The Impact of Gut Microbial Diversity in Preterm Infant Infections

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Abstract: Certain factors bring about microbial preterm infections, including mode of delivery, socio-economic/geographic factors, gut microbial diversity etc. Consequent colonization of the gut by these microorganisms can have malevolent aftermaths. In the same vein, premature infections such as necrotizing entero colitis (NEC) and sepsis are among the crucial infections causing morbidity and mortality among preterm infants. This review is aimed at highlighting the impact of gut microbial diversity in premature infections. During this review, we employed the use of online published articles from peer review journals, google scholar and other accepted published conference proceedings. The use of chemoprophylaxis, empirical chemotherapeutic regimens and the administration of probiotics are the ways put forward to manage the infections. Gut microbial diversity is one of the prime factors in preterm infections and subsequent neonatal death, and as such is an important checkpoint in preventing preterm infections. Identification of this microbial diversity and the environmental needs of microbes is hence paramount.

Keywords: Gut, Diversity, Microbes, Premature and Infection

INTRODUCTION

An estimated 15 million babies are born preterm, and the survival gap between those born in high and low income countries is widening, with one million deaths a year due to direct complications of preterm birth, and around one million more where preterm birth is a risk factor, especially among those who are also growth restricted. Most premature babies (>80%) are between 32 and 37 weeks of gestation, and many die needlessly for lack of simple care (Ahumada *et al.*, 2016).

Care for every newborn comprises of support for immediate and exclusive breastfeeding, hygienic code and skin care. Rapid neonatal resuscitation is crucial in case of respiratory complications. Extra care, including feeding support, can halve mortality in babies weighing <2000 g. Case management of newborns with signs of infection, safe oxygen management and supportive care for those with significant jaundice are all critical, and are especially dependent on competent nursing care. For health systems in low and middle income settings with increasing facility births, district hospitals are the key frontiers for improving obstetric and neonatal care, and some large scale programmes now include specific newborn care strategies (Lawn *et al.,* 2013).

The established determinants of gut colonization by microorganisms include: delivery mode, perinatal antibiotics and infant diet. Weaning onto solid diet containing non-digestible carbohydrates and cessation of breastfeeding are key stages in the microbial colonization process. In addition, the microbiome of the placenta, amniotic fluid, and breast milk, alongside vaginal and faecal bacteria, may aid the transfer of maternal bacteria to the infant (Brown and Allen-Vorce, 2011).

Several hundred bacterial species and a total of 10^{14} cells colonize the human gastrointestinal (GI) tract in a mutualistic relationship with the host and its immune system (Round and Mazmanian, 2009). A healthy gut microbiota is stable and serves various useful functions such as metabolizing barely digestible polysaccharides, detoxifying toxic products, serving as a barrier against pathogens, and aiding in the development of the host immune system. However, recent studies highlight that dysbiosis, in which the symbiotic relationship between the host and gut microbiota is altered, is associated with various diseases (Claus *et al.,* 2016).

Knowledge of the bacteria in the human gut has increased substantially since the elucidation and elaboration of the microbiome - the collective genome of the gut microbiota, in different populations (De Filippo *et al.,* 2010; Suzuki and Woreby, 2014).

Invasive candidiasis in neonates has become an increasing problem over the past decade in Neonatal Intensive Care Units (NICUs). This results in significant morbidity and mortality of low-birth-weight infants. Premature neonates often have compromised skin integrity, gastrointestinal tract disease, chronic malnutrition, central venous catheters, long-term endotracheal intubation, and other factors that lead to increased risk of acquiring such infections.

Among the microbial infections indicted in preterm infants, infections with fungi (particularly candidal species) and with coagulase-negative *Staphylococci* (CoNS) are especially prevalent (Benjamin *et al.,* 2000). *Candida spp*. is the third most common pathogen of nosocomial acquired blood stream infections in premature infants, and they are associated with the second highest mortality rate. Despite antifungal treatment, mortality from all *Candida species* in premature infants has been consistently reported at 20% by large multicenter studies. Prematurity and low birth weight is strongly associated with the development of neonatal nosocomial bloodstream infections (Chapman *et al.,* 2003; Roilides *et al.,* 2004; Smiths *et al.,* 2005). The aim of this review is to highlight the impact of gut microbial diversity in premature infections.

Method

The review involves the systematic analysis of online published articles, Conference proceedings, related reviews among others were employed. The results of the analysis where summarized and presented.

