Published in final edited form as:

Clin Perinatol. 2019 March ; 46(1): 29–38. doi:10.1016/j.clp.2018.10.003.

Necrotizing enterocolitis pathophysiology: how microbiome data alters our understanding

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Keywords

Necrotizing enterocolitis; Microbiome; Antibiotic stewardship; Genomics

Introduction

The pathophysiology of necrotizing enterocolitis (NEC) is a complex and multifactorial process that has been the topic of many areas of research in neonatology. NEC is a significant cause of morbidity and mortality in premature neonates [1,2], as well as a major player in the economics of hospital care and medicine [3,4]. In North America, NEC affects approximately 7% of very low birth weight infants (birth weight < 1500g), with as many as 20–30% of these patients dying of this major gastrointestinal disease [5,6]. Survivors of NEC are at risk for a range of complications, including short-gut syndrome and neurodevelopmental delays [7]. Although the exact mechanism of NEC is still unknown, many aspects of the disease have been observed and discussed in literature, including the role of the gut microbiome as it relates to the evolution of NEC. Research in this area is expanding, and with advances in bacterial analysis, our understanding of the relationship between the microbiome and NEC continues to grow.

Neonatal microbiome

The human microbiome is considered the “sum of microbial life living in and on the human body [8].” Its study has been largely facilitated by advances in the field of genomics, and research continues to show the importance between the microbiome and its host. The microbiome seems to play a role in a broad range of essential functions, including metabolism, nutrition, and the immune system. It begins to develop from birth, and as will be discussed below, perhaps even earlier *in utero*, and continues to evolve throughout life in order to maintain homeostasis with its host [9]. The gut microbiome is of particular interest

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DISCLOSURE STATEMENT

We have no financial interests to disclose.

in neonatal research as there appear to be multiple identifiable and modifiable factors that may alter both the establishment and development of this commensal community of bacteria.

Techniques in microbiome analysis

Recent advances in research surrounding microbiome analyses have been spurred on by the increasing use of nonculture-based techniques in the evaluation of intestinal bacteria. As many bacteria found in stool samples cannot be analyzed using traditional culture-based methods, the role of genomic sequencing has opened up this area of research. Two of the more commonly utilized approaches are 16S rRNA sequencing and metagenomics. The 16S rRNA component of ribosomal RNA is of particular utility for studies related to the microbiome, as genes encoding this region can be highly conserved or variable, and therefore facilitate taxonomic classification of bacteria [10]. Metagenomic sequencing may provide even more information as to function, as this approach is not limited to the 16S rRNA regions. These methods allow for a more comprehensive approach to analysis of intestinal microbial communities.

Role and development of the commensal microbiome

The human gastrointestinal tract assumes many critical roles, including providing a connection between the internal and external environments of the body. This large immune organ must find the delicate balance between protecting against harmful pathogens, while serving as host to commensal bacteria [11]. This paradigm seems especially relevant to the pathogenesis of NEC, in that there appears to be an association between the perturbation of the makeup and homeostasis of the gut microbiome and the uncontrolled inflammation seen in NEC. Commensal bacteria are important in a number of gastrointestinal functions, including digestion [12] and the adaptive immune response [13]. Therefore, the establishment of the host microbiome in a newborn is a delicate and essential process in their development.

Intrauterine origin of the microbiome—Although there is still debate regarding exactly when the newborn gut begins to acquire commensal bacteria, there is increasing evidence that this may begin *in utero*. In a study by Ardisson et al. [14], meconium was collected from infants 23 to 41 weeks gestation, analyzed using 16S rRNA sequencing methods, and compared to previously studied bacterial profiles of amniotic fluid. The investigators found multiple bacterial genera in common between meconium and amniotic fluid. This idea that the maternal uterine environment may affect the infant gut microbiome is of particular interest when looking at studies that demonstrate microbial dysbiosis in mothers with preterm premature rupture of membranes (PPROM). In a study by Baldwin et al. [15], investigators attempted to characterize amniotic fluid discharge and the vaginal microbiome by taking serial vaginal swabs from the onset of PPRM until delivery. The samples were analyzed using 16S rRNA sequencing, and results showed that although there was substantial variation between the microbiomes of PPRM subjects, overall trends indicated that the bacterial makeup of the vagina and amniotic fluid discharge was significantly influenced by exposure to latency antibiotics. This study showed the persistent microbial dysbiosis present in mothers with PPRM from onset until delivery, and if indeed

