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Modulation of gut microbiota: An emerging consequence in neonatal sepsis

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ABSTRACT

Introduction: Billions of microorganisms reside in gut altering its homeostasis and leading to various diseases. The initial days of life are crucial for developing gut flora that helps in gut maturation and neonatal health. This review summarizes the evidence on seeding of neonatal gut microbiota, modulation of gut microbiota in neonatal sepsis, antimicrobial resistance, and role of probiotics or other therapies to re-establish altered microbiota.
Conclusion: Gut microbiota regulates host physiological homeostasis mediators, including the gut barrier function, and disease susceptibility pathways. Maintenance or restoration of microbiota and metabolite composition may be a therapeutic or preventative target against critical illness.

1. Introduction

Neonatal sepsis is a dysregulated host response to infection leading to life-threatening organ dysfunction.[1] The Global Burden of Disease (GBD) Study (2016–17) reported a global incidence of 1.3 million cases per year of neonatal sepsis with 203 000 sepsis-attributable deaths, which comes at the cost of $10 billion to $469 billion.[2] Neonates from low-income and middle-income countries (LMICs) are mostly affected due to the high burden of infectious disease, poor access to healthcare resources, maternal malnutrition etc.[3] Although it is challenging to quantify neonatal sepsis's long-term effects as clinical outcomes range from death due to sepsis to long-term disability with post-infectious hydrocephalus and neurodevelopmental impairment with cerebral palsy.[4]

The gut microbiota plays a protective role by acting as a barrier against harmful pathogens, a metabolic function by helping in the digestion and metabolism of breast milk, colostrum, and other formulas and a trophic role by helping in the growth and differentiation of the intestinal lumen epithelial cells, and the homeostatic maintenance of the immune system.[5] The gut flora of neonates is constantly altered by billions of microorganisms with the age of the neonates and has implications for various diseases. Numerous factors, such as gestation period, delivery time, mode of delivery, dietary patterns, weaning, antibiotic administration, etc., could perturb the gut flora.[6]

A preterm infant's immature intestine predisposes it to infection and inflammation, as it has immature immunity, barrier function, and peristalsis. One such disease that could arise from alteration in the gut microbiota is neonatal sepsis. Few studies indicate intestinal dysbiosis precedes neonatal sepsis development.[7,8] This review describes various aspects of gut colonization in neonates and the pathophysiology (modulation of gut microbiota due to sepsis). It also sheds light on the role of gut microbiota in antibiotic resistance development. This review emphasizes the other factors influencing microbiota composition, like breastfeeding patterns, mode of delivery, antibiotic treatment, and a potential source of novel therapeutics for modulating gut microbiota in neonates.

2. Seeding of neonatal gut microbiota

Seeding of microorganisms in the new-born gut through maternal transmission may have an impact on long-term health. Gut microbiota and its formation are affected by different factors, including the type of delivery, diet pattern, genetic factors, and intake of probiotics, prebiotics and antibiotics.[5] In immunocompromised premature neonates, microbial colonization patterns of the gut may form the basis for long-term disorders of immune modulation. In healthy full-term neonates, the gut system gets colonized by numerous microflora within ten days of life, whereas in the microflora in preterm neonates, it takes more

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than ten days.[6]

The host and gut microbiota have unique and cryptic interlinkage; the formation of an individual's gut microbiota starts right from birth and is shaped by various factors. At birth, microbes colonize the neonate's gut from the mother, who is a source of this inoculum. In adults, human microbiota or the community of bacteria is very stable, and there is not much change in the pattern of microbes. In newborns, the bacteria are essentially not present at the time of delivery; babies are colonized with these bacteria over weeks and months at the beginning of life. The kind of bacteria a baby gets exposed to depends on the mode of delivery Fig. 1. If the neonate is born by vaginal delivery, it gets a dose of microbes from the mother's vagina. If a baby is delivered by cesarean section, then microorganisms could potentially be transmitted from the skin surface of involved personnel and caregivers. Then gradually, numerous factors affect intestinal microbial colonization, such as the type of feeding (breast or formula), genetic predisposition, and possibly administration of pro, pre-and antibiotics.[4]

Several studies have been published from the genesis of the Human Microbiome Project (HMP), enumerating microbial seeding in term and preterm neonates. Although a huge number of data is generated, there are still numerous unanswered queries as there are no worldwide agreements on how microbiota affects human health.[9] Human bacterial colonization begins during fetal life,[10] in contrast to the previous theory of the "sterile womb", which suggests that the human fetal environment is sterile and microbial colonization begins at birth.[11] Several studies have detected microbial components in the placenta,[12,13] cord blood,[14] amniotic fluid[15] and even in uncomplicated pregnancies with healthy-term newborns.[11] Each has a unique microbiota, which is influenced by maternal health and habits and significantly impacts pregnancy and offspring outcomes.[16]

