preterm infants using bacterial DNA techniques demonstrated a predominance of Actinobacteria, which decreased over time in infants who subsequently developed chronic lung disease (gestational age 26.2 $\pm$ 1.9 weeks) but remained stable over time in the infants who did not (gestational age 28.9 $\pm$ 1.4 weeks). In the former group, *Staphylococcus* increased over time and bacterial diversity was lower.[66] A study of 10 infants with birth weight 500 to 1250 g who were intubated for more than 21 days demonstrated a predominance of *Staphylococcus, Ureaplasma, Pseudomonas, Enterococcus,* and *Escherichia*.[67] In both culture-based and DNA-based studies there appear to be differences in the tracheal microbiota between infants who subsequently develop chronic lung disease and those who do not; however, distinguishing between colonization of the airway and infection remains challenging.

Environmental Microbes and Disease Risk

The impact of the NICU environment on colonization, immune responses, and risk for nosocomial infection in the extremely preterm infant has not been fully characterized. The composition of the surface and airborne microbiota is influenced by building design and utilization with hospital surfaces more likely to contain human pathogens than other office settings.[68] Two studies of NICU surfaces using bacterial DNA techniques, found significant diversity between NICUs and demonstrated common neonatal pathogens (eg, *Enterobacter, Pseudomonas, Streptococcus, Staphylococcus, Escherichia, Enterococcus, Acinetobacter*, and *Candida albicans*) on NICU surfaces.[69,70] Intensive cleaning has been shown to significantly reduce the total microbial load and reshape the diversity toward nonpathogenic organisms. Interestingly, many of the common NICU enteric genera (*Enterococcus, Klebsiella, Escherichia,* and *Pseudomonas*) were not significantly altered by an intensive cleaning regimen, and routine cleaning of environmental surfaces with antibacterial wipes may be just as effective to reduce potentially pathogenic bacteria.[70]

Examples of environmental studies of NICU infectious outbreaks are abundant. In one NICU, during high-risk respiratory syncytial virus season, 4% of clothing swabs, and 9% of environmental "high-touch" surface swabs (beds, side tables, countertops, chairs, tables, and computers) tested positive for the virus by PCR.[71] Using DNA sequencing, a sink drain was shown to be the source of a *Pseudomonas aeruginosa* outbreak and replacing the sink and plumbing appeared to eradicate the outbreak.[72] A *Burkholderia cepacia* outbreak, in which 12 neonates developed clinical and/or laboratory evidence of sepsis was traced to contaminated intravenous solution and water for humidification of ventilator circuits.[73] A cluster of *Bacillus cereus* colitis cases,[74] an extended-spectrum beta-lactamase *E coli* outbreak,[75] and case reports of Group B *Streptococcus* septicemia in preterm infants[76] have all been attributed to contaminated breast milk. *Cronobacter* species have been identified as a contaminant of powdered milk formulas, with sporadic outbreaks linked to NEC, bacteremia, and

Table 3
Studies of the fecal microbiota before the onset of NEC (only studies that included infants with gestational age <28 weeks are included)

	Gestational Age at Birth[a]	NEC	Controls	Meconium	Early Stools	Just Before NEC Onset
De la Cochetiere et al,[43] 2004	24–29	3	9		*Clostridium perfringens* ↑	
Mai et al,[41] 2011	23–29	9	9		Firmicutes ↑ Actinobacteria ↓ Bacteroidetes ↓	Proteobacteria ↑
Stewart et al,[44] 2012	24–28	7	21		Coagulase-negative staphylococci ↑ Enterococci ↓	
Smith et al,[45] 2012	23–30	15	128	No differences at 3 time points: 0–5 d, day 10, and day 30		
Morrow et al,[40] 2013	25.5 (1.8)	11	21	Propionibacterium ↓	Staphylococci ↑ Enterococci ↑ Enterobacteriaceae ↑	
Normann et al,[46] 2013	22–25	10	16		Trends: Enterobacteriaceae ↑ Bacillales ↑ Enterococci ↓	
Torrazza et al,[47] 2013	27.4 (2.6)	18	35	Klebsiella-like sp ↑	Proteobacteria ↑ Actinobacteria ↑ Bifidobacteria ↓ Bacteroidetes ↓	
Jenke et al,[48] 2013	24–27	12	56		Lactobacilli ↑ *Escherichia coli* ↓	*E coli* ↑
McMurtry et al,[49] 2015	27.2 (2.8)	21	74			Actinobacteria ↓ Clostridia ↓ *Veillonella* ↓ Streptococci ↓