there is a connection between amniotic fluid and the infant microbiome, then antenatal antibiotic exposure may play a role in the development of the neonatal gut microbiota.

Other studies have also identified microbial DNA sequences in meconium samples of newborns, supporting the idea of an intrauterine origin of the gut microbiome [16, 17]. Further corroboration of this idea is provided by research looking at the presence and profile of bacteria in placental cultures [18], although these studies do not specifically look at any potential relationship between the microbial makeup of the placenta compared to the newborn gastrointestinal tract. In contrast, a study by Lauder et al. [19] concluded that a distinctive microbial profile could not be found in placental samples when compared to contamination controls, countering the idea of a “placental microbiome.” Ultimately, these studies show that the role the intrauterine environment plays in the development of the neonatal microbiome seems relevant and requires further study and analysis.

Extrauterine development of the microbiome—Many factors appear to play a role in the development of the newborn gut microbiome in the extrauterine environment, including mode of delivery (cesarean versus vaginal) and type of enteral feeds (formula versus breast milk) [20,21]. Preterm infants admitted to a neonatal intensive care unit encounter an additional set of variables in that they may be exposed to certain medications (namely antibiotics), require feeding tubes, and have enteral feeds introduced more slowly. The ICU environment itself may also influence a patient’s microbiome, from hand hygiene practices to central line care [22].

Mode of delivery: The cesarean delivery rate in the United States in 2016 was 31.9% [23]. Although this is the lowest rate since 2007, cesarean deliveries are still a significant portion of overall births, and several studies have shown differences in the microbiome of newborns after cesarean and vaginal deliveries [24]. In a more recent non-culture-based study by Dominguez-Bello et al. [25], investigators showed that samples taken from the skin, oral mucosa, and nasopharyngeal aspirates of newborns delivered vaginally reflected their mother’s vaginal microbiota, namely *Lactobacillus*, *Prevotella*, or *Sneathia* species. In contrast, samples taken from newborns delivered via cesarean section showed bacteria more commonly found on the skin surface, including *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* species. Although bacteria related to cesarean deliveries seems to have more pathogenic potential to the host, there have been no studies showing an association between delivery mode and the development of NEC to date.

Enteral exposures including type of feeds and probiotics: Multiple studies have shown that an exclusive human milk diet decreases the incidence of both medical and surgical NEC in preterm infants [26–29]. The understanding of the benefits of human milk are also reflected in the American Academy of Pediatrics Policy Statement from 2017 which recommends donor milk for preterm infants (particularly those < 1500g) when the mother’s milk is not available [30]. Many components of human milk seem to be related to the decreased incidence of NEC, including immunoglobulins, lactoferrin, lysozyme, and human milk oligosaccharides (HMOs). HMOs make up the third largest component of human milk and may play a significant role in the development of the newborn microbiome [31] through selective consumption by commensal gut bacteria [32]. In healthy term infants that are

receiving an exclusive human milk diet, *Bifidobacteria* and *Bacteroidetes* are able to thrive secondary to their ability to digest HMOs, while pathogenic bacteria such as *Enterobacteriaceae* are unable to utilize HMOs to promote their proliferation [33]. Interestingly, administration of probiotics containing *Bifidobacteria* to preterm infants leads to a decreased risk of NEC [34].

