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Necrotizing enterocolitis and preterm infant gut bacteria

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Summary

Necrotizing enterocolitis remains an intractable consequence of preterm birth. Gut microbial communities, especially bacterial communities, have long been suspected to play a role in the development of necrotizing enterocolitis. Direct-from-stool nucleic acid sequencing technology now offers insights into the make-up of these communities. Data are now converging on the roles of Gram-negative bacteria as causative agents, despite the dynamic nature of bacterial populations, the varying technologies and sampling strategies, and the overall small sample sizes in these case–control studies. Bacteria that confer protection from necrotizing enterocolitis have not been identified across studies. The beneficial effect of probiotics is not apparent in infants with birth weights <1000 g (these infants are at highest risk of, and have the highest case fatality rate from, necrotizing enterocolitis). Further work should be directed to the modulating gut microbes, or the products they produce, to prevent this devastating complication of preterm birth.

Keywords

Necrotizing enterocolitis; Gammaproteobacteria

1. Introduction

The newborn gut microbiome is an area of intense and growing interest in perinatology. There is emerging appreciation of the roles played by gut microbes in intestinal health, and, indeed, in lifelong health. Most relevant to preterm infants in neonatal intensive care units (NICUs), several very important disorders are likely to originate from either abnormal proportions of microbial content (dysbiosis), or when a vulnerable host encounters a specific pathogen. In this review, we focus on early-in-life bacterial population assembly in the preterm infant gut, recent data on the biology and ecology of the bacterial community, and

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the role these microbes play in the development of necrotizing enterocolitis (NEC). Experimental data are mentioned as examples that corroborate or extend observations from the human host.

2. The in-population of the human infant gut by microbes

Classic teaching holds that the human gut (i.e., the meconium) contains no bacteria, or at least no viable bacteria, at birth. However, recent data prompt reconsideration of this dogma. Mshvildadze et al. [1] identified bacterial sequences in freshly produced meconium, as have Heida et al. [2], and Ardissone et al. [3]. Stout et al. [4] have identified bacterial bodies on electron microscopy in the basal plate of placentas delivered via cesarean section. Aagaard et al. [5] reported bacterial 16S and metagenomic sequences in placentas, finding similarities between these sequences and those of bacteria resident in the mouth. Additional studies have identified bacterial sequences in amniotic fluid from term pregnancies delivered by elective cesarean section [6]. Notably, these papers rarely present evidence of viable bacteria in specimens putatively colonized based on nucleic acid sequencing (Table 1).

The possibility that the fetal gut is colonized by bacteria before maternal membranes rupture is intriguing. However, low grade bacteremia occurs independent of pregnancy in healthy adults after brushing or flossing teeth, or defecation [11]. The finding of bacterial sequences in or on a newborn infant immediately after rupture of membranes may reflect colonization of the newborn while passing through the birth canal or being delivered through the skin. These sequences may also reflect nucleic acid remnants of viable bacteria that circulated in the mother's blood, but which have no replicative potential or relevance to the assembly of the earliest-in-life members of the bacterial community of the infant gut. An additional argument against prenatal colonization of the gut with bacteria acquired in utero is the observation that germ-free animals, which generally require immersion in iodine solution during the derivation process, are generated from mothers (usually mice) who are not free of germs. Iodine immersion would not sterilize the colonized gut, so if the gut is an intergenerational habitat for viable bacteria, we would expect that it would be impossible to derive germ-free animals.

Recent publications illuminate rules of assembly for the human infant gut microbiome. Among healthy term and near-term infants, early gut colonization patterns are driven largely by delivery route and feeding patterns [12,13], with emerging data suggesting a role for the gut virome [10]. Infants born very preterm (<32 weeks), however, have a distinct set of exposures compared to those born near term. Parenteral antibiotic use is nearly universal during the first several days of life in preterm infants, feeding tubes are placed early, and enteral nutrition is commenced cautiously. In the days and weeks after birth, preterm infants reside almost exclusively in NICUs. These environments are designed to limit microbial transmission, and contact with bacteria is controlled to the extent possible. Visitors are restricted and often only parents and professional staff are permitted to touch the infant. Hand hygiene is stressed, line care is protocolized, and nutrition is either human breast milk (mother of infant or pasteurized donor pool), or sterilized liquid formula. There is no exposure to pets, or physical contact with other relatives. This microbiologically constrained biosphere offers a rich opportunity to study the transition of the neonatal gut from sterility or

near sterility at birth to an organ that houses, for the rest of the life of the host, the greatest density of microbes in the human body. Not unexpectedly, bacterial communities assemble differently in the preterm gut than in the term infant gut.