Preterm Infants and Preterm Birth: Definitions and Associated Risk Factors

The World Health Organization (WHO) defined the term preterm infant as a neonate born after 20 weeks and before 37 weeks of gestation (WHO, 2010). Several factors are associated with premature birth but the aetiology is not fully known. The risk factors implicated with preterm birth include: high catecholamine levels in the maternal urine, anaemia, history of preterm birth, tobacco consumption, premature rupture of membrane, lack of prenatal care and preeclampsia (Ahumada-Barrios and Alvarado, 2016).

Infections Associated with Preterm Neonates

Neonates born at < 37 weeks gestation age are more susceptible to various infections due to their weak immune system (Goldenberg, 2009). Haematological cardiorespiratory, gastroenterologic infection, meningitis and neurological disorders are some of the diseases associated with preterm babies. The dysbiosis in the gut of infant is likely to be a frequent risk factor that contributes to premature infection. Sepsis, necrotizing enterocolitis (NEC) are the most seen infection in the preterm with a high morbidity and mortality rate. Bacteria and certain fungi, such as *Candida albicans*, are known to cause a variety of premature infections in newborns, such as sepsis, necrotizing enterocolitis, meningitis, etc (Abdulkadir, 2016).

Gut Microbiome

Gut microbiome refers to the collection of microorganisms including bacteria, archaea, viruses and fungi found within the gastrointestinal tract (GIT) together with their complete genetic material (Actis, 2014; Galland, 2014). It is estimated that the GIT comprises of 70% of most of the microbes found in the human body, making it complex environment with a large population of microorganisms (Brown and Allen-Vercoe 2011). GIT plays a significant role in health as well as affecting the physiological functions and psychological changes in our lives (Actis, 2014; Christian, 2015). A recent report demonstrated that the human gut colonisation may be initiated in-utero by distinct microbial communities already

present in the placenta and amniotic fluid (Collado *et al.,* 2016).

Colonisation and Composition of Gut Microbiomes

Before and subsequent to birth, a variety of both beneficial and pathogenic microorganisms colonize the gut of the infant. Bacteria, specifically the benevolent *Bifidobacteria* and *Lactobacilli*, and the potentially pathogenic microbes mainly Enterobacteriaceae members, *Escherichia, Enterococcus, Bacteriodes, Streptococcus,* and pathogenic microbes which include *Staphylococcus* and *Clostridia* are all reported as common gut inhabitants (Westerbeek, 2006). Many environmental factors influence the composition and colonisation of the GIT by microorganisms including: geography, medication and general life style. The gut microbial colonisation by vaginal and faecal bacteria starts during and immediately after delivery such that the early gut microbiome resembles that of the maternal microbiota (Rigon, 2012). It has been reported recently that the composition of infants gut microbiota begins to resemble that found in colostrum (Collado *et al.,* 2016).

In infants delivered by caesarean section, the microbiome is significantly influenced by maternal skin contact and from the environment (Nyangale *et al.,* 2012; Mshvildadze, 2010). In particular, some literature show that the colonisation of the preterm gut microbiome differs over time and between hospital environments which could be relevant to patient's outcomes (Taft, 2014). Interestingly, after weaning, the composition of the gut microbiota becomes almost identical to that of adults and remains relatively stable throughout life depending on other environmental factors (Thompson-Chagoyán *et al.,* 2007). Factors associated with premature delivery can affect the composition of gut microbiota (Stewart *et al.,* 2012). There is limited information related to the microbial communities and their subsequent evolution and dynamics from meconium during the early life of preterm infants (Moles, 2013). Facultative anaerobes during the first day(s) of life are the prevalent, due to absence of oxygen in the GIT; however obligate anaerobic bacteria isolates are observed (Ventura *et al.,* 2012).

Microbiome and Preterm Gut

The gut of an unborn child is regarded as sterile (Rigon. 2012) and recent researches show that colonization begins as soon as the unborn child swallows amniotic fluid containing microbes from the gut of the mother, this is evidenced by meconium samples that are not sterile but harbour diverse microbial communities. However, antibiotic treatment in pregnant mothers affects the colonisation of their infants gut microbiota.