The idea that the gut microbiome may be altered by certain enteral exposures is also reflected in research surrounding probiotics. Probiotics may be a way in which beneficial commensal strains of certain bacteria can be introduced into an infant's gut microbiome in order to potentially provide protection against inflammation and ultimately NEC [11]. A meta-analysis of 20 randomized-controlled trials demonstrated that probiotics reduced both severe NEC and all-cause mortality [35]. Although the two most commonly used probiotic agents are *Bifidobacteria* and *Lactobacillus*, the optimal type of probiotic supplement has not yet been determined [36], and concerns for the risk of infection and sepsis continue. The complicated nature of the use of probiotics as a therapeutic agent is also reflected in the intricate balance of the gut microbiome. Maintaining the complex homeostasis of the infant intestinal microbiota may not be as simple as exposing the gastrointestinal tract to one or two genera of "beneficial bacteria," and may require a more wholistic approach to the microbiome.

Antibiotics and other medications: It is often standard practice in the neonatal intensive care unit (NICU) to initiate empiric antibiotic therapy for preterm infants upon delivery for the potential for early-onset sepsis (EOS). While the morbidity and mortality related to EOS is high, the actual rate of EOS is low at 0.98 per 1000 live births [37]. This supports the idea that judicious use of antibiotics in certain lower-risk preterm patients may be done safely, potentially allowing for the maintenance of the commensal gut microbiome in these infants. Multiple studies have shown the association between prolonged antibiotic therapy and increased risk of NEC [38–40]. In a retrospective cohort analysis of extremely low birth weight infants, Cotten et al. [38] showed that prolonged empiric antibiotic therapy (started in the first 3 postnatal days with sterile culture results, 5 days duration) was associated with increased odds of NEC. Similarly, in a retrospective case-controlled study Alexander et al. [40] showed that duration of antibiotic therapy was associated with increased risk of NEC in neonates without previous sepsis. As it is generally understood that antibiotics cause alterations in the makeup of both commensal and pathogenic microbiota, these studies support the idea that variables (such as antibiotic exposure) that affect gut bacteria may also affect the pathogenesis of NEC.

Although there have not been many large cohort studies looking at the association between specific antibiotic regimens and their effects on the makeup of the gut microbiome, some smaller studies have shown interesting taxonomic patterns of the bacteria from neonatal stool samples in patients exposed to antibiotics [41–43]. In a study by Greenwood et al. [41], investigators utilized 16s rRNA sequencing to show that early empiric antibiotic use in preterm infants led to an increased abundance of *Enterobacter*, lower bacterial diversity of the gut microbiome overall in the second and third weeks of life, and more cases of NEC. The initial decrease in bacterial diversity following empiric antibiotic exposure (in this case, ampicillin and gentamicin) in this study contrasts with a study done by Tanaka et al. [42]

which showed no significant differences of the gut microbiota in infants 36 weeks gestation in the first month following therapy with oral cephalixin. Although the two studies are difficult to compare due to multiple differing variables, they both illustrate the fact that larger studies are required to look at factors such as specific antibiotic classes and route of administration in relation to the gut microbiome, and ultimately the risk of NEC [44]. Similar to antibiotics, H2-blocker medications such as ranitidine have been associated with an increased risk of NEC [45, 46]. As in the Greenwood et al. study, Gupta et al. [47] also utilized 16s rRNA sequencing to show lower microbial diversity and increase in relative abundance of Enterobacteriaceae in stool samples taken from preterm infants exposed to H2-blockers.

Intestinal microbiota and inflammation

The intestinal mucosa carries out multiple complex functions, including serving as a first line barrier defense from potential pathogens, as well as modulation of the innate immune system [13, 48, 49]. Both commensal and pathogenic gut bacteria contain microbial associated molecular patterns (MAMPs) [50] that are recognized by specific pattern recognition receptors (PRRs) present on the intestinal mucosa. Microbial cell wall products such as lipopolysaccharides and peptidoglycans may serve as MAMPs that are recognized by PRRs on human intestinal cells, such as Toll-like receptors (TLRs). These intricate interactions lead to signaling pathways involved in critical gastrointestinal tract functions such as homeostasis and inflammatory effects [11]. The makeup of the gut microbiome is therefore extremely relevant as certain MAMPs present on specific bacteria may activate versus deactivate inflammatory pathways, which in turn may affect the risk of NEC.