Whereas the in-population of the infant gut with bacteria is a fascinating ecologic event worthy of study in its own right, accumulating experimental data suggest that the earliest-in-life gut bacteria affect the future well-being of their hosts [14]. Hence, the study of these communities in infants is justifiable in order to determine whether the animal data are relevant for humans. However, pertinent to infants born very prematurely, there are additional compelling reasons to study the gut microbiome because of the high frequency with which these infants experience complications of premature birth that are plausibly associated with this biomass. The two most dire consequences in which gut bacteria could play major roles in outcome are NEC and late-onset neonatal bloodstream infections (P.I. Tarr and B.B. Warner, Chapter 4, this issue).

The “normal” preterm infant gut microbiome has been characterized among infants born very preterm who were at risk of developing NEC, but who did not experience this event, i.e., controls. Until recently, these analyses used culture-based technology, or polymerase chain reaction amplification of DNA extracted from stool and testing for mobility in a gel. Most recently, advances in sequencing technology, expansion of ribosomal RNA gene databases, and metagenomic capabilities (DNA sequencing not confined to 16S rRNA gene regions) have made feasible the direct-from-stool amplification of extracted bacterial DNA. These approaches provide a less circuitous, and deeper and more economical, portrayal of bacterial populations in polymicrobial substances. In the targeted approach, conserved regions of the 16S ribosomal RNA gene of bacteria are primed and amplified, a technique employed in the NIH-sponsored Human Microbiome Project [15]. This targeted approach enables deep “censusing” of bacterial populations, as all such mass readouts are confined to the regions of the bacterial chromosome that identify the organism from which they are derived.

We are aware of six publications from NICUs in eight different centers in which bacterial community assembly in “normal” preterm infants has been interrogated in depth using direct-from-stool sequencing. For the purposes of this review, our criteria for including such studies are those that included at least 100 stools from at least 25 subjects who did not develop NEC, and that the enumeration technology employed 16S rRNA gene or metagenomic sequencing (Table 2).

Even though only one of the papers in Table 2 exclusively focused on defining the pattern of progression in children without NEC [20], data supplied in the others [16,18,21] were sufficient to confirm the findings of La Rosa et al. [20]. In that comprehensive study of preterm infants, 16S rRNA gene sequencing demonstrated a remarkably choreographed pattern: namely, the early-in-life gut bacterial content is predominated by Bacilli (despite their name, Bacilli are Gram-positive cocci such as staphylococci, streptococci, and enterococci). Bacilli are soon overtaken by Gram-negative facultative organisms (a diversity of genera and species within the Gammaproteobacteria class). This surge in Gammaproteobacteria is counterbalanced by a gradually increasing abundance of Clostridia

(many genera and species) and Negativicutes (predominantly *Veillonella*). Overall, four bacterial classes (Bacilli, Gammaproteobacteria, Clostridia, and Negativicutes) account for >90% of the taxa present. Compared to the gut content of older children and adults, these preterm infant gut bacterial populations have much higher content of Gammaproteobacteria (one to two orders of magnitude difference), and approximately half the density of obligate anaerobes.

There is a convergence on a consensus community structure by the equivalence of 33–36 weeks postmenstrual age (the sum of gestational age at birth, and day of life on which the sample was obtained). The content of this consensus community at this postmenstrual age (but not earlier) is independent of gestational age at birth. In particular, anaerobic bacteria gain abundance more rapidly in the gastrointestinal tracts of infants born least prematurely. This choreographed progression is punctuated unpredictably and substantially by short-lived changes in composition, before the communities self-revert to the choreographed progression. Such abrupt changes have been noted in older children and adults [12,13,15,22]. Unexpectedly, the factors believed to be influential in microbial community assembly (at least in children born after full-term gestation), namely mode of delivery (vaginal vs cesarean section), antibiotic administration in the aggregate, and feeds (breast milk), were either not determinative of bacterial content, or had only minimal or temporary influence on this progression.