Sim et al,[50] 2015	25–28	12	36		*Klebsiella* ↑	*Klebsiella* ↑ Clostridia ↑
Zhou et al,[39] 2015	24–31	12	26			Clostridia ↑ Staphylococci ↓
Heida et al,[51] 2016	24–29	11	22	*C perfringens* ↑ *Bacteroides dorei* ↑		*C perfringens* ↑ Staphylococci ↓
Warner et al,[22] 2016[b]	26.0 (24.7–27.9)	46	120			γ-Proteobacteria ↑ Negativicutes ↓ Clostridia ↓
Ward et al,[52] 2016	26 (23–28)	7	37		No differences in samples from days 3–16. Days 17–22: Uropathogenic *E coli* ↑ *Veillonella* ↓	
Twin studies						
Stewart et al, 2013[53]	26–30	5	5			*Escherichia* ↑
Claud et al,[54] 2013		1	1			Proteobacteria ↑ *Veillonella* ↓

Arrows represent significant differences in NEC compared with control specimens.

Abbreviation: NEC, necrotizing enterocolitis.

[a] Range or mean (SD) or median (interquartile range).

[b] The associations were most strong for infants with gestational age at birth <27 weeks with strong time-by-NEC interactions.

meningitis.[77,78] Bacteria also can colonize unlikely sources. *B cepacia* and *Enterobacter cloacae* have the ability to hydrolyze, render inactive, and proliferate in parabens, which are esters of para-hydroxybenzoic acid that are usually antimicrobial and used as preservatives in ultrasound gel (implicated in a *B cepacia* outbreak in a NICU).[79,80] *Serratia marcescens* outbreaks in NICUs have been linked to stored water and incubator surfaces,[81] the exit port of a high-frequency oscillatory ventilator,[82] contaminated parenteral nutrition,[83] soap dispensers,[84] and baby shampoo.[85]

MANIPULATING THE MICROBIOTA OF THE EXTREMELY PRETERM INFANT

Diet

Breast-fed *term* infants generally become colonized in the first weeks after birth with gut microbes that are able to consume HMOs and other human milk glycans (bifidobacteria and *Bacteroides*), whereas formula-fed infants tend to become colonized with a more diverse mixture of microbes. As noted previously, in the extremely preterm infant, the provision of human milk does not have a marked influence on the fecal microbiota with "human milk–consuming" microbes consistently either absent or present in low abundance across multiple studies. In a small study, neither the addition of a mixture rich in HMOs to preterm infant formula nor the "all-human diet" (human milk fortified with a fortifier made from donor human milk) led to a significant change in the fecal microbiota.[86] Nevertheless, careful analysis of the composition of HMOs in ingested milk and undigested HMOs in feces in preterm infants showed that different HMO structures are differentially consumed in the preterm gut with increased fucosylated HMOs in milk associated with a decrease in Proteobacteria in the infant feces.[87]

Probiotics

To date, a total of 41 randomized placebo-controlled trials of probiotics in preterm infants have been published in English; 37 of these trials included NEC, sepsis, and/or death as an outcome. In spite of differences in probiotic choice and dose administered, several meta-analyses have reached the same conclusion: probiotics decrease the risk of NEC, death, and sepsis in preterm infants and decrease the time to full enteral feeding in preterm infants receiving human milk.[88–90] In addition, there have been 11 cohort studies published in English comparing periods of no probiotic to periods of universal probiotic administration in preterm infants with a meta-analysis of these studies demonstrating a decrease in NEC and mortality with probiotic administration.[91] **Table 4** summarizes the nonweighted results of the randomized controlled trials and the cohort studies.[92–96] In spite of this astounding level of evidence and the relative lack of risk, routine probiotic administration is not recommended in the United States due to concerns from the Food and Drug Administration and other experts regarding the lack of commercial probiotic products that meet high standards of purity and viability. Whether these recommendations are justifiable given the incidence, cost, and severity of NEC and the relative paucity of evidence of harm associated with probiotic administration is hotly debated. In addition, it has been widely reported that although probiotic products appear to be beneficial for preterm infants with birth weight greater than 1000 g, data supporting a benefit for extremely low birth weight infants are lacking.[21] **Tables 5** and **6** summarize the data available from the randomized controlled trials and the cohort studies for the smallest preterm infants, including unweighted totals and percentages.[96–114] Although the level of support is not as compelling as that for larger preterm infants, these data suggest potential benefit and certainly no convincing evidence of harm for this population.