The relationship between MAMPs and their TLRs is highly specific [51]. A well-known signaling pathway that may be involved in the pathogenesis of NEC is the activation of TLR-4 by lipopolysaccharides present on Gram-negative bacteria [52, 53]. Studies have shown that TLR-4 is involved in the phagocytosis and translocation of these Gram-negative bacteria across the intestinal mucosal barrier [54]. The activation of receptors like TLR-4 can also lead to the activation of nuclear factor κ B (NF- κ B) and caspases, which can then lead to induction of pro-inflammatory cytokines such as certain tumor necrosis factors (TNF) and interleukins (IL) [11]. It has been postulated that an end result of this pathway may lead to the development of NEC [55]. Although this pathway is just an example of a component of the likely pathophysiology of NEC, it illustrates the overarching idea that patterns of microbial colonization in the preterm gut are related to the expression of regulation of such pathways, and are therefore correlated to the risk of NEC.

Gut bacterial assembly in preterm infants

As mentioned previously, there seem to be many factors that play into the development of the newborn gut microbiome. There have been a number of studies looking at the intestinal microbiota of preterm infants without NEC using either 16s rRNA or metagenomic sequencing [56–61]. Overall, the data from these studies support the paradigm that colonization of the newborn gut occurs in a relatively orderly process. In a study by La Rosa et al. [61], investigators showed a reproducible pattern in the evolution of the preterm gut microbiome in infants without NEC. Initial samples were predominantly of the Bacilli class

(Gram-positive cocci such as *Staphylococci*, *Streptococci*, and *Enterococci*). These were then overtaken by Gram-negative facultative bacteria of the Gammaproteobacteria class, and this second surge was then counterbalanced by about 20 days of life with Clostridia. An additional interesting observation by the investigators of this study was that the overall bacterial composition of preterm infant (median gestational age 27 weeks) stool samples studied differed from the intestinal bacterial composition that would be expected in term infants, older children, and adults. The preterm stool samples had higher percentages of Gammaproteobacteria and less obligate anaerobes. Although direct causality between factors such as a specific class of bacteria or timing of postnatal predominance of a certain group of bacteria and NEC has not been shown, it is interesting to note that the preterm infant gut microbiome differs in composition from term newborns.

Analysis of intestinal microbiome in infants with NEC

A number of studies comparing the intestinal microbiota of preterm infants with and without NEC have pointed towards an association between Gammaproteobacteria and increased risk for NEC [56, 60, 62]. This data correlates with what has been mentioned previously regarding TLRs and the activation of pro-inflammatory pathways by MAMPs presents on Gram-negative bacteria. Although this data is compelling, research shows that the pathogenesis of NEC is extremely complex, and studies utilizing non-culture based bacterial sequencing techniques continue to produce highly variable results. Some postulate that the wide variation in microbiota composition seen across different studies may be center-specific [63]. Others point to limitations in the sample size of most studies, and the difficulties inherent in capturing data related to such a dynamic disease process. This is reflected in those studies showing the progression of intestinal bacterial colonization of the preterm gut, and the seemingly abrupt shifts that can occur from phase to phase [61].

Summary

NEC is a devastating disease that continues to play a significant role in the mortality and morbidity of preterm infants. Although many layers of the pathophysiology of this complex gastrointestinal disorder have been studied, the role of the intestinal microbiome and the evolution of NEC seems to be one of the most promising areas of research. Newer genetic sequencing techniques have allowed for a more elegant, specific, and practical approach to the study of microbiota, and these findings must continue to be discussed and applied to what is already known about the disease. The idea of the intestinal microbiome playing a role in the development of NEC appears to be significant starting even *in utero*, and may be related to many of the postnatal variables that have been shown to relate to NEC, including type of enteral feeds and antibiotic exposure. There continues to be a high degree of variability in the genetic sequencing studies of the intestinal microbiota, therefore stressing the importance of continued research in this field.