These non-associations between diverse exposures, each of which could logically be considered to influence bacterial community structure in the gut, prompts us to interpret that in the preterm human infant, the major driver of bacterial population assembly is intrinsic host biology or succession ecology rather than exogenous factors. However, we offer two caveats. First, as described above, the microbial exposures of very preterm infants differ considerably from those of infants born at term, in whom mode of delivery and breast-milk feeding appear to influence gut bacterial population assembly. Second, we wish to note that in a subsequent study of the St Louis Children's Hospital cohort [19], specific antibiotics (meropenem, cefotaxime, and ticarcillin–clavulanate), which were used in few subjects in La Rosa et al. [20], were associated with substantial directional changes in microbial content. It is also noteworthy that when metagenomic sequencing technology was applied [19], bacterial community population characteristics as previously defined by 16S rRNA gene sequencing were recapitulated [20].

3. The gut microbiome and NEC

Multiple lines of circumstantial evidence suggest that NEC is influenced by bacteria in the very preterm infant gut. Most notably, NEC does not occur in utero, and, in fact, rarely occurs before approximately day of life 10, after bacterial populations start to assemble in the newborn gut. Also, NEC is statistically and independently associated with increased antibiotic use, especially prolongation of antibiotics during the first week of life [23–25]. Moreover, H2 blockers, which could affect gut microbial populations by reducing the gastric acid line of defence against bacterial colonization, are associated with increased NEC risk [26].

Multiple studies suggest that probiotics prevent NEC (reviewed in [27]), but this benefit accrues chiefly to infants who weigh <1000 g at birth, and who are at lesser risk of experiencing NEC, and of dying from NEC, than those whose birthweights are <1000 g. Indeed, a recent large and well-conducted multi-center randomized control trial of *Bifidobacterium breve* BBG-001 failed to demonstrate any protective effect against NEC in a population in which most of the children weighed ≤1000 g at birth [28]. Whereas this failure might represent the use of a single probiotic instead of a combination of probiotics, and though the choice of the probiotic intervention could be subject to debate, it seems unlikely that we will soon identify viable microbes that can exert a profoundly protective effect against NEC, especially among infants whose birth weights are ≤1000 g.

A review of the many taxa that have been associated with NEC is beyond the scope of this article. However, the diversity of incriminated species, the overall small numbers of subjects in these studies, and small effect sizes reported (often only in subgroup analysis), cast doubt on the existence of a specific mono-microbial driver of NEC [29]. Nonetheless, as reviewed above, direct-from-stool sequencing of DNA now offers new opportunities to compare cases with NEC to controls, to determine whether microbial populations are associated with this outcome. In the past decade, multiple groups have attempted to apply direct-from-stool sequencing to identify bacteria that might cause NEC. Some such attempts are summarized in Table 3, focusing on studies that utilized 16S rRNA amplification methods rather than culture or gel electrophoresis-based methods.

These studies suggest that diverse bacterial taxa are associated with either risk of, or protection from, developing necrotizing enterocolitis among preterm infants. One interpretation is that there are center-specific differences in microbial drivers of NEC, as described for variability in gut microbial populations before the onset of bloodstream infections [35]. An alternative explanation is that the population biology of bacteria in the gut is exceptionally dynamic in the interval during which NEC occurs, which obligates the assembly of exceptionally large cohorts to study this disorder, and the need to interrogate an abundance of specimens prior to the event. Indeed, only one of the studies in Table 3 reported the analysis of >100 pre-NEC specimens.

The dynamism of bacterial populations poses immense challenges. In the first 60 days of life, as described above, there is a week-by-week aggregate progression from Bacilli to Gammaproteobacteria predominance, while Clostridia slowly rise in abundance. In reality, the Clostridia class described by La Rosa et al. contains Clostridia and Negativicutes, because Negativicutes (Gram-negative obligate anaerobic bacteria) have recently been assigned their own class [20]. NEC generally does not present until after the second week of life, and risk extends to approximately day of life 60, with infants born most prematurely developing NEC later in this period of vulnerability [29]. To illustrate this challenge, stools from a case occurring on day of life 25 would ideally be compared to stools from a control group of infants produced on day of life 25, these controls having been born after the same gestational duration. However, control specimens for a case of NEC that occurs on day of life 45 would greatly differ in content from controls chosen on day of life 25, even if controlling for gestational age at birth. That is to say, the norm changes throughout the interval of risk, during which NEC can occur at any time. When one also takes into account