Implication of Gut Microbiome in Health and Diseases

The Microbial community of the human gastrointestinal tract (GIT) plays a vital role in human health due to its significance in digestion, nutrition and maintenance of host physiology (Brown and Allen-Vercoe, 2011). Preterm gut microbiome has been reported to have short term health effects immediately after birth at NICU and long-term effects during post discharge (Stewart *et al.,* 2012). The gut microbiome has significant impacts on the health by stimulating the bacterial proteins to interact with human antigens to affect the responses of the adaptive immune system and production of neurotoxic metabolites (D-lactic acid and ammonia) by bacterial enzymes (Galland 2014; Cong *et al.,* 2015). GIT microbiota play a vital role in regulating adaptive immune functions, but its role against systemic viral infections is not clear. Additionally, the gut microbiome produce hormones that influence microbial growth and virulence (Galland, 2014), it also stimulate afferent neurones of the enteric nervous system to send signals to the brain through different mechanisms which help to shape the psychological behaviour of the host (sleep, stress, mood and cognition) (Cong *et al.,* 2015; Christian, 2015). On the other hand, the GI flora has also been implicated in the pathogenesis of disease

(Claud *et al.,* 2013; Magne*.,* 2005). Premature infants are particularly vulnerable to infections and neonatal sepsis due to the fact that, they have low immune system and yet fully matured organs as well as having small number of beneficial microbes (Singh *et al.,* 2015).

Factors Affecting the Preterm Gut Microbiome

i- Postnatal

Postnatally, many factors can influence intestinal bacterial colonization as well as the responses to colonization. Some studies have shown that mode of delivery (vaginal vs C-section), type of milk (human milk vs formula), and antibiotics influence the intestinal flora (Jernberg *et al.,* 2007). Those infants born via C-section, fed formula milk and exposed to antibiotics have a decrease in diversity of intestinal microbiota and abnormal patterns of colonization with suppression of healthy bacteria such as *Lactobacillus* and *Bifidobacteria*.

ii- C-Section vs. Vaginal Delivery

A review of recent epidemiologic data showing a relationship between increased C-section rate and risk for development of subsequent diseases was recently reviewed, showing relative association between high risk of infection and C-section deliveries. In association with "the trend of increasing C-section deliveries, there has been an epidemic of both autoimmune diseases such as type 1 diabetes, Crohn's disease, multiple sclerosis and allergic diseases, such as asthma, allergic rhinitis, and atopic dermatitis. The occurrence of these diseases is higher in more affluent, Western, industrialized countries". As Neu and Rushing (2011) reviewed, several previous studies have demonstrated differences in microbial colonization after C—section and vaginal deliveries. During vaginal delivery, the contact with the mother's vaginal and intestinal flora is an important source of the infant's colonization with a predominance of *Lactobacillus, Prevotella* and other *Bifidobacterium spp*.During cesarean delivery, direct contact of the mouth of the newborn with vaginal and intestinal microbiota is absent, and non-maternally derived environmental bacteria have a more important role in the infants intestinal colonization, which has a less diverse flora and a bacterial community similar to those found on the skin surface dominated by *Staphylococcus* and with a delayed intestinal colonization by *Lactobacillus, Bifidobacterium* and *Prevoltella* (Dominguez-Bello *et al.,* 2010).

iii- Human Milk vs Infant Formula

Studies in animals and premature infants have shown that human milk decreases the incidence of NEC. Beneficial factors of breast milk include immunoglobulins, cytokines, lactoferrin, lysozyme, growth factors, and Human milk oligosaccharides (HMO) (Hanson *et al.,* 2003). Human milk oligosaccharides (HMOs) contain a lactose core and act as prebiotics stimulating growth of *Bifidobacterium* species (Ward *et al.,* 2006). At birth a rapid colonization of intestinal flora occurs in the newborn with aerobic or facultative anaerobic bacteria such as *Enterobacteria, Enterococci* and *Staphylococc*i. These consume oxygen during growth thereby allowing the proliferation of anaerobic bacteria such as *Bacteroides, Bifidobacteria* and *Clostridia* (Bjorkstrom *et al.,* 2009). In formula fed infant, this transition does not occur and the newborn intestinal flora differs in its pattern of colonization with a predominance of Gram negative bacteria and fewer anaerobes (Penders *et al.,* 2006). If grouped by phyla breast-fed infants have a predominance of 'healthy' Firmicutes, mainly *Lactobacillus, Bacteroides* and *Actinobacteria (Bifidobacterium).* Formula fed infants have a predominance of Proteobacteria such as *Escherichia coli,* and *Firmicute*s some of which have pathogenic characteristics such as *Clostridia* and *Staphylococcus*. In one study of preterm infants it was speculated that the abnormal pattern of colonization at birth coupled with a delayed bloom of more pathogenic bacteria could potentially lead to the development of NEC (Abdulkadir, 2016).