Table 4
Unweighted summary of probiotic studies in preterm infants

Number Enrolled		NEC Cases Stage 2 or 3		Culture-Positive Sepsis		Deaths	
Probiotic	Control	Probiotic	Control	Probiotic	Control	Probiotic	Control
7 randomized placebo-controlled trials with 200 or more preterm infants in each arm							
2520	2554	98	151	236	244	129	169
% of those reporting the outcome		3.9	5.9	10	11	5.1	6.6
7 cohort studies with 200 or more preterm infants in each group							
6779	5099	201	299	648	530	498	434
% of those reporting the outcome		3.0	5.9	11	13	7.3	8.5
37 randomized placebo-controlled trials (includes the infants in the 7 larger trials above)							
4710	4675	153	283	475	548	224	315
% of those reporting the outcome		3.3	6.2	12	14	5.1	7.2
11 cohort studies (includes the infants in the 7 larger studies above)							
7742	7592	224	408	737	667	556	493
% of those reporting the outcome		2.9	5.3	12	14	7.7	9.0

Details of 33 of the randomized controlled trials and 10 of the cohort studies are presented in Tables 1 and 2 of Ref.[90] with the additional studies in Refs.[91–94,108]

Antibiotics

Five clinical trials of oral administration of antibiotics that are unlikely to be absorbed systemically (gentamicin, kanamycin, or vancomycin) demonstrated a decrease in the incidence of NEC.[115] Although this approach has been adopted in some NICUs, the concerns of emergence of resistant organisms have precluded widespread adoption. A recent report of the emergence of colistin-resistant extended-spectrum beta-lactamase–producing Enterobacteriaceae following oral administration to preterm infants for NEC prophylaxis underscores the validity of these concerns.[116]

Buccal Colostrum

Administration of colostrum directly into the buccal pouch has been proposed as oral hygiene to decrease the risk of ventilator-associated pneumonia in intubated neonates. To date, studies have shown an impact on the oropharyngeal lymphatic tissues[117] and the oral microbiota,[27] a decrease in clinical sepsis,[117] but no clear decrease in pneumonia. A multicenter trial of this intervention is under way.[118]

Cleaning Agents

Early studies of the value of environmental disinfection as a strategy to decrease hospital-acquired infections were limited and disappointing.[119] More recent studies suggest that novel interventions may be helpful in interrupting and preventing infectious hospital outbreaks.[120] Demonstrations that chlorhexidine bathing is associated with decreased risk of hospital-acquired infections compared with soap and water[121] have prompted widespread adoption of this practice for children and adults. For similar reasons, frequent use of hand-sanitizing gels and foams among health care providers has become widespread. Unfortunately, we have no data about the

Table 5
Randomized controlled trials evaluating probiotics published in English and specifically evaluating infants <1 kg

			n <1 kg		NEC Cases ≥ stage 2		Culture + Sepsis		Deaths	
Author	Country	Probiotic Species	Pro	Pla	Pro	Pla	Pro	Pla	Pro	Pla
Costeloe et al,[98] 2016	UK	*Bifidobacterium breve*	317	327	50	53	63	61	46	53
Kanic et al,[93] 2015[a]	Slovenia	*Lactobacillus acidophilus* + *Enterococcus faecium* + *Bifidobacterium infantum*	13	17	0	5	8	6	3	3
Van Niekirk et al,[99] 2015[a]	South Africa	*Bifidobacterium infantis* + *Lactobacillus rhamnosus*	43	49	0	4	–	–	5	5
Sangtawesin et al,[97] 2014[a]	Thailand	*L acidophilus* + *Bifidobacterium bifida*	3	4	1	1	2	1	0	0
Tewari et al,[100] 2015[a]	India	*Bacillus clausii*	23	22	0	0	6	8	8	9
Oncel et al,[101] 2014	Turkey	*Lactobacillus reuteri*	93	103	5	9	6	19	11	17
Patole et al,[102] 2014[a]	Australia	*B breve*	28	29	–	–	11	6	0	0
Totsu et al,[103] 2014[a,b]	Japan	*Bifidobacterium bifidum*	76	66	0	0	5	10	2	0
Jacobs et al,[104] 2013[c]	Australia + NZ	*B infantis* + *Streptococcus thermophilus* + *Bifidobacterium lactis*	235	239	10	14	53	58	–	–
Al-Hosni et al,[105] 2012	US	*B infantis* + *L rhamnosus*	50	51	2	2	13	16	3	4
Mihatsch et al,[106] 2010[d]	Germany	*B lactis*	91	89	2	4	28	29	2	1
Rouge et al,[107] 2009	France	*Bifidobacterium longum* + *L rhamnosus*	16	22	–	–	12	14	–	–
Underwood et al,[96] 2009	US	*L rhamnosus* OR combination (*L acidophilus* + *B infantis* + *B longum* + *B bifidum*)	9	7	1	0	4	0	0	0
Lin et al,[108] 2008[e]	Taiwan	*L acidophilus* + *B bifidum*	102	79	4	7	28	14	0	6
Wang et al,[109] 2007[a]	Japan	*B breve*	11	11	0	0	–	–	–	–
Bin-Nun et al,[110] 2005[a]	Israel	*B infantis* + *S thermophilus* + *B lactis*	25	17	2	6	4	10	6	9
Total			1140	1137	77	106	248	254	86	107
Percentage of those reporting the outcome			–	–	6.8	9.5	23	24	9.8	12

