Gut Microbiota and Probiotics in Neonate

Anucha Thatrimontrichai, M.D.

Division of Neonatology, Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.
E-mail: tanucha@medicine.psu.ac.th

Abstract:

The collaboration and symbiotic relationship between microbiota and humans is complex. Development of the gut microbiota in infants is a dynamic process that is dependent on maternal–fetal–neonatal determinants. Vast collaborative enterprises of cooperative, co–dependent, and competitive ecologies are enormously powerful when merged together as one discrete entity or organ. This forgotten and invisible organ has both benefits and harm in health throughout the life cycle and across the next generations for the long term. Rapid advances in microbiology, genomic research and metagenomics analysis have uncovered the microbial contribution in both the hugeness and diversity of the microbial sphere. As a result, simultaneous findings have repercussions for microbial growth and health in the neonatal period and extend into childhood and adulthood. Pioneer microbiota in and on the neonate may find 'windows of opportunity' to promote health and prevent diseases. Oral probiotics is one choice to alter and balance the gut microbiota. However, the heterogeneity of research outcomes cannot be applied routinely in premature infants.

Keywords: microbiome, microbiota, necrotizing enterocolitis, newborn, premature infant, probiotics
Introduction

The human gut is an extremely complex ecosystem where microbiota (microorganisms that live inside and on humans or microbial taxa associated with humans), 1,2 nutrients, and host cells interact extensively. Early bacterial colonization gradually diversifies to make a symbiotic ecosystem by host–microbe interactions in the gut. 3 The microbiome (genomes of microbial symbionts or catalog of microbes and their genes) is composed of an enormous number of microbes (at least 100 trillion of bacteria, viruses, and fungi are in and on us), their genes, and their metabolites that function optimally in our gut, brain, respiratory, and immune systems. 4 Pioneer bacteria are an imperative determinant of both innate and adaptive immunity and may impact the development of disease across childhood and the entire adult life cycle. 5-7 If a homeostatic imbalance of healthy gut microflora and significant intestinal dysbiosis (disruption of the normal intestinal flora) 8 are able to be linked with inflammatory bowel disease and susceptibility to enteropathogens, 9 metabolic syndrome and obesity, 10 there may be a gradual alteration of brain function and behavior through neural, endocrine, and immune pathways. 11 In the early years of life, technological know–how of the microbiota may clarify the ‘windows of opportunity’ for strategies that may promote health and prevent diseases, especially through the use of probiotics (live microorganisms, which when consumed in adequate amounts, confer health to the host). 12 Organic therapy by probiotics is a new innovation to build a healthy environment and control enteropathogens in the intestine.

Maternal–fetal–neonatal microbiota

Pregnancy and birth, co-development and the origin of a new community, are the “passage from one set of symbiotic relationships to another”. 13 Both maternal and neonatal physiologies change in their ecological and reciprocal relationships. Crucially, the maternal microbiome results in various pregnancy outcomes and preterm birth. 14 Even though it has been long thought that the uterus, amniotic fluid, the fetus, and breast milk are sterile, this has been an incorrect hypothesis. Placenta and amniotic fluid sampling demonstrated the existence of bacteria from the maternal gut and oral microorganisms whose mechanism of transfer is unknown. It is postulated that the transmission occurs via the lymphatic system in the intestine 15 or the hematologic system secondary to gingival inflammation. 16 In addition, breast milk has hundreds of bacterial strains and varies over the duration of lactation 17,18 as well as some materials in the mother’s milk for the bacteria but not for the infant. 13 Among the many components of milk that modulate the infant gut microbiota are the milk glycans which are composed of free oligosaccharides, glycoproteins, and glycolipids which are the drivers of microbiota development, overall gut health, and also act as prebiotics (nondigestible oligosaccharides that stimulate the growth of healthy bacteria). 19,20 The development of the microbiome in the neonatal period and infancy should be considered as a developing “forgotten microbial organ”. The collective metabolic activity of the gut flora is equal to a “virtual organ within an organ” and there is a significant overlap with the maternal microbiome. For example, a high incidence of resistomes (antibiotic-resistant genes) 21 transferred from the maternal birth canal was discovered in the meconium and fecal samples of infants. 22 Also, non–pathogenic commensal microbiota has been demonstrated in in–utero colonization of the infant gut. 23,24 Most microbes were identified as members of the Enterobacteriaceae family along with lactic acid bacteria in the meconium. 25 Microbial dysbiosis from mode of delivery, maternal influence, and maternal or neonatal antibiotic use can be associated with obesity 26 and allergies 27,28 in children and adults.
Gut microbiota