References

1. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med. 2011;364(3):255–264. [PubMed: 21247316]

2. Lin PW, Stoll BJ. Necrotising enterocolitis. *Lancet*. 2006;368(9543):1271–1283. [PubMed: 17027734]
3. Stey A, Barnert ES, Tseng CH, et al. Outcomes and costs of surgical treatments of necrotizing enterocolitis. *Pediatrics*. 2015;135(5):e1190–1197. [PubMed: 25869373]
4. Bisquera JA, Cooper TR, Berseth CL. Impact of necrotizing enterocolitis on length of stay and hospital charges in very low birth weight infants. *Pediatrics*. 2002;109(3):423–428. [PubMed: 11875136]
5. Fitzgibbons SC, Ching Y, Yu D, et al. Mortality of necrotizing enterocolitis expressed by birth weight categories. *J Pediatr Surg*. 2009;44(6):1072–1075; discussion 1075–1076. [PubMed: 19524719]
6. Holman RC, Stoll BJ, Curns AT, Yorita KL, Steiner CA, Schonberger LB. Necrotising enterocolitis hospitalisations among neonates in the United States. *Paediatr Perinat Epidemiol*. 2006;20(6):498–506. [PubMed: 17052286]
7. Hintz SR, Kendrick DE, Stoll BJ, et al. Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis. *Pediatrics*. 2005;115(3):696–703. [PubMed: 15741374]
8. Fricke WF. The more the merrier? Reduced fecal microbiota diversity in preterm infants treated with antibiotics. *J Pediatr*. 2014;165(1):8–10. [PubMed: 24750805]
9. Gritz EC, Bhandari V. The human neonatal gut microbiome: a brief review. *Front Pediatr*. 2015;3:17. [PubMed: 25798435]
10. Torrazza RM, Neu J. The altered gut microbiome and necrotizing enterocolitis. *Clin Perinatol*. 2013;40(1):93–108. [PubMed: 23415266]
11. Patel RM, Denning PW. Intestinal microbiota and its relationship with necrotizing enterocolitis. *Pediatr Res*. 2015;78(3):232–238. [PubMed: 25992911]
12. Guarner F, Malagelada JR. Gut flora in health and disease. *Lancet*. 2003;361(9356):512–519. [PubMed: 12583961]
13. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol*. 2009;9(5):313–323. [PubMed: 19343057]
14. Ardisson AN, de la Cruz DM, Davis-Richardson AG, et al. Meconium microbiome analysis identifies bacteria correlated with premature birth. *PLoS One*. 2014;9(3):e90784.
15. Baldwin EA, Walther-Antonio M, MacLean AM, et al. Persistent microbial dysbiosis in preterm premature rupture of membranes from onset until delivery. *PeerJ*. 2015;3:e1398.
16. 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(1):20–25. [PubMed: 19783002]
17. Heida FH, van Zoonen A, Hulscher JBF, et al. A Necrotizing Enterocolitis-Associated Gut Microbiota Is Present in the Meconium: Results of a Prospective Study. *Clin Infect Dis*. 2016;62(7):863–870. [PubMed: 26787171]
18. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. *Sci Transl Med*. 2014;6(237):237ra265.
19. Lauder AP, Roche AM, Sherrill-Mix S, et al. Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota. *Microbiome*. 2016;4(1):29. [PubMed: 27338728]
20. 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(5):690–703. [PubMed: 25974306]
21. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol*. 2007;5(7):e177.
22. Warner BB, Tarr PI. Necrotizing enterocolitis and preterm infant gut bacteria. *Semin Fetal Neonatal Med*. 2016;21(6):394–399. [PubMed: 27343151]
23. Martin JA, Hamilton BE, Osterman MJK, Driscoll AK, Drake P. Births: Final Data for 2016. *Natl Vital Stat Rep*. 2018;67(1):1–55.
24. Neu J, Rushing J. Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. *Clin Perinatol*. 2011;38(2):321–331. [PubMed: 21645799]

25. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A*. 2010;107(26):11971–11975.
26. Lucas A, Cole TJ. Breast milk and neonatal necrotising enterocolitis. *Lancet*. 1990;336(8730):1519–1523. [PubMed: 1979363]
27. Sullivan S, Schanler RJ, Kim JH, et al. An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. *J Pediatr*. 2010;156(4):562–567 e561. [PubMed: 20036378]
28. Cristofalo EA, Schanler RJ, Blanco CL, et al. Randomized trial of exclusive human milk versus preterm formula diets in extremely premature infants. *J Pediatr*. 2013;163(6):1592–1595 e1591. [PubMed: 23968744]
29. Kantorowska A, Wei JC, Cohen RS, Lawrence RA, Gould JB, Lee HC. Impact of Donor Milk Availability on Breast Milk Use and Necrotizing Enterocolitis Rates. *Pediatrics*. 2016;137(3):e20153123.
30. Committee On N, Section On B, Committee On F, Newborn. Donor Human Milk for the High-Risk Infant: Preparation, Safety, and Usage Options in the United States. *Pediatrics*. 2017;139(1).
31. Underwood MA, Gaerlan S, De Leoz ML, et al. Human milk oligosaccharides in premature infants: absorption, excretion, and influence on the intestinal microbiota. *Pediatr Res*. 2015;78(6):670–677. [PubMed: 26322410]
32. Sela DA, Mills DA. Nursing our microbiota: molecular linkages between bifidobacteria and milk oligosaccharides. *Trends Microbiol*. 2010;18(7):298–307. [PubMed: 20409714]
33. Yu ZT, Chen C, Newburg DS. Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. *Glycobiology*. 2013;23(11):1281–1292. [PubMed: 24013960]
34. Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. *Pediatrics*. 2010;125(5):921–930. [PubMed: 20403939]
35. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev*. 2014(4):CD005496.
36. Wang Q, Dong J, Zhu Y. Probiotic supplement reduces risk of necrotizing enterocolitis and mortality in preterm very low-birth-weight infants: an updated meta-analysis of 20 randomized, controlled trials. *J Pediatr Surg*. 2012;47(1):241–248. [PubMed: 22244424]
37. Stoll BJ, Hansen NI, Sanchez PJ, et al. Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. *Pediatrics*. 2011;127(5):817–826. [PubMed: 21518717]
38. 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(1):58–66. [PubMed: 19117861]
39. 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(5):720–725. [PubMed: 21784435]
40. Alexander VN, Northrup V, Bizzarro MJ. Antibiotic exposure in the newborn intensive care unit and the risk of necrotizing enterocolitis. *J Pediatr*. 2011;159(3):392–397. [PubMed: 21489560]
41. Greenwood C, Morrow AL, Lagomarcino AJ, et al. Early empiric antibiotic use in preterm infants is associated with lower bacterial diversity and higher relative abundance of Enterobacter. *J Pediatr*. 2014;165(1):23–29. [PubMed: 24529620]
42. Tanaka S, Kobayashi T, Songjinda P, et al. Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. *FEMS Immunol Med Microbiol*. 2009;56(1):80–87. [PubMed: 19385995]
43. Tagare A, Kadam S, Vaidya U, Pandit A. Routine antibiotic use in preterm neonates: a randomised controlled trial. *J Hosp Infect*. 2010;74(4):332–336. [PubMed: 19926166]
44. Gibson MK, Crofts TS, Dantas G. Antibiotics and the developing infant gut microbiota and resistome. *Curr Opin Microbiol*. 2015;27:51–56. [PubMed: 26241507]