the additional abrupt changes in populations, it is clear that a substantial number of subjects and specimens must be assembled to characterize the microbial population in children at risk in a case–control study. Notably, the larger studies tend to lean towards a predominance of Gram-negative bacteria as being drivers of NEC. Consensus protective organisms have generally not emerged from these large studies. A final complicating note is that specimens obtained immediately before NEC is apparent may reflect changes of NEC that are already under way before infants become visibly affected. It therefore seems prudent to "censor" sequence data from the hours preceding the onset of clinical NEC if trying to identify signatures well in advance of NEC that could be associated with this disorder. Interestingly, in one study in which specimens were analyzed late (tissue at resection) [30] or early (meconium) [2], anaerobic bacteria were associated with NEC.

In the largest study (in terms of numbers of cases and numbers of pre-NEC stools analyzed) reported to date, an overrepresentation of Gammaproteobacteria was associated with NEC, whereas anaerobic bacteria, especially Negativicutes and secondarily Clostridia, were associated with control status (i.e., protection). Gammaproteobacteria risk has been suggested in several smaller studies [16,18,34]. In contrast, however, several publications employing direct-from-stool sequencing have not identified overabundant Gram-negative bacteria as a prelude to NEC [2,33].

Indirect data support that Gram-negative bacteria are causal in NEC pathogenesis. In animal models, toll-like receptor 4, the ligand for lipopolysaccharide, is believed to play a central role in mucosal injury [36], and antibiotics active against Gram-negative bacteria confer protection [37]. Moreover, anaerobic bacteria, in response to microbiota-accessible carbohydrates, generate anti-inflammatory short-chain fatty acids, notably acetate, propionate, and butyrate [38]. Several literature reviews [39,40] have evaluated studies in which infants were administered oral aminoglycosides in attempts to prevent NEC. Aminoglycosides would be active against Gammaproteobacteria in the gut, but not suppress anaerobic bacterial populations. In the aggregate, these studies [41–44] support the use of oral aminoglycosides to prevent NEC. However, because of concerns about selecting for aminoglycoside resistance [45] and of absorption of the oral aminoglycosides from the gut (albeit confined to very early in life before the incidence of NEC increases [46]), enteral antibiotics to prevent NEC are not widely used. It is interesting to note that the oral aminoglycosides were often discontinued in these studies before the time of life at which the most premature infants develop NEC. This timing raises the possibility that the beneficial effects of antibiotics in these studies might have been understated.

Bacterial diversity – defined as the number of different taxa present, weighed according to their proportionality – is considered to reflect a healthy luminal microbial community in inflammatory bowel disease and *C. difficile* infections [47,48]. Even before these associations between lack of diversity and gut inflammation were reported, Claud and Walker proposed the hypothesis that diminished diversity of the premature infant gut could result in NEC [49]. Subsequent studies failed to find an association between lack of bacterial diversity and development of NEC [16–18,31,33,34,50], though again, as for NEC microbial associations, the numbers were limited. However, in a recent study [21], an association between subsequent development of NEC and comparatively lower gut bacterial diversity

was noted. The difference appeared to be related to delayed or suppressed maturation of microbial diversity in infants who subsequently developed NEC, compared to those who did not. In other words, diversity slowly increased over the first 60 days of life in the controls but not the cases. However, this association is not straightforward: gut bacterial communities are exceptionally non-complex in preterm infants. Therefore, a change in the proportionality of one taxon is necessarily counterbalanced by a change in one or more of the few other taxa present. With only four dominant taxonomic “degrees of freedom,” it is difficult to attribute NEC to lack of gut bacterial diversity per se, versus an increase or a decrease in one or another taxon. In other words, it cannot be stated that lack of diversity is the driver of risk for NEC, versus an overrepresentation of Gammaproteobacteria, which directly ordains the lack of diversity in these sample sets. The role of bacterial diversity in protecting from NEC remains an intriguing hypothesis, however.

4. Conclusion

NEC remains a catastrophic disorder. It is concerning that we have not had meaningful and durable improvements in incidence or outcomes of NEC in the nearly four decades since widespread recognition of this entity permeated neonatology. The finding of a microbial signature prior to development of NEC, and/or a protective signature in the form of obligate anaerobic bacteria, now offers new opportunities to prevent this devastating consequence of preterm birth.

