

Effects of Antibiotics on the Development and Colonization of Preterm Gut Microbiota: A Short Review

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Abstract

The gut microbiota plays a vital role in the development of the immune system, nutrient absorption, and resistance to pathogen colonization. Antibiotics are among the many factors that affect and influence the establishment of the microbiota. Neonates, particularly those born prematurely, represent an interesting population because they receive early and often extensive antibiotic therapy in the first year of life. It is important to understand the effects of these antibiotics in reshaping and colonization of intestinal microbiome. Antibiotic therapy in preterm infants can dramatically affect the gut microbiome. Early establishment of the gut microbiome is suspected to have a particularly profound impact in protecting the gut from infectious disease and on long-term subsequent health by predisposing individuals to atopic or autoimmune disease later in life. This review gathers relevant literatures on the effects of antibiotics on the preterm gut microbiota both on their composition and development. This review indicates that some antibiotic treatments are associated with decreased species richness and diversity. Treatment with antibiotic encouraged resistance genes and proliferation of multidrug resistant organisms. Antibiotics regimens bring about population shifts and reshape the abundant microbial colonization. We therefore, demonstrated the impact of antibiotics on the composition of microbial community and its establishment from the gut of preterm infants during their early days of life.

Key Words: Antibiotics, Colonization, Gut, Microbiota, Neonate and Preterm.

INTRODUCTION

The preterm gut microbiota refers to diverse population of bacteria, archaea and fungi that reside in the gastrointestinal tract of preterm babies (Willing, *et al.*, 2011). Antibiotics are compounds of natural, semi-synthetic, or synthetic origin which inhibit growth of microorganisms without significant toxicity to the human or animal host. The gut microbiota plays an important role in human health by providing a barrier for colonization of pathogens, by exerting important metabolic functions (fermentation of non-digestible fibers, salvage of energy as short-chain fatty acids, and production of vitamin k), and by stimulating the development of the immune system (Penders *et al.*, 2008). The microbial community of preterm infants is known to consist of dramatically fewer beneficial species, lower bacterial diversity, and more pathogens than observed in healthy term infants, but it is not known to what extent this observation can be attributed to the high use of antimicrobial agents (Greenwood *et al.*, 2014). Considering that preterm babies are prone to infections due to their immature immune system and that current diagnostic tests for

neonatal sepsis have poor positive predictive values, clinicians often prescribe antibiotics to them shortly after birth, especially after the onset of chorioamnionitis in mothers and premature rupture of membranes. While in general antibiotics should be discontinued after 48 hours if the laboratory tests indicate a low probability of sepsis, prolonged use of antibiotics is very common due to various concerns. Nowadays, increasing attention has been paid to the adverse effects of antimicrobial therapy in the composition of preterm gut microbiota (Zhu *et al.*, 2017). Unrestricted antimicrobial use can have persistent, unintended consequences, including reduced diversity of the microbial community, and after use is discontinued, recovery of a healthy microbiome is not assured. Early empiric antibiotic use in preterm infants is associated with increased risk of necrotizing enterocolitis (NEC), sepsis, and death (Greenwood *et al.*, 2014). This paper is aim to provide a short narrative reviews on recent researches on the effects of antibiotics on preterm infants babies and the composition and development of their gut microbiota.

Gastro Intestinal Tract Microbiota in Utero

The GI tract has traditionally been considered sterile until it is colonized by microorganisms residing in the environment at birth. However, recent study has revealed the presence of microorganisms in amniotic fluid, fetal membranes, umbilical cords, placentas, and meconium. Gosalbes *et al.* (2013) showed that meconium microbiotas could be classified into two types: the first is less diverse and dominated by bacteria in the family Enterobacteriaceae, and the other is more diverse and dominated by bacteria in the phylum Firmicutes, especially lactic acid bacteria. These microbial communities differ from the microbiotas of the vagina, feces, or skin of a pregnant woman, but resemble the microbiota of the amniotic fluid, suggesting that microbes in the meconium originate from the uterus of the mother. A fetus's GI tract appeared to be colonized by bacteria through amniotic fluid that was swallowed. Further, the type of microbiota in the meconium was associated with maternal factors such as history of allergies and could have consequences for childhood health (Tanaka & Nakayama, 2017).

