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Gut Microbiota and Probiotics in Neonate

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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

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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 microbiomes 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).[12,19] 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)[20] transferred from the maternal birth canal was discovered in the meconium and fecal samples of infants.[21] Also, nonpathogenic commensal microbiota has been demonstrated in in–utero colonization of the infant gut.[7,22,23] Most microbes were identified as members of the *Enterobacteriaceae* family along with lactic acid bacteria in the meconium.[7,24] Microbial dysbiosis from mode of delivery, maternal influence, and maternal or neonatal antibiotic use can be associated with obesity[25,26] 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.[23] Cesarean section delivery is less exposed to vaginal microbial transfer thus increasing the risk of celiac disease, type 1 diabetes, asthma, and obesity.[26] 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*).[7,23,29] 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.[30] Comparisons between breast- or formula-fed infants show larger populations of "*Bifidobacterium* and *Lactobacillus*" in those who were breast-fed[23,31] and "*Atopobium*, *Clostridium difficile* and *Clostridium coccoides*, *Bacteroides vulgatus*, *Bifidobacterium longum subsp. longum*, *Enterococcus*, *Streptococcus*, and *Veillonella*" in those who were formula-fed.[7,23,31] After neonates were treated with antibiotics, *Bifidobacteria* and *S. aureus* colonization decreased.[32-34] On the other hand, *Enterococcus* and *Enterobacteriaceae* colonization increased.[33] 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.[35] Additionally, female preterm neonates were more likely to have a higher abundance of *Clostridium* and a lower abundance of *Enterobacteriaceae* than males during infancy.[35] Finally, a typical adult gut microbiome does not occur until 3 years of age (range 1-4 years).[3,36,37]

Gut microbial dysbiosis may result in necrotizing enterocolitis (NEC)[12,38,39] 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.[40] 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.[41,42]

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.[43] 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.[44-47]

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.[48] Microbial dysbiosis in the neonatal lungs has shown an association with the incidence of bronchopulmonary dysplasia. The gastrointestinal microbiota has a regulatory influence on the immunology of the lung.[49,50] 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.[50] 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.[50-52]

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.[20] Due to prolonged hospitalization, VLBW infants may develop neonatal intensive care unit flora[53] 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.[20,54,55] Evaluating suspected sepsis in preterm infants is difficult due to nonspecific 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.[56-59]

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),[12] lactoferrin, and human milk feeding.[12,38] 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.[12,60] 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.[61] The etiology of NEC remains unclear; however, preterm, hyperosmolar diet, and intestinal microbiota dysbiosis may be the risk factors.[8,62,63] Evidence-based medicine, probiotics, and synbiotics can decrease NEC but not prebiotics alone.[64] Previous meta-analyses concluded that probiotics had a protective effect in reducing the incidence of "NEC stage II or greater", "all-cause mortality",[65-68] and "late-onset sepsis"[69] in preterm infants. Strain-specific sub-meta-analyses showed a significant effect of *Bifidobacteria* and probiotic mixtures on NEC.[66] 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*[70] or *Bifidobacterium lactis* and/or *Bifidobacterium longum*[71]) 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.[72]

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.[73]

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.[74] 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".[75] 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.[76] 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.[61,77] 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.[77]

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 health-care professionals before routine application in premature care.

References

1. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007; 449: 804 - 10.
2. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. Nutr Rev 2012; 70 (Suppl 1): S38 - 44.
3. Wopereis H, Oozeer R, Knipping K, Belzer C, Knol J. The first