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References

1. Mshvildadze M, Neu J, Shuster J, Theriaque D, Li N, Mai V. Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. J Pediatr. 2010; 156:20–5. [PubMed: 19783002]
2. Heida FH, van Zoonen AG, Hulscher JB, et al. A necrotizing enterocolitis-associated gut microbiota is present in the meconium: results of a prospective study. Clin Infect Dis. 2016; 62:863–70. [PubMed: 26787171]
3. Ardissone AN, de la Cruz DM, Davis-Richardson AG, et al. Meconium microbiome analysis identifies bacteria correlated with premature birth. PloS One. 2014; 9:e90784. [PubMed: 24614698]
4. Stout MJ, Conlon B, Landeau M, et al. Identification of intracellular bacteria in the basal plate of the human placenta in term and preterm gestations. Am J Obstet Gynecol. 2013; 208:226 e1–7. [PubMed: 23333552]
5. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med. 2014; 6:237ra65.
6. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep. 2016; 6:23129. [PubMed: 27001291]
7. Jimenez E, Fernandez L, Marin ML, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr Microbiol. 2005; 51:270–4. [PubMed: 16187156]

8. DiGiulio DB, Romero R, Amogan HP, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PloS One. 2008; 3:e3056. [PubMed: 18725970]
9. Rautava S, Collado MC, Salminen S, Isolauri E. Probiotics modulate host–microbe interaction in the placenta and fetal gut: a randomized, double-blind, placebo-controlled trial. Neonatology. 2012; 102:178–84. [PubMed: 22776980]
10. Lim ES, Zhou Y, Zhao G, et al. Early life dynamics of the human gut virome and bacterial microbiome in infants. Nature Med. 2015; 21:1228–34. [PubMed: 26366711]
11. Nikkari S, McLaughlin IJ, Bi W, Dodge DE, Relman DA. Does blood of healthy subjects contain bacterial ribosomal DNA? J Clin Microbiol. 2001; 39:1956–9. [PubMed: 11326021]
12. Backhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe. 2015; 17:690–703. [PubMed: 25974306]
13. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. PLoS Biol. 2007; 5:e177. [PubMed: 17594176]
14. Arrieta MC, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The intestinal microbiome in early life: health and disease. Front Immunol. 2014; 5:427. [PubMed: 25250028]
15. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. Nature. 2012; 486(7402):207–14. [PubMed: 22699609]
16. Zhou Y, Shan G, Sodergren E, Weinstock G, Walker WA, Gregory KE. Longitudinal analysis of the premature infant intestinal microbiome prior to necrotizing enterocolitis: a case–control study. PloS One. 2015; 10:e0118632. [PubMed: 25741698]
17. Ward DV, Scholz M, Zolfo M, et al. Metagenomic sequencing with strain-level resolution implicates uropathogenic E. coli in necrotizing enterocolitis and mortality in preterm infants. Cell Rep. 2016; 14:2912–24. [PubMed: 26997279]
18. Sim K, Shaw AG, Randell P, et al. Dysbiosis anticipating necrotizing enterocolitis in very premature infants. Clin Infect Dis. 2015; 60:389–97. [PubMed: 25344536]
19. Gibson MK, Wang B, Ahmadi S, et al. Developmental dynamics of the preterm infant gut microbiota and antibiotic resistome. Nature Microbiol. in press.
20. La Rosa PS, Warner BB, Zhou Y, et al. Patterned progression of bacterial populations in the premature infant gut. Proc Natl Acad Sci USA. 2014; 111:12522–7. [PubMed: 25114261]
21. Warner BB, Deych E, Zhou Y, et al. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case–control study. Lancet. 2016; 387(10031):1928–36. [PubMed: 26969089]
22. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science. 2009; 326(5960):1694–7. [PubMed: 19892944]
23. Cotten CM, Taylor S, Stoll B, et al. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. Pediatrics. 2009; 123:58–66. [PubMed: 19117861]
24. Kuppala VS, Meinzen-Derr J, Morrow AL, Schibler KR. Prolonged initial empirical antibiotic treatment is associated with adverse outcomes in premature infants. J Pediatr. 2011; 159:720–5. [PubMed: 21784435]
25. Alexander VN, Northrup V, Bizzarro MJ. Antibiotic exposure in the newborn intensive care unit and the risk of necrotizing enterocolitis. J Pediatr. 2011; 159:392–7. [PubMed: 21489560]
26. Guillet R, Stoll BJ, Cotten CM, et al. Association of H2-blocker therapy and higher incidence of necrotizing enterocolitis in very low birth weight infants. Pediatrics. 2006; 117:e137–42. [PubMed: 16390920]
27. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2014; (4):CD005496. [PubMed: 24723255]
28. Costeloe K, Hardy P, Juszczak E, Wilks M, Millar MR. Probiotics in Preterm Infants Study Collaborative Group. Bifidobacterium breve BBG-001 in very preterm infants: a randomised controlled phase 3 trial. Lancet. 2016; 387(10019):649–60. [PubMed: 26628328]
29. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med. 2011; 364:255–64. [PubMed: 21247316]