Infancy and the Gut Microbiota

Much of what is known about the infant microbiome has been from research on term infants, and fewer studies have focused on the preterm and low birth weight infants. Term and preterm infants were thought to be born essentially sterile, but new evidence of bacterial translocation in utero and the presence of microbial life in preterm infants' meconium may refute this. Observed lipopolysaccharide (LPS) in the cord blood of preterm infants provides evidence of this translocation process. While these findings are still controversial, they point to a profound ignorance of the founding of our microbiota. When born vaginally, infants are quickly colonized by trillions of maternal vaginal, enteric, skin, and milk microorganisms. The microbiota of neonatal skin, oral mucosa, and nasopharyngeal aspirates of vaginally delivered term infants are similar to their mother's vaginal microbiota, while Caesarean section (C-section) infants harbor bacterial communities similar to skin microbiota (Groer *et al.*, 2014)

Compositional and Functional Development of The Gut Microbiota During Infancy

After birth, the gut microbiota of a newborn is transiently dominated by Enterobacteriaceae and Staphylococcus. Thereafter, an infant's gut

microbiota is dominated by Bifidobacterium and some lactic acid bacteria. Bifidobacterium-dominated microbiota, called "Bifidus flora," is maintained until the introduction of solid food. After weaning, *bifidus* flora is outcompeted by adult-type microorganisms, represented mainly by bacteria in the genera *Bacteroides*, *Prevotella*, *Ruminococcus*, *Clostridium*, and *Veillonella*, which colonize an infant's intestines. Eventually, by about three years of age, a typical adult-like gut microbiota is established. The functions of the gut microbiota also change greatly before and after the introduction of weaning foods. The functional repertoire of an infant's microbiota changes during the first year of life, as the early microbiota before weaning is enriched in bacteria with genes that facilitate lactate utilization, whereas, after weaning, solid foods promote the growth of bacteria enriched in genes coding to allow the utilization of a larger variety of carbohydrates, vitamin synthesis, and xenobiotic degradation. (Tanaka & Nakayama, 2017)

Establishment and Evolution of The Neonatal Gut Microbiome

The first, and most important, contribution to the genesis of the microbiome is vertical transmission of maternal microbiota. Colonization of mucosa in the digestive, respiratory, urogenital tracts, as well as the skin begins at, or perhaps even before, the time of birth when a newborn is exposed to a mother's microbiota. It was previously thought that the in utero environment was largely sterile and that a fetus was not colonized with bacteria until the time of birth. Recent studies suggest the presence of a microbiome within the placenta as well as fetal meconium, suggesting that the colonization process begins well before delivery. Aagaard *et al.* (2014) have recently characterized a placental microbiome profile, composed of non-pathogenic commensal microbiota from the Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla which, interestingly, shares some similarities with the human oral microbiome (Gritz & Bhandari, 2015).

Dysbiosis of Neonatal Microbiota

Even after the microbiome is well established in healthy infants, dysbiosis, or shifts in microbial composition or diversity can occur in the setting of dietary changes, antibiotic exposure, or infection.

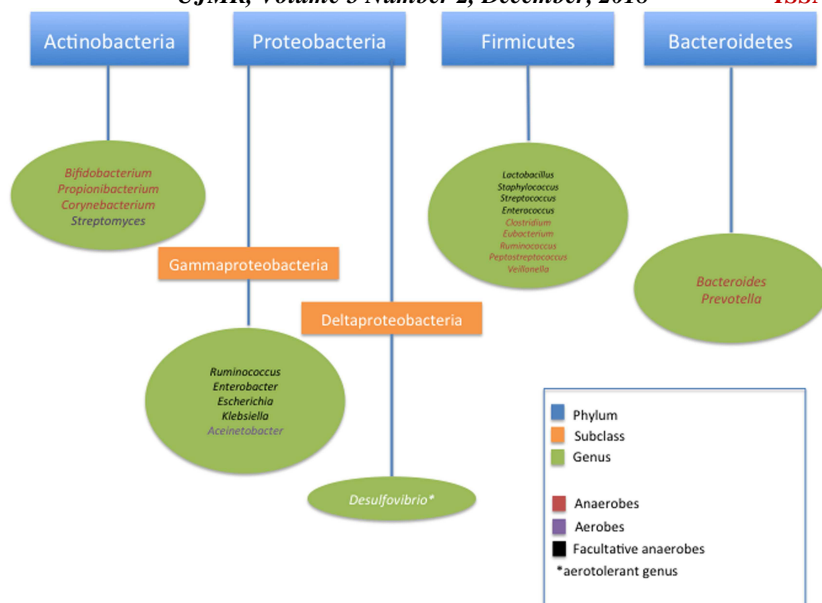


FIGURE 1 | Classification of common bacteria found in neonatal intestinal microbiome (Greenwood *et al.* 2014).