The microbial profile of early infants is significantly dependent on a range of maternal factors, and consequently, as maturation of the neonatal gut microbiota develops. After birth, colonization by *Bifidobacterium*, *Lactobacillus*, and *Bacteroides species* was influenced by mode of delivery, type of feeding, and presence of siblings. Cesarean section delivery is less exposed to vaginal microbial transfer thus increasing the risk of celiac disease, type 1 diabetes, asthma, and obesity. Vaginal delivery promotes infant colonization by maternal vaginal and fecal bacteria (*Lactobacillus*, *Bacteroides*, *Fusobacteria*, and *Bifidobacteria*), whereas neonates born by Cesarean section have a greater number of microbes associated with the skin and hospital environment (*Staphylococcus*, *Corynebacterium*, *Propionibacterium*, *Enterococcus*, and *C. perfringens*). The highest incidence of beneficial microbiota in the gut, defined as the highest counts of *Bifidobacteria* and lowest numbers of *Clostridium difficile* and *Escherichia coli*, occurs among term neonates born vaginally and exclusively breastfed. Comparisons between breast- or formula-fed infants show larger populations of "*Bifidobacterium and Lactobacillus*" in those who were breast-fed and "*Atopobium, Clostridium difficile and Clostridium cocoides, Bacteroides vulgatus, Bifidobacterium longum subsp. longum, Enterococcus, Streptococcus, and Veillonella*" in those who were formula-fed. After neonates were treated with antibiotics, *Bifidobacteria* and *S. aureus* colonization decreased. On the other hand, *Enterococcus* and *Enterobacteriaceae* colonization increased. Preterm infants fed on mother’s own breastmilk had a higher diversity of gut microbiome and number of *Clostridium* and *Lactobacillus* than infants fed on non-mother’s breastmilk. Additionally, female preterm neonates were more likely to have a higher abundance of *Clostridium* and a lower abundance of *Enterobacteriaceae* than males during infancy. Finally, a typical adult gut microbiome does not occur until 3 years of age (range 1–4 years).

Gut microbial dysbiosis may result in necrotizing enterocolitis (NEC), although no specific microbe has been identified as a causal factor in NEC. Preterm infants with NEC revealed a low diversity of specific gut microbial species compared to full-term controls. In addition, a relative intenseness of *Gamma-proteobacteria* (Gram-negative facultative bacilli, for examples, *Escherichia, Shigella, Salmonella, Haemophilus, Vibrio, Pseudomonas*) and relative paucity of strict anaerobic bacteria (especially *Negativicutes or Firmicutes*) occurs in very low birthweight (VLBW) infants with NEC.

Recent knowledge of the gut–brain axis indicates a role for the microbiota regulating development and social behaviors. Microbes in the placenta of the preterm predicts white matter damage and later cerebral palsy. The microbiota–gut–brain interrelationship has been associated with neonatal sepsis and NEC with long-term psychomotor morbidity as well as altered nervous development and behavior, and impacts cognitive function, stress management, neurodevelopmental disorders, social impairment, and diverse neurodevelopmental patterns, such as autism spectrum disorders.

Equilibrium of the intricate ecological microbiota in the respiratory system, similar to the gut microbiota, must be maintained to resist bacterial overgrowth and respiratory infections later. Microbial dysbiosis in the neonatal lungs has shown an association with the incidence of broncho-pulmonary dysplasia. The gastrointestinal microbiota has a regulatory influence on the immunology of the lung. Gut microbes are digested by dendritic cells either by translocation through M cells or from the lumen to the gut-associated lymphoid tissue (GALT). Dendritic cells enhance activated T cells within the mesenteric lymph nodes (MLN).
and make plentiful regulatory cytokines. Activated cells of the GALT and MLN in the gut lead the respiratory mucosa to promote protective and anti-inflammatory reactions. The production of various bacterial metabolites also affects the gut-lung axis and can alter the levels of inflammation. Factors affecting neonatal airway and lung bacterial colonization include antibiotic use, delivery mode, chorioamnionitis, bowel or nasopharyngeal colonization, and feeding method. A reduced diversity of the lung microbiome may develop bronchopulmonary dysplasia (similar to gut microbiome with NEC) in the preterm neonate by immune responses, mediators, and cytokines.

Very preterm or VLBW infants developed a sparse microbiome compared to full-term infants. Exposure to many risk factors as prenatal (prenatal maternal illness, infections such as bacterial vaginosis and chorioamnionitis, smoking, and physiological stress), antenatal (rapid vaginal or Cesarean section deliveries reducing exposure to maternal vaginal and enteric microbiota), and postnatal (formula feeding, invasive procedures, antibiotics, medications that alter gastric pH, and hospital environment) insults may shape the gut microbiota in the preterm infant. Due to prolonged hospitalization, VLBW infants may develop neonatal intensive care unit flora that may be antibiotic resistant microbes. Gut microbiota is a large reservoir for resistomes by two important mechanisms: (i) conjugative transfer of plasmids and transposons and (ii) transduction. Evaluating suspected sepsis in preterm infants is difficult due to non-specific signs. Broad-spectrum empirical antibiotic therapy must start as soon as possible after taking blood and/or cerebrospinal fluid or urine for culture. Following the evolution of bacteria, only resistant pathogens will survive. Therefore, since the development of bacterial resistance is directly associated with antibiotic selection pressure, multidrug resistance is troublesome in neonatal sepsis and pneumonia.