3. Modulation of gut microbiota in neonatal sepsis

Early life is crucial for the neonatal gut to promote gut maturation. Neonatal sepsis due to the translocation of intestinal bacteria is one of the main reasons for morbidity and mortality in neonates. Depleting gut microflora in neonates, especially in vulnerable preterm neonates, may elevate the risk of neonatal infections.[17] Few commensal gut microbes have the potential to be pathogenic. The commensal gut microbiota may translocate into the intestinal epithelium, reaching blood circulation. Enterobacteriaceae, Staphylococci, Enterococci, and Lactobacilli are some translocating bacteria.[10]

Bassetti et al. rightly narrated in their article that sepsis and the microbiome have an 'incompletely understood bi-directional relationship'.[18] Literature reiterates that a stable gut microbiome is fundamental to attaining immunity against pathogenic microorganisms invading the gut and other organs.[8,10] Any imbalance in the gut flora can cause dysbiosis, potentially increasing susceptibility to various infections, especially in neonates who are more vulnerable to infections. The microbiota in a neonate is in the weaning stage with reduced acid secretion and low protective mucous levels in the gut, particularly in premature and low birth weight infants.[19]

Gut microbes convert dietary nutrients such as vitamins, amino acids, or dietary fibre into metabolites, which affect the regulatory functions by activating biologically active molecules in the host. These include amino-acid-derived metabolites such as serotonin or gamma-aminobutyric acid (GABA), short-chain fatty acids (SCFAs), biogenic amines (such as histamine), and others. These products mentioned above may induce changes in microbial composition and may be active biologically during disease states.[20] S Graspeunter et al. (2019) reported a distinct intestinal microbiome pattern of gut dysbiosis preceding Late-Onset Sepsis (LOS), characterized by an accumulation of Bacilli and an absence of anaerobic bacteria. The researchers concluded that early microbiome and metabolic patterns could be a biomarker to prevent LOS in high-risk neonates.[21]

4. The emergence of antimicrobial resistance

Numerous studies have shown that antibiotic consumption causes dysbiosis and disturbance of the gut microbiome in infants, children, and adults.[22,23] Consequently, this may lead to various disorders such as diabetes, obesity, inflammatory bowel disease, asthma, rheumatoid arthritis, depression, autism, and infection, especially in immunocompromised patients.[24] Although antibiotics are targeted to kill only pathogens, they may adversely affect commensals by destroying them or inhibiting their activity. Antibiotic exposure in and around the delivery period is one of the causes of the remarkable depletion of the range and spectrum of intestinal microbiota, as it delays the onset of beneficial commensal microbes in neonates.[14] About 40% of expectant mothers are administered perinatal antibiotics.[25]

Antibiotics are associated with lower levels of commensal bacteria, delayed colonization with Bifidobacteria and Bacteroidetes, and an increase in potential pathogens.[26] However, after antibiotic use, probiotic treatments used to restore healthy microbiota even caused prolonged