Dysbiotic conditions can favor invasion and growth of pathogenic species and can disrupt the finely tuned regulatory circuits of the immune system that maintain a system of pro- and anti-inflammatory checks and balances. The neonatal microbiome, in healthy full-term infants and especially in preterm infants given its dynamic nature, is fragile and impressionable. As such, the microbiome is extremely susceptible to external influences that can dramatically affect the short- and long-term health of the host. The development of NEC in the preterm population is a multifactorial, devastating, and as yet poorly understood disease process. (Gritz & Bhandari, 2015) A link between NEC and a microbial etiology has been recognized for decades and has been corroborated by outbreaks in neonatal intensive care unit the presence of pneumatosis intestinalis as a likely byproduct of bacterial fermentation, and the often-concomitant presence of bacteremia. As such, NEC is increasingly thought to be, at least in part, related to a perturbation of intestinal immune homeostasis, and a generalized disturbance of normal colonization patterns within the developing gut, rather than growth of a single pathogen (Gritz & Bhandari, 2015)

The Effect of Antibiotic Usage on Gastrointestinal Microbial Development In Infants

The use of antibiotics is more prevalent in infants born via C-section and in those born preterm when compared to term infants born

vaginally. Maternal and infant exposure to antibiotics during the perinatal period has been linked to increased risks of later onset diseases, such as asthma, obesity, inflammatory bowel disease, and other allergic/inflammatory conditions in children. Antibiotic exposure during the prenatal, perinatal, and postnatal periods has also been hypothesized to cause a delay in microbial maturation from 6 to 12 months after birth (Yieh, *et al.*, 2018).

Intrapartum antimicrobial prophylaxis (IAP) is believed to be the most frequent source of antibiotic exposure in neonates. IAP (penicillin, ampicillin, or ampicillin plus erythromycin) is administered to mothers who are positive for group B Streptococcus during labour to reduce the risk of early-onset neonatal infections, such as pneumonia, septicaemia, and meningitis. Two cohort studies of full term vaginally-born babies have reported reduced alpha diversity in faecal samples from infants that are exposed to maternal IAP when compared to non-IAP exposed infants. Absolute levels of Actinobacteria and Bacteroidetes were lower in IAP-exposed infants in two different studies that performed 16s rRNA amplicon sequencing using the Illumina and Ion Torrent sequencing platforms (Yieh *et al.*, 2018). Significantly lower levels of Bifidobacteriaceae were also observed in IAP-exposed infants. By contrast, the Firmicutes and Proteobacteria phyla recorded increased numbers ($p < 0.05$ and $p < 0.062$, respectively) in IAP-exposed infants.

Exposure of the maternal system to IAP impacts upon the early GI microbial composition in infants. However, the magnitude of the effect and its relationship to the duration (i.e., short- and long-term) and timing of the exposure is yet to be determined. Despite this, the impact on certain bacterial counts within faecal samples from neonates in early life is affected by the combined effect of IAP exposure, postpartum feeding mode, and birth mode. Studies conducted to date to understand the effects of these interactions on microbiota maturation have focused on term born babies in western countries. Therefore, future studies should focus on the combined effects of these factors on GI microbial maturation processes, taking into account gestational age and the ethnicity of the infants (Yieh *et al.*, 2018).

Antibiotics Disrupt the Richness and Composition of Preterm Gut Microbiota

Gut microbial species richness, defined as the number of species present in a microbiota, is widely used as a measure of microbiome health. Decreased species richness in infancy has been associated with a number of host pathologies. To identify factors that significantly contribute to species richness, Gibson *et al.* (2015) developed a generalized linear mixed model with individual included as a random effect, leveraging available metadata