30. Brower-Sinning R, Zhong D, Good M, et al. Mucosa-associated bacterial diversity in necrotizing enterocolitis. PloS One. 2014; 9:e105046. [PubMed: 25203729]
31. Mai V, Young CM, Ukhanova M, et al. Fecal microbiota in premature infants prior to necrotizing enterocolitis. PloS One. 2011; 6:e20647. [PubMed: 21674011]
32. McMurtry VE, Gupta RW, Tran L, et al. Bacterial diversity and Clostridia abundance decrease with increasing severity of necrotizing enterocolitis. Microbiome. 2015; 3:11. [PubMed: 25810906]
33. Raveh-Sadka T, Thomas BC, Singh A, et al. Gut bacteria are rarely shared by co-hospitalized premature infants, regardless of necrotizing enterocolitis development. eLife. 2015; 4
34. Torrazza RM, Ukhanova M, Wang X, et al. Intestinal microbial ecology and environmental factors affecting necrotizing enterocolitis. PloS One. 2013; 8:e83304. [PubMed: 24386174]
35. Taft DH, Ambalavanan N, Schibler KR, et al. Center variation in intestinal microbiota prior to late-onset sepsis in preterm infants. PloS One. 2015; 10:e0130604. [PubMed: 26110908]
36. Hackam DJ, Afrazi A, Good M, Sodhi CP. Innate immune signaling in the pathogenesis of necrotizing enterocolitis. Clin Dev Immunol. 2013; 2013:475415. [PubMed: 23762089]
37. Jensen ML, Thymann T, Cilieborg MS, et al. Antibiotics modulate intestinal immunity and prevent necrotizing enterocolitis in preterm neonatal piglets. Am J Physiol Gastrointest Liver Physiol. 2014; 306:G59–71. [PubMed: 24157972]
38. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013; 341(6145):569–73. [PubMed: 23828891]
39. Bury RG, Tudehope D. Enteral antibiotics for preventing necrotizing enterocolitis in low birthweight or preterm infants. Cochrane Database Syst Rev. 2001; (1):CD000405. [PubMed: 11279690]
40. Bell EF. Preventing necrotizing enterocolitis: what works and how safe? Pediatrics. 2005; 115:173–4. [PubMed: 15629996]
41. Rowley MP, Dahlenburg GW. Gentamicin in prophylaxis of neonatal necrotising enterocolitis. Lancet. 1978; 2(8088):532.
42. Grylack LJ, Scanlon JW. Oral gentamicin therapy in the prevention of neonatal necrotizing enterocolitis. A controlled double-blind trial. Am J Dis Child. 1978; 132:1192–4. [PubMed: 362900]
43. Egan EA, Mantilla G, Nelson RM, Eitzman DV. A prospective controlled trial of oral kanamycin in the prevention of neonatal necrotizing enterocolitis. J Pediatr. 1976; 89:467–70. [PubMed: 784926]
44. Boyle R, Nelson JS, Stonestreet BS, Peter G, Oh W. Alterations in stool flora resulting from oral kanamycin prophylaxis of necrotizing enterocolitis. J Pediatr. 1978; 93:857–61. [PubMed: 361939]
45. Grylack L, Neugebauer D, Scanlon JW. Effects of oral antibiotics on stool flora and overall sensitivity patterns in an intensive care nursery. Pediatr Res. 1982; 16:509–11. [PubMed: 7050868]
46. Grylack L, Boehnert J, Scanlon J. Serum concentrations of gentamicin following oral administration to preterm newborns. Dev Pharmacol Therapeut. 1982; 5:47–52.
47. Wills ES, Jonkers DM, Savelkoul PH, Masclee AA, Pierik MJ, Penders J. Fecal microbial composition of ulcerative colitis and Crohn's disease patients in remission and subsequent exacerbation. PloS One. 2014; 9:e90981. [PubMed: 24608638]
48. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal microbiome in recurrent Clostridium difficile-associated diarrhea. J Infect Dis. 2008; 197:435–8. [PubMed: 18199029]
49. Claud EC, Walker WA. Hypothesis: inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. FASEB J. 2001; 15:1398–1403. [PubMed: 11387237]
50. Morrow AL, Lagomarcino AJ, Schibler KR, et al. Early microbial and metabolomic signatures predict later onset of necrotizing enterocolitis in preterm infants. Microbiome. 2013; 1:13. [PubMed: 24450576]