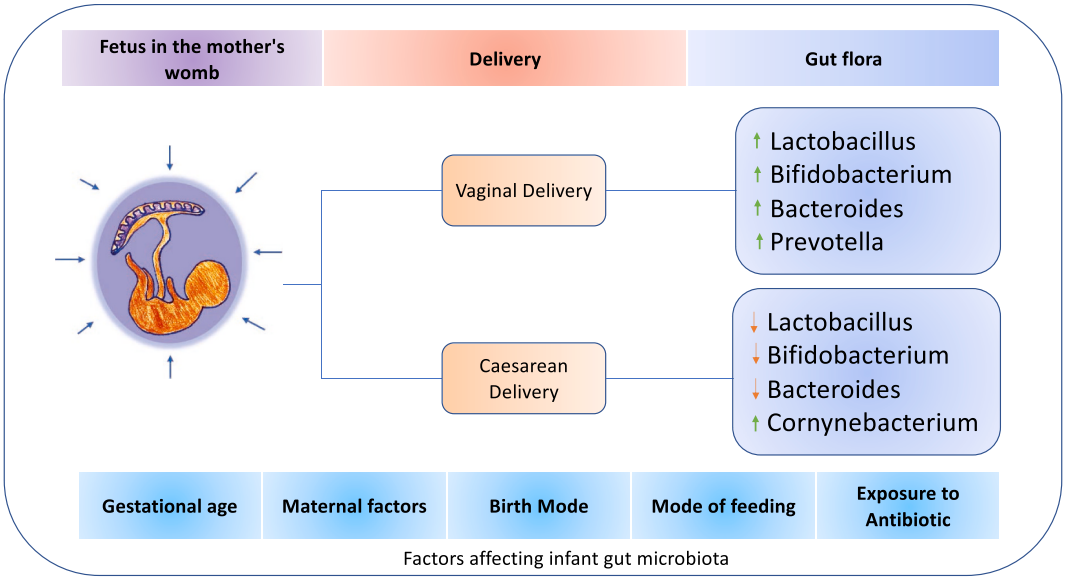

Fig. 1. Establishment of early life gut microbiota.

dysbiosis in healthy neonates.[27] In a systematic review by et al., Dierikx TH observed the effect of prepartum and intrapartum antimicrobial use on the new-born gut flora and stated that it significantly impacts infant gut colonization, leading to a decrease in diversity of the phyla Actinobacteria and Bacteroidetes and an increment in Proteobacteria. The increase in diversity was more significantly observed in vaginally delivered neonates and lasted throughout the first year of life.[28] According to Yassour et al. antibiotics significantly decreased the variety of strains, leading to a less stable microbiota. Additionally, bacteria have been developing genes for resistance to antibiotics resulting in the emergence of antibiotic resistance.[27]

5. Antibiotic resistance and neonatal infection

The rapid emergence of antibiotic resistance is a growing challenge in treating neonatal infections. Records suggest that 90% of neonates are resistant to first-line antibiotics in India, which is a matter of concern.[29] According to the Centre for Disease Dynamics, Economics and Policy, every year in India, approximately one million neonates die in the first four weeks of life, of which 190 000 deaths are caused by sepsis. Of these, 30% of sepsis deaths are due to antibiotic resistance. The primary cause of antibiotic resistance in neonates is the overuse of antibiotics during pregnancy.[30] 30% of the gut bacteria are affected by broad-spectrum antibiotics, which leads to a rapid decrease in taxonomic richness and diversity of good commensal gut bacteria contributing to the loss of colonization resistance.[30,31]

Antibiotic-resistance genes can be spread horizontally among bacteria through conjugation, transduction, and transformation. Karami et al. and their colleagues investigated the acquired ampicillin resistance by an *Escherichia coli* strain in neonates' gut. Their observations concluded a dynamic adaptation by gut commensal bacteria in response to antibiotic treatment. Their results also showed an unequivocal demonstration of gene transfer between two strains co-residing in the human gut.[32] Due to infection, there will be dysbiosis of the resident commensal bacteria causing mutation in susceptible genes and leading to antibiotic resistance. Moreover, antibiotic-resistance genes are more readily spread across bacterial species, leading to a quick spread of antibiotic resistance in other gut microbiota members.

6. Role of the gut microbiome in modulating antibiotic resistance

Low-abundance microorganisms found in the gut microbiome are often labelled as a reservoir. These are used for antibiotic-resistance genes. Due to antibiotics' overuse, particularly broad-spectrum antibiotics, microbes are repeatedly exposed to the antibiotic and become resistant to it. Casaburi (2019) et al., in their clinical trial, characterized the effect of an intervention with *B. infantis* EVC001 on the abundance of antibiotic resistance in breastfed infants. He concluded that there was a significant reduction in antibiotic resistance using probiotic *B. infantis* EVC001. The number of potentially pathogenic bacteria was also considerably reduced.[33] The ability for microbes to replicate and survive despite the presence of antibiotics confers resistance to them, thus leading to persistent infection despite antibiotic use. The vital role that the microbiota play in enhancing host immunity through multiple metabolic pathways is an unarguable fact.

7. Revamp: probiotics or other novel therapies can re-establish an altered microbiota in neonates

The intestinal microbial composition significantly impacts neonatal gut health from birth through the first stages of weaning.[33] It is crucial to understand how adding specific microbial species and prebiotic additives may restore the gut microbiota and ameliorate the risk of infection in neonates.