including infant health (e.g. CRIB II (Clinical Risk Index for Babies) score, day of life. Antibiotics are the most prescribed medications in the neonatal intensive care unit. Data adapted from Clark *et al.* Pediatrics 2006. Frequency defined as the number of times a unique medication name was reported in the medications table in 220 NICUs in the United States and Puerto Rico between January 1996 and April 2005. gestational age, birthweight, delivery mode, and presence of positive culture), medications (e.g., antibiotics, caffeine, and iron), and maternal health (e.g. preeclampsia and premature membrane rupture). While postmenstrual age and breast milk significantly contribute to increased species richness, the opposite was true with high CRIB II score, meropenem, cefotaxime, and ticarcillin-clavulanate treatment significantly contributing to decreased species richness. Cumulative antibiotic exposure in infants was associated with a significant reduction in species richness and, with the exception of gentamicin, all antibiotics were associated with reduced species richness. This decrease in species richness occurred over intervals during which the gut microbiota of age matched, antibiotic-naive preterm infants was increasing in richness, and was accompanied by disruption of microbiota composition (Gasparrini *et al.*, 2016).

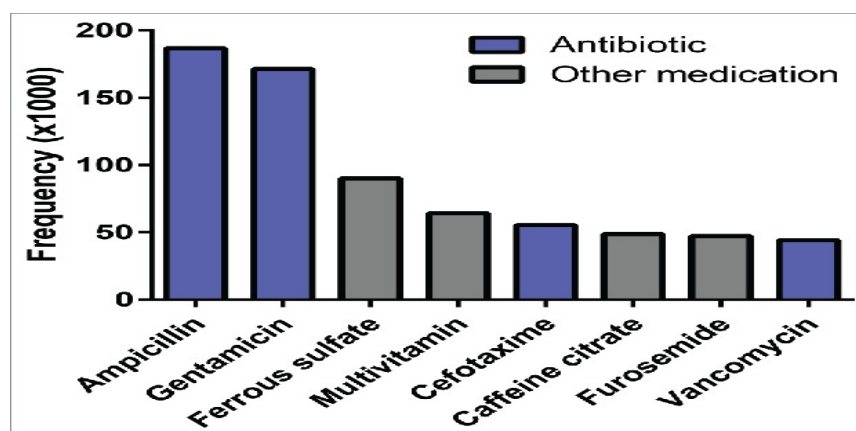


FIGURE 2: Antibiotics are the most prescribed medications in the neonatal intensive care unit. Data adapted from Clark *et al.* Pediatrics 2006. Frequency defined as the number of times a unique medication name was reported in the medications table in 220 NICUs in the United States and Puerto Rico between January 1996 and April 2005.

The Predictable Response of The Preterm Infant Gut Microbiota to Antibiotic Therapy

To quantify changes to the gut microbiota before and after antibiotic treatment Gibson *et al.* (2015) utilized metagenomic shotgun sequencing to interrogate the phylogenetic

architecture of the bacterial community. While meropenem, cefotaxime, and ticarcillin-clavulanate each significantly and immediately reduced species richness, gentamicin and vancomycin (2 of the most commonly used antibiotics in preterm infant populations, and

which are often co-administered) resulted in variable species richness responses. Using random forests classification, the direction of species richness response to vancomycin and gentamicin treatment can be predicted based on the relative abundance of only 2 species (*Staphylococcus aureus* and *E. coli*) and 2 antibiotic resistance genes (cpxR and cpxA) with an error rate of only 15%. It was hypothesized that this finding might be due to the innate vancomycin resistance of Gram-negative bacteria such as *E. coli* and the innate vancomycin-susceptibility of *S. aureus*, coupled with the ability of cpxR and cpxA to confer gentamicin resistance to *E. coli* (Gasparrini *et al.*, 2016)

These analyses reveal significant antibiotic-specific microbial and compositional responses of the preterm infant gut microbiota. For a commonly prescribed antibiotic regimen, co-therapy with vancomycin and gentamicin, Gibson *et al.* (2015) identified important species and antibiotic resistance gene biomarkers that can predict with high accuracy

the short-term species richness response and therefore potential for disruption of the developing preterm infant gut microbiota (Gasparrini *et al.*, 2016).

CONCLUSION

The early establishment of the gut microbiome is suspected to have a particularly profound impact protecting the gut from infectious disease and on long-term subsequent health by predisposing individuals to atopic or autoimmune disease later in life. This review established that meropenem, ticarcillin-clavulanate, and cefotaxime treatments were associated with decreased species richness, while gentamicin and vancomycin had variable effects on species richness. Nonetheless, all antibiotic treatments enriched the presence of resistance genes and multidrug resistant organisms. Treatment with different antibiotics further resulted in unique population shifts of abundant organisms and selection for different sets of resistance genes.

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