Practice points

- The causes of NEC are unknown.
- Judicious use of antibiotics and promotion of human milk use might lower the risk of NEC, but these interventions are unlikely to categorically reduce disease incidence, and are justifiable for multiple additional reasons.

Research directions

- How can we anticipate the microbial community changes that lead to NEC?
- How can we modulate the gut microbial community to reduce bacteria-associated processesthat might lead to NEC?

Table 1

Data in support of prenatal colonization of the new-born gut with microbes.

Study	Subject of study	Comments
Jimenez et al. [7]	Cord blood cultures of term neonates born by elective cesarean section	Enterococci, streptococci, staphylococci, and propionibacterium recovered from cord blood
Mshvildadze et al. [1]	Meconium by 16S rRNA gene sequencing	Viable bacteria not sought
DiGiulio et al. [8]	Amniotic fluid cultures and 16S rRNA gene sequencing in preterm deliveries	16S rRNA gene sequences identified in, and *Mycoplasma hominis*, *Ureaplasma* sp., *Streptococcus agalactiae*, *Lactobacillus* sp., *Prevotella* sp., *Fusobacterium nucleatum*, coagulase-negative *Staphylococcus* sp., *Bacillus* sp. (not anthrax), *Peptostreptococcus* sp., and *Gardnerella vaginalis* recovered from the amniotic fluid
Rautava et al. [9]	Bacterial DNA detected in amniotic fluid at time of elective cesarean section by 16S rRNA gene sequencing	Viable bacteria not sought
Stout et al. [4]	Electron microscopy of placenta	Bacteria identified in basal plate, no attempt to culture
Aagaard et al. [5]	16S rRNA gene sequences and metagenomic sequences	Bacterial sequences identified and reflected periodontal microbes, no attempt to culture
Lim et al. [10]	First in life stool (days 1–4) subjected to 16S rRNA gene sequencing and virome analysis	Few bacterial species, many bacteriophages, based on sequence analysis

Table 2

Studies of gut bacterial assembly in preterm infants without necrotizing enterocolitis (NEC).

Study and location	Sequencing technology	No. Of subjects without NEC	No. of specimens	Conclusions about pattern of bacterial community assembly
Zhou et al. [16], Brigham and Women's Hospital, Boston, MA, USA	16S rRNA gene sequencing	26	111	Increasing proportion of *Clostridia* over time, balanced by slowly diminishing proportion of Gram-negative genera, with little effect of antibiotics on this trend
Ward et al. [17], Cincinnati, OH, and Birmingham, AL, USA	Metagenomic sequencing	89	185	Clostridia class increases over time (specifically veillonella and *C. freundii*), with consistently high Proteobacteria (specifically *E. coli*)
Shaw et al. [18], St Mary's Hospital, Queen Charlotte's and Chelsea Hospital, London,UK[a]	16S rRNA gene sequencing	44	369	Bifidobacteria and klebsiella increased in proportionality, and Gram-positive bacteria decreased in proportionality, over time
Gibson et al. [19], St Louis Children's Hospital, St Louis, MO, USA	Metagenomic sequencing	84	401	Some of these subjects and specimens were also analyzed in La Rosa et al. [21]. Notably, metagenomic sequencing recapitulated the 16S sequence analysis of this cohort in these two companion publications.
La Rosa et al. [20], St Louis Children's Hospital, St Louis, MO, USA	16S rRNA Gene sequencing	58	922	Bacterial classes proceed from Bacilli to Gammaproteobacteria to Clostridia in these infants, but these populations are prone to changes in content. When infants near 33–36 weeks postconceptional age (i.e., an interval that is equivalent to the 3rd to the 12th week of age, in view of the wide range of gestational ages in this cohort), the populations converge on a consensus community, with ~40% of the bacteria being obligate anaerobes (especially Clostridia and Negativicutes), and an equal percentage being Gammaproteobacteria. There was little or no effect of use of postnatal antibiotics, mode of delivery, or breast milk, and the community composition at this point.
Warner et al. [21], St Louis Children's Hospital, St Louis, MO; Children's Hospital at Oklahoma University, Oklahoma City, OK; Kosair Children's Hospital, Louisville, KY, USA	16S rRNA gene sequencing	120	2720	Includes the 58 subjects without NEC and their 922 stools in La Rosa et al. [21]. Patterns in NICUs in Oklahoma City and in Louisville recapitulate those in St Louis cohort