Prebiotic supplementation in neonates encourages the development and spread of probiotic bacteria in their gastrointestinal tract by enhancing intestinal motility and permeability and improving the integrity of the epithelial surface of the intestines. Prebiotics aid in the maturation of the intestinal mucosa and prevent pathogen overgrowth.[34] Lactoferrin supplementation is one prebiotic to prevent LOS in preterm infants. Lactoferrin is an iron-binding glycoprotein that plays an important role in the innate immune response of mammals.[35] Low to moderate quality evidence suggests that lactoferrin supplementation which reduces LOS in preterm infants were associated with a decreased rate of sepsis in neonates.[35–37] Faecal microbiota transplant is another emerging novel practice for restoring depleted gut flora.[38]

Probiotics are defined as live microorganisms administered in adequate amounts that confer a health benefit on the host and have been considered potential LOS prevention tools.[39] Probiotics help revive the disrupted gut microbiota and prevent inflammation and other intestinal diseases.[40] Researchers are looking forward to ways to rebuild the disrupted microbiota through a probiotic supplement.[41,42] Several systematic reviews conducted to determine the effects of single-strain and multi-strain probiotic formulations to prevent LOS have been listed in Table 1. A systematic review conducted by Balasubramanian et al. among preterm infants in India showed a significantly lower risk of blood culture-positive LOS with a risk ratio of 0.56 (95% CI: 0.45, 0.7) after 48 h of birth in the probiotic group.[31] Panigrahi et al. used *Lactobacillus Plantarum* with fructooligosaccharide as a probiotic in newborns in rural India. They observed a significant decrease in neonatal sepsis (risk ratio 0.60, 95% confidence interval 0.48–0.74). Their findings suggested that sepsis in newborns in developing nations could be substantially reduced using *L. Plantarum* ATCC-202195.[42]

Probiotics decrease the overgrowth of pathogenic bacteria in the gut of preterm neonates, thus reducing the frequency of nosocomial infections in the Neonatal Intensive Care Unit (NICU).[43] Probiotics may decrease the risk of sepsis through diverse mechanisms, such as the up-regulation of host anti-inflammatory genes, suppression of inflammation through the nuclear factor-B signalling pathway, relief from hypoxemic injury, production of short-chain fatty acids to lower intestinal pH, support intestinal epithelial cell function, elimination of pathogenic organisms and modifying host reaction to antimicrobial metabolites.[44]

8. Conclusion

Knowing the patterned progression of neonates' bacterial population, one can identify the bacteria missing in the disease condition and repair what's damaged by comparing them to a healthy preterm population with a very optimal health outcome. A relative reduction in the Bifidobacteriaceae counts and an increase in Enterobacteriaceae and Clostridiaceae may be a good criterion for defining dysbiosis in the initial months of life. Understanding the effects of sepsis in new-borns is crucial because disruptions to the microbiota during this critical developmental period may impact both immediate and long-term health outcomes. It can also explain gut colonization after birth, which can be the basis for introducing nutritional strategies targeted at the microbiota. These dietary interventions, mainly based on pre-and probiotics, may be used favourably to alter the gut microbiome in neonatal sepsis. Dysbiosis of the gut microbiome invites the expansion of the pathobiont population, which may lead to sepsis in neonates. The initial days of life are crucial for an adequate microbiome that facilitates gut maturation and neonatal health.

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Table 1
Published systematic reviews/metanalysis on probiotics for prevention of sepsis.

Author, Year, Reference	Inclusion criteria (Gestational age, Birthweight)	Number of infants	Number of trials	Sepsis (RR; 95% CI)	Conclusion
Chi et al., 2021[45]	<37 weeks, <2500 g	11351	38	0.77 (0.68, 0.88)	The results suggest the rate of sepsis may be reduced by combined use of any two of Lactobacillus, Bifidobacterium, and prebiotic.
Deshmukh and Patole et.al 2021[46]	<37 weeks	7976	9	0.67 (0.45, 1.00)	Probiotic supplement was not associated with a significant reduction in LOS
Aceti et al., 2017[47]	<37 weeks	5868	37	0.79 (0.71, 0.88)	Probiotic supplementation resulted in a significantly lower incidence of LOS
Hu et al., 2017[48]	<37 weeks, <2500 g	1371	7	0.64 (0.46–0.88)	Probiotic supplementation can reduce the risk of invasive fungal sepsis in preterm neonates in NICUs.
Rao et al., 2016[49]	<37 weeks, <2500 g	9416	37	0.86 (0.78, 0.94)	Probiotic supplementation results in statistically significant benefits in reducing LOS
Zhang et.al., 2016[43]	<37 weeks, <2500 g	6104	25	0.83 (0.73–0.94)	Probiotic supplementation is safe, and effective in reducing the risk of LOS in preterm neonates in neonatal intensive care units

LOS: Late onset of sepsis, NICU: Neonatal intensive care unit, RR: Risk ratio.

Declaration of competing interest

No conflicts of interest are involved with this review. This present review is an original work and has never been published elsewhere.

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