Premature Infections

i- Necrotizing Enterocolitis (NEC)

Necrotizing Enterocolitis (NEC) is an enigmatic disease that has been recognized for over a century. With the advent of neonatal intensive care, it has become one of the most common and devastating diseases in neonates (Obladen, 2009). Complicating the literature is the fact that its causes are multifactorial and it is not a single disease (Young *et al.,* 2011). For example, when an infant born at term with a hypoplastic left ventricle presents with *pneumatosis intestinalis* at 2 days of age, the etiology and pathophysiology of this baby's "NEC" is likely different than the 25 weeks gestation preterm who presents with *Pneumatosis intestinalis* at 5 weeks of age. The first is more likely related to ischemic injury due to hemodynamic insufficiency and hypoxic–ischemic injury rather than a coalescence of factors that result in intestinal inflammation and injury related to intestinal immaturity, as the preterm infant. In the latter case, sometimes referred to as "classic" NEC, the interactions of a predisposing genetic background, an immature intestinal barrier and a microbial environment that is conducive to the development of NEC are thought to play an interactive and critical role in pathogenesis. Additional observations showing clusters of cases, outbreaks in institutions, the finding of *Pneumatosis intestinalis*, which likely represents submucosal gas produced by bacterial fermentation, and the common findings of bacteremia and endotoxinemia in affected neonates, supports a microbial role in the pathogenesis of this disease. Numerous bacteria have been related to NEC, but none of them have been found to fulfill Koch's postulates because they are commonly found among patients without NEC. Viruses have also been implicated in the pathogenesis of NEC and Coronavirus within fecal samples and resected intestinal segments were reported in patients with NEC, but their actual role in the causation of the disease has not been substantiated (Neu and Walker, 2011).

ii- Sepsis

Neonatal sepsis is a major cause of morbidity and mortality during the early days of preterm infant's life (Camacho-Gonzalez *et al.,* 2013). It usually occurs as a result of bacteremia. Sepsis can be associated with subsequent sequelae including prolonged ventilation and need for intravascular access, bronchopulmonary dysplasia, NEC, and an increased length of hospital stay (Satar and Özlü, 2012). Different microorganisms are responsible for the neonatal sepsis depending on the age at onset (Satar and Özlü, 2012). It can cause long-term complications to the new-born and premature infants during their stay at intensive care unit (Tappero and Johnson, 2010). The initial signs and symptoms are non-specific and can easily be confused with the other conditions from the infants (Tappero and Johnson, 2010). The prevalence of the disease seems to occur in smaller preterm infants (Russell, 2011), due to their immature organs and the compromised nature of their immune system. Almost 20% - 30% of all very-low-birth-weight (VLBW; <1500g) that have been hospitalised in NICU will suffer from sepsis at some stage; however, the risk increases up to 35% in preterm infants belonging to the extremely-low-birth-weight (ELBW; <1000g) category, and the figure reaches 50% in infants of less than 750g birth weight (Stoll *et al.,* 2004).

Types of Neonatal Sepsis

The neonatal sepsis can be classified in to two types depending on the onset of symptoms (Stefanovic, 2011), with different aetiological causes; these are: early on-set sepsis (EOS) and late on-set sepsis (LOS).

i- Early On-set Sepsis (EOS)

Early on-set sepsis (EOS) normally occur during the first 1-3 days of life (Vergnano and Heath, 2013). They are associated with very low birth weights of <1500g and the pathogens implicated are those that crossed the placenta (Zuhair, 2012). Among the pathogens are the Group B Streptococci and *Escherichia coli* (Hornik, 2012). Other

organisms have been reported to cause the sepsis such as *Klebsiella pneumonia*, coagulase negative *Staphylococcus*, *Pseudomonas spp.*, *Micrococcus spp.,* and *Alcaligenes faecalis,* depending on the region and environment. Transmission of the pathogens occurs mostly during labour and EOS infection is characterised by symptoms of respiratory disorder or fever during the early hours of life (Samuelsson *et al.,* 2014). Sometimes the symptoms are delayed more especially if the mother has been treated with antibiotics.

ii- Late On-set Sepsis (LOS)

Late onset sepsis (LOS) occurs between 48 hrs to 3 months after birth. These infections are most common in very low birth weight preterm infants, or preterm infants that have long stay at neonatal intensive care unit. Studies show that preterm infants are more susceptible than full term babies and the mortality rate is greatest in infections that occur soon after birth. Gram positive organisms are the implicated pathogens responsible for LOS (Hornik, 2012; Zuhair, 2012). The most prevalent pathogens include: coagulase negative *Staphylococci* and Enterobacteriaceae such as *Escherichia coli, Klebsiella pneumoniae* and *Acinetobacter baumannii*. Complications during birth, prolonged labour, ventilation, exposure to antibiotics and parenteral nutrition and others are the associated risk factors that aggravate the incidence (Hornik*,* 2012).