NICU, neonatal intensive care unit.

[a]Based on data from Supplemental Table 1 in [18].

Table 3

Summary of studies in which DNA sequences obtained directly from stools have been used to associate bacterial risk and development of necrotizing enterocolitis (NEC), listed in ascending order according to number of pre-NEC stools sequenced.

Study	Sequencing technology	No. of subjects without NEC	No. of specimens from subjects without NEC	No. of subjects with NEC	No. of pre-NEC specimens from subjects who subsequently developed NEC	Comments
Brower-Sinning et al. [30], Pittsburgh, PA, USA	**16S rRNA gene sequencing**	**10**	**10**	**9**	**9**	**Tissue analysis only, no pre-NEC samples; Proteobacteria, Clostridia associated with risk, as was diminished bacterial diversity**
Mai et al. [31], three University of Florida-affiliated NICUs, FL, USA	16S rRNA gene sequencing	9	18	9	18	Case stools demonstrated an increase in Proteobacteria, and a decrease in Firmicutes in the second of the paired samples (i.e., week before NEC)
McMurtry et al. [32], Louisiana State University Health Sciences Center, Touro Infirmary, East Jefferson General Hospital and Children's Hospital of New Orleans, LA, USA	16S rRNA gene sequencing	74	74	21	21	Bacterial diversity and relative abundance of Clostridia was significantly lower in NEC specimens compared to controls
Raveh-Sadka et al. [33] Pittsburgh, PA, USA	Metagenomic sequencing	5	34	5	21	No clear association between bacterial content as identified by metagenomics and outcome; no microbiologic evidence of time–space clustering
Heida et al. [2], Groningen, The Netherlands	16S rRNA gene sequencing of meconium and subsequent stools	22	57	11	30	*Clostridium perfringens* and *Bacteroides dorei* associated with risk, and staphylococci associated with protection.
Torrazza et al. [34], Gainseville, FL, USA	16S rRNA gene sequencing	35	77	18	40	Novel sequence matching closest to *Klebsiella pneumoniae* during week 1 associated with subsequent development of NEC
Ward et al. [17], Cincinnati, OH, and Birmingham, AL, USA	Metagenomic sequencing	89	185	27	60	Specific sequence types of E. coli associated with NEC
Sim et al. [18], St Mary's Hospital, Queen Charlotte's and Chelsea Hospital, London, UK	16S rRNA gene sequencing	44	369	22	88	Klebsiella, clostridium associated with risk; no microbiologic evidence of time–space clustering
Zhou et al. [16], Brigham and Women's Hospital, Boston, MA, USA	16S rRNA gene sequencing	26	111	10	88	Age-specific differences identified, with early- and late-onset NEC having an association with Clostridia and Gammaproteobacteria, respectively
Warner et al. [23], St Louis Children's Hospital, St Louis, MO; Kosair Children's Hospital, Louisville, KY; Children's Hospital at Oklahoma University, Oklahoma City, OK, USA	16S rRNA gene sequencing	120	2720	46	866	Gammaproteobacteria associated with risk, and Negativicutes associated with protection; lack of diversity is associated with risk

NICU, neonatal intensive care unit.