**Probiotics**

Strategies to prevent NEC and make a balance in the intestinal microbiota include probiotics, prebiotics, synbiotics (a combination of probiotics and prebiotics to enhance beneficial health effects in the intestinal tract more than either agent administered alone), lactoferrin, and human milk feeding. From bottles to diapers, synbiotics act as functional foods to mediate immune responses, modulate the number of gut microbial, and reduce the incidence and severity of NEC. Probiotics can change the gut flora leading to the development of flora metabolites that can affect intestinal health through 1 of 3 mechanisms: (i) direct antimicrobial effects, (ii) promotion of mucosal barrier integrity, and (iii) immune modulation. The etiology of NEC remains unclear; however, preterm, hyperosmolar diet, and intestinal microbiota dysbiosis may be the risk factors. Evidence-based medicine, probiotics, and synbiotics can decrease NEC but not prebiotics alone. Previous meta-analyses concluded that probiotics had a protective effect in reducing the incidence of “NEC stage II or greater”, “all-cause mortality”, and “late-onset sepsis” in preterm infants. Strain-specific sub-meta-analyses showed a significant effect of *Bifidobacteria* and probiotic mixtures on NEC. However, recent and large randomized clinical trials found no difference in the incidence of NEC, sepsis, growth, or death in preterm infants who were given probiotics (*Bifidobacterium breve* or *Bifidobacterium lactis* and/or *Bifidobacterium longum*) compared to placebo. Besides the prevention of NEC, preterm infants had fewer fussing and crying episodes among the prebiotic and probiotic groups than in the placebo group (19% vs 19% vs 47%, respectively; p-value=0.020), whereas the frequency of stools (>3 stools/day) tended to be higher in the prebiotic than in the probiotic and placebo groups.
Prebiotics are nondigestible oligosaccharides, such as fructooligosaccharides, galactooligosaccharides, lactulose, and inulin, which have the potential to stimulate growth in beneficial gut microbiota, particularly *Lactobacillus* and *Bifidobacteria*. Because of their composition, prebiotics cannot be adsorbed until they reach the colon, where they can be fermented by a specific microbe into short-chain fatty acids and lactate. From a meta-analysis, prebiotic supplementation alone did not result in a decreased incidence of NEC, late onset sepsis or time to full enteral feeds but resulted in a significantly higher growth of probiotics.

Lactoferrin is an iron–binding glycoprotein and is present in most biological fluids. Colostrum and mammalian milk have especially high levels of lactoferrin. Lactoferrin can promote host defense with antimicrobial, immunostimulatory, and immunomodulatory abilities. More than 70% of bovine lactoferrin is homologous with human lactoferrin. Fecal levels of probiotics (*Lactobacillus* and *Bifidobacteria*) correlated directly with the concentration of fecal lactoferrin at birth in preterm infants. From a meta-analysis, oral lactoferrin supplementation decreased “NEC stage II or greater” by 5% [505 participants, risk ratio (RR) 0.30, number needed to treat (NNT) 20], “late–onset sepsis” by 9% (678 participants, RR 0.49, NNT 11), and “all–cause mortality” by 5% (505 participants, RR 0.30, NNT 20). Oral lactoferrin with probiotic supplementation decreased “NEC stage II or greater” by 5% (496 participants, RR 0.04, NNT 20) and “late–onset sepsis” by 13% (321 participants, RR 0.27, NNT 8) but not “all–cause mortality”. However, the previous studies had moderate to low-quality evidence and limited information on adverse effects and long-term neurological outcomes.

From a meta-analysis, donor human milk can reduce the incidence of NEC compared with formula milk in preterm infants. Human milk stimulates the growth of a well–balanced and diverse microbiota environment. A switch is then made from the predominant intrauterine T helper 2 (TH2) environment to a TH1/TH2 balanced reaction. Breast milk–stimulated specific organisms (*Bifidobacteria, Lactobacillus*, and *Bacteroides*) also activate T–regulatory cells after birth. Prebiotics are fermented by colonic probiotics that produce an acid for bacterial proliferation and growth. In addition, short–chain fatty acids in breast milk activate receptors on T–regulatory cells and microbiome which preferentially mediate intestinal tight junction expression and anti–inflammation.

Apart from probiotics, prebiotics, and lactoferrin in breast milk, they also prevent immune–mediated diseases (asthma, inflammatory bowel disease, type 1 diabetes) later in life through a balanced initial immune response.

**Conclusion**

The early microbe–host (microbiota–gut–brain–lung–immune) interrelationship is a crucial component not only for microbial and metabolic programming, but also in human health and disease as well as preterm infants. From the meta–analyses, probiotics can decrease NEC; however, the recent randomized clinical trials showed no significant difference in the use of probiotic supplementation to reduce NEC rates. We should refrain from pooling data on different probiotics to avoid misleading patients and healthcare professionals before routine application in premature care.

**References**