45. 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(2):e137–142. [PubMed: 16390920]
46. Terrin G, Passariello A, De Curtis M, et al. Ranitidine is associated with infections, necrotizing enterocolitis, and fatal outcome in newborns. *Pediatrics*. 2012;129(1):e40–45. [PubMed: 22157140]
47. Gupta RW, Tran L, Norori J, et al. Histamine-2 receptor blockers alter the fecal microbiota in premature infants. *J Pediatr Gastroenterol Nutr*. 2013;56(4):397–400. [PubMed: 23254444]
48. Sharma R, Young C, Neu J. Molecular modulation of intestinal epithelial barrier: contribution of microbiota. *J Biomed Biotechnol*. 2010;2010:305879.
49. Murgas Torrazza R, Neu J. The developing intestinal microbiome and its relationship to health and disease in the neonate. *J Perinatol*. 2011;31 Suppl 1:S29–34. [PubMed: 21448201]
50. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell*. 2004;118(2):229–241. [PubMed: 15260992]
51. Rhee SH. Basic and translational understandings of microbial recognition by toll-like receptors in the intestine. *J Neurogastroenterol Motil*. 2011;17(1):28–34. [PubMed: 21369489]
52. Lu P, Sodhi CP, Hackam DJ. Toll-like receptor regulation of intestinal development and inflammation in the pathogenesis of necrotizing enterocolitis. *Pathophysiology*. 2014;21(1):81–93. [PubMed: 24365655]
53. Yazji I, Sodhi CP, Lee EK, et al. Endothelial TLR4 activation impairs intestinal microcirculatory perfusion in necrotizing enterocolitis via eNOS-NO-nitrite signaling. *Proc Natl Acad Sci U S A*. 2013;110(23):9451–9456. [PubMed: 23650378]
54. Neal MD, Leaphart C, Levy R, et al. Enterocyte TLR4 mediates phagocytosis and translocation of bacteria across the intestinal barrier. *J Immunol*. 2006;176(5):3070–3079. [PubMed: 16493066]
55. Leaphart CL, Cavallo J, Gribar SC, et al. A critical role for TLR4 in the pathogenesis of necrotizing enterocolitis by modulating intestinal injury and repair. *J Immunol*. 2007;179(7):4808–4820. [PubMed: 17878380]
56. 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(3):e0118632.
57. 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(12):2912–2924. [PubMed: 26997279]
58. Sim K, Shaw AG, Randell P, et al. Dysbiosis anticipating necrotizing enterocolitis in very premature infants. *Clin Infect Dis*. 2015;60(3):389–397. [PubMed: 25344536]
59. Gibson MK, Wang B, Ahmadi S, et al. Developmental dynamics of the preterm infant gut microbiota and antibiotic resistome. *Nat Microbiol*. 2016;1:16024.
60. 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):1928–1936. [PubMed: 26969089]
61. La Rosa PS, Warner BB, Zhou Y, et al. Patterned progression of bacterial populations in the premature infant gut. *Proc Natl Acad Sci U S A*. 2014;111(34):12522–12527. [PubMed: 25114261]
62. Wang Y, Hoenig JD, Malin KJ, et al. 16S rRNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis. *ISME J*. 2009;3(8):944–954. [PubMed: 19369970]
63. 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(6):e0130604.

KEY POINTS

- Alterations in the balance of the commensal gut microbiome in neonates may be related to risk for necrotizing enterocolitis
- The newborn gut microbiome seems to develop in the setting of both pre and post-natal exposures
- Colonization of the gut microbiome occurs in a relatively orderly and step-wise process
- The preterm intestinal microbiota seems to differ from term infants, and points to the relevance of the microbiome in the development of necrotizing enterocolitis

SYNOPSIS

Necrotizing enterocolitis (NEC) is a major cause of mortality and morbidity in the preterm infant population. The gut microbiome is of particular interest in research surrounding NEC, as variations in the intestinal microbiota seem to correlate with the risk of inflammation and disease. Recent advances in non-culture-based genomic sequencing have also allowed for more intricate analyses of the intestinal microbiome. Its evolution seems to be influenced by both intrauterine and extrauterine factors, ranging from antenatal antibiotic exposure to type of enteral feeds. Ultimately, these alterations in the gut microbiome have the potential to result in devastating diseases like NEC.