iii- Invasive Fungal Infections

Invasive fungal infections are usually caused by pathogenic fungi most commonly *Candida* species (Koh 2013; Kaufman, 2014). Some studies demonstrated that fungal infections accounts for almost 30% of infections in preterm infants with very low birth weight that result in mortality (Koh, 2013). The incidence of invasive fungal sepsis is rapidly increasing and becoming common among preterm babies receiving neonatal care. This is due to the ability of fungi to colonize the skin surface, mucosal membrane and vascular catheters associated with VLBW (Aydemir*.,* 2011). Preterm infants that are immunosuppressed, and or require invasive therapies, are exposed to parenteral feeding and broad spectrum antibiotics are vulnerable to fungal infection (Kaufman*,* 2014). In some healthcare systems, prophylactic antifungal and topical prophylaxis are routinely prescribed to reduce the risk of invasive fungal infections (Kaufman *,* 2014).

Clinical Diagnosis and Treatment of Necrotizing Enterocolitis (NEC)

NEC diagnosis is done by careful observations from clinicians to look for swelling, pain and tenderness as well as conducting abdominal X-ray, radiography and ultrasound for the symptoms of inflammation. Laboratory stool tests can be conducted to see the presence of blood (Ng *et al.,* 2015). Blood tests for measuring the white blood cells and platelets level can also be helpful in the diagnosis of NEC.

The treatment and prevention of NEC remains challenging, since the exact pathogenesis of the disease is yet to be confirmed (Lin and Stoll, 2006). NEC treatment depends on the following factors: the severity of the infection, the gestational age of infants and the general health condition of the baby. The treatment can include dietary interventions (antibiotics and probiotics), intravenous fluids or sometimes surgery in severe cases (Bozeman*,* 2013). Moreover, cessation or delaying in enteral feeds, gastric decomposition with intermittent suction and prompt antibiotic therapy can be effective in treating the NEC cases. Administering oral feeds with human milk and probiotics supplementation has also been reported to be effective in the prevention of NEC (Torrazza *et al.,* 2013). Furthermore, the maternal breast milk is one of the important key factor considered as a natural prevention of NEC and facilitating the healthy gut microbiome (Roger, 2010), this is because it composed of many immuno-protective and growth factors, prebiotics oligosaccharides, bioactive immune-modulatory cells and other 'immunonutrients' including amino acids, fatty acids, lysozyme,\ lactoferrin, minerals and metals such as zinc.

Table-1: Clinical Diagnosis and Treatment of Neonatal Sepsis

Diagnosis Appraches	Reference	Treatment Options	Reference
Review of suspected symptoms and patient history during the onset may give a diagnostic clue to the physician.	Stefanovic, (2011)	Since empirical antibiotics therapy remains an effective treatment for suspected cases of LOS; hence, antibiotic treatment for a minimum of 5 days is advised.	Dong and Speer, (2015)
Blood culture techniques	Satar and Özlü, (2012)	Antibiotics are usually prescribed to a preterm who show signs of infections and may help in reducing the incidence and severity of the infections.	Tappero and Johnson, (2010)
LOS is diagnosed by the manifestation of various clinical symptoms including hyperglycaemia, abnormal white blood count, feeding intolerance among others.	Samuelsson *et al.,* (2014)	Intrapartum antibiotic prophylaxis is very effective against Group B Streptococci; Sepsis can also be treated using antifungal prophylaxis for at least 5 days for the management of fungal mediated sepsis	Stefanovic, (2011)
		Oral lactoferrin has also been used in the prevention of neonatal sepsis	Venkatesh and Abrams, (2010)
		Administration of probiotics and the use of antibiotic is reported to play a role in the management of neonatal sepsis	Angelakis *et al.,* (2013)

CONCLUSION

Preterm infants are neonates born after >20 weeks but <37 weeks of normal gestation period. Those neonates are facing great challenges manifesting as various severe infections due to their weak immune system. Certain factors bring about microbial preterm infections, including mode of delivery, socio-economic/geographic factors, among others. Consequent colonization of the gut by these microorganisms can have malevolent aftermaths. In the same vein, premature infections such as NEC and sepsis are among the crucial infections causing morbidity and mortality among the preterm infants. The use of chemoprophylaxis, empirical chemotherapeutic regimens and the administration of probiotics are the ways put forward to manage the infections.

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