Abbreviations: NEC, necrotizing enterocolitis; Pla, placebo; Pro, probiotic.
[a] Personal communication from the author.
[b] Culture-positive sepsis at greater than 7 days of life.
[c] In regression model, reduction of NEC significant in subgroup analysis of less than 1 kg infants (RR [relative risk] 0.73).
[d] These infants were less than 29 weeks (birth weight <1.16 kg).
[e] Death and NEC were significantly lower in the probiotic group for infants 500 to 750 g ($P = .02$).

Table 6
Cohort studies evaluating probiotics published in English and specifically evaluating infants <1 kg

			n <1 kg		NEC Cases ≥ Stage 2		Culture + Sepsis		Deaths	
Author	Country	Probiotic Species	Pro	Con	Pro	Con	Pro	Con	Pro	Con
Guthman et al,[111] 2016	Switzerland	*Lactobacillus acidophilus* + *Bifidobacterium infantis*	238	250	6	16	–	–	16	26
Janvier et al,[112] 2014	Canada	*Bifidobacterium bifidum* + *Bifidobacterium breve* + *B infantis* + *B longum* + *Lactobacillus rhamnosus*	98	109	10	18	30	38	14	27
Hunter et al,[113] 2012	US	*Lactobacillus reuteri*	79	232	2	35	18	72	–	–
Luoto et al,[114] 2010	Finland	*L rhamnosus*	218	879	17	45	–	–	–	–
Total			633	1470	35	114	48	110	30	53
% of those reporting the outcome			–	–	5.5	7.8	27	32	8.9	15

Abbreviations: Con, control; NEC, necrotizing enterocolitis; Pro, probiotic.

long-term impact of these interventions on the skin microbiota or systemic absorption of these products for either the health care provider or the patient (particularly for highly vulnerable patients like the extremely preterm infant).

Emollients

Topical application of ointments, oils, or other emollients to the skin of the preterm infant has not demonstrated any significant decrease in rates of invasive infection or death.[122] Studies of the impact of this approach on the skin microbiota are limited to culture studies, with 1 study showing no differences[123] and 2 studies showing nonspecific changes.[30,124]

Functionalized Surfaces

Creation of novel surfaces that are resistant to colonization with potentially pathogenic microbes is a promising approach. Although this field is still in its infancy, the most promising result may be decreased surface contamination with viruses.[125] Isolettes with pathogen-resistant surfaces and medical devices coated with commensal or probiotic organisms may someday be commonplace.

SUMMARY

The study of microbes that colonize extremely preterm infants and the devices and surfaces with which they come in contact holds great promise for decreasing the high morbidity and mortality in this evolutionarily new population. Understanding

and preventing dysbiosis may be crucial to the prevention of common and devastating processes such as NEC, chronic lung disease, and sepsis, but also may impact growth, development, immune function, and risk for a broad variety of chronic diseases and conditions.

Best Practices

What is the current practice?

- The American Academy of Pediatrics recommends mother's own milk for preterm infants and pasteurized donor human milk if the mother is unable to provide sufficient milk
- In many countries, prophylactic probiotic supplements are routine for preterm infants

What changes in current practice are likely to improve outcomes?

- Increased utilization of probiotics that reach high standards of purity and viability will decrease NEC in infants with birth weight greater than 1000 g (Centre for Evidence-Based Medicine, Oxford, 1a)
- Increased utilization of probiotics may decrease NEC and sepsis in smaller preterm infants (Centre for Evidence-Based Medicine, Oxford, 1b) and may decrease risk of childhood and adult onset diseases (Centre for Evidence-Based Medicine, Oxford, 5)
- Development of targeted approaches to decrease dysbiosis of the mouth, stomach, intestines, skin, and trachea may decrease diseases of extremely preterm infants associated with acute or chronic inflammation (Centre for Evidence-Based Medicine, Oxford, 5)

Data from Oxford Centre for Evidence-based Medicine – Levels of Evidence (March 2009). Centre for Evidence Based Medicine. Available at: http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/. Accessed March 6, 2017.

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