thousand days – intestinal microbiology of early life: establishing a symbiosis. Pediatr Allergy Immunol 2014; 25: 428 – 38.
4. Miller WB Jr. The eukaryotic microbiome: origins and implications for fetal and neonatal life. Front Pediatr 2016; 4: 96.
5. Hand TW, Vujkovic-Cvijin I, Ridaura VK, Belkaid Y. Linking the microbiota, chronic disease, and the immune system. Trends Endocrinol Metab 2016; 27: 831 – 43.
6. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science 2012; 336: 1268 – 73.
7. Koleva PT, Kim JS, Scott JA, Kozyrskyj AL. Microbial programming of health and disease starts during fetal life. Birth Defects Res C Embryo Today 2015; 105: 265 – 77.
8. Elgin TG, Kern SL, McElroy SJ. Development of the neonatal intestinal microbiome and its association with necrotizing enterocolitis. Clin Ther 2016; 38: 706 – 15.
9. Chassaing B, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory owel diseases. Gastroenterology 2011; 140: 1720 – 28.
10. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. J Clin Invest 2011; 121: 2126 – 32.
11. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A 2011; 108: 3047 – 52.
12. Johnson-Henry KC, Abrahamsson TR, Wu RY, Sherman PM. Probiotics, prebiotics, and synbiotics for the prevention of necrotizing enterocolitis. Adv Nutr 2016; 7: 928 – 37.
13. Gilbert SF. A holobiont birth narrative: the epigenetic transmission of the human microbiome. Front Genet 2014; 5: 282.
14. Fox C, Eichelberger K. Maternal microbiome and pregnancy outcomes. Fertil Steril 2015; 104: 1358 – 63.
15. Donnet-Hughes A, Perez PF, Dore J, Leclerc M, Levenez F, Benyacoub J, et al. Potential role of the intestinal microbiota of the mother in neonatal immune education. Proc Nutr Soc 2010; 69: 407 – 15.
16. Leon R, Silva N, Ovalle A, Chaparro A, Ahumada A, Gajardo M, et al. Detection of Porphyromonas gingivalis in the amniotic fluid in pregnant women with a diagnosis of threatened premature labor. J Periodontol 2007; 78: 1249 – 55.
17. Hunt KM, Foster JA, Forney LJ, Schutte UM, Beck DL, Abdo Z, et al. Characterization of the diversity and temporal stability of bacterial communities in human milk. PLoS One 2011; 6: e21313.
18. Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. Am J Clin Nutr 2012; 96: 544 – 51.
19. Pacheco AR, Barile D, Underwood MA, Mills DA. The impact of the milk glycobiome on the neonate gut microbiota. Annu Rev Anim Biosci 2015; 3: 419 – 45.
20. Groer MW, Luciano AA, Dishaw LJ, Ashmeade TL, Miller E, Gilbert JA. Development of the preterm infant gut microbiome: a research priority. Microbiome 2014; 2: 38.
21. Gosalbes MJ, Valles Y, Jimenez-Hernandez N, Balle C, Riva P, Miravet-Verde S, et al. High frequencies of antibiotic resistance genes in infants' meconium and early fecal samples. J Dev Orig Health Dis 2016; 7: 35 – 44.
22. Funkhouser LJ, Bordenstein SR. Mom knows best: the universality of maternal microbial transmission. PLoS Biol 2013; 11: e1001631.
23. Martin R, Makino H, Cetinyurek Yavuz A, Ben-Amor K, Roelofs M, Ishikawa E, et al. Early-life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. PLoS One 2016; 11: e0158498.
24. Jimenez E, Marin ML, Martin R, Odriozola JM, Olivares M, Xaus J, et al. Is meconium from healthy newborns actually sterile? Res Microbiol 2008; 159: 187 – 93.
25. Thompson AL. Developmental origins of obesity: early feeding environments, infant growth, and the intestinal microbiome. Am J Hum Biol 2012; 24: 350 – 60.
26. Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG. The infant microbiome development: mom matters. Trends Mol Med 2015; 21: 109 – 17.
27. Murk W, Risnes KR, Bracken MB. Prenatal or early-life exposure to antibiotics and risk of childhood asthma: a systematic review. Pediatrics 2011; 127: 1125 – 38.
28. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, et al. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. EMBO Rep 2012; 13: 440 – 7.
29. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, 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: 11971 – 5.

30. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics 2006; 118: 511 – 21.
31. Fallani M, Young D, Scott J, Norin E, Amarri S, Adam R, et al. Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics. J Pediatr Gastroenterol Nutr 2010; 51: 77 – 84.
32. Fouhy F, Guinane CM, Hussey S, Wall R, Ryan CA, Dempsey EM, et al. High-throughput sequencing reveals the incomplete, hort-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. Antimicrob Agents Chemother 2012; 56: 5811 – 20.
33. Tanaka S, Kobayashi T, Songjinda P, Tateyama A, Tsubouchi M, Kiyohara C, et al. Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. FEMS Immunol Med Microbiol 2009; 56: 80 – 7.
34. Lindberg E, Adlerberth I, Matricardi P, Bonanno C, Tripodi S, Panetta V, et al. Effect of lifestyle factors on *Staphylococcus aureus* gut colonization in Swedish and Italian infants. Clin Microbiol Infect 2011; 17: 1209 – 15.
35. Cong X, Xu W, Janton S, Henderson WA, Matson A, McGrath JM, et al. Gut microbiome developmental patterns in early life of preterm infants: impacts of feeding and gender. PLoS One 2016; 11: e0152751.
36. Ringel-Kulka T, Cheng J, Ringel Y, Salojarvi J, Carroll I, Palva A, et al. Intestinal microbiota in healthy U.S. young children and adults--a high throughput microarray analysis. PLoS One 2013; 8: e64315.
37. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature 2012; 486: 222 – 7.
38. Vongbhavit K, Underwood MA. Prevention of necrotizing enterocolitis through manipulation of the intestinal microbiota of the premature infant. Clin Ther 2016; 38: 716 – 32.
39. Heida FH, van Zoonen AG, Hulscher JB, te Kiefte BJ, Wessels R, Kooi EM, et al. A necrotizing enterocolitis-associated gut microbiota is present in the meconium: results of a prospective study. Clin Infect Dis 2016; 62: 863 – 70.
40. Torrazza RM, Neu J. The altered gut microbiome and necrotizing enterocolitis. Clin Perinatol 2013; 40: 93 – 108.
41. Mai V, Young CM, Ukhanova M, Wang X, Sun Y, Casella G, et al. Fecal microbiota in premature infants prior to necrotizing enterocolitis. PLoS One 2011; 6: e20647.
42. Warner BB, Deych E, Zhou Y, Hall-Moore C, Weinstock GM, Sodergren E, et al. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. Lancet 2016; 387: 1928 – 36.
43. Leviton A, Allred EN, Kuban KC, Hecht JL, Onderdonk AB, O'Shea TM, et al. Microbiologic and histologic characteristics of the extremely preterm infant's placenta predict white matter damage and later cerebral palsy. the ELGAN study. Pediatr Res 2010; 67: 95 – 101.
44. O' Mahony SM, Stilling RM, Dinan TG, Cryan JF. The microbiome and childhood diseases: focus on brain-gut axis. Birth Defects Res C Embryo Today 2015; 105: 296 – 313.
45. Dinan TG, Stilling RM, Stanton C, Cryan JF. Collective unconscious: how gut microbes shape human behavior. J Psychiatr Res 2015; 63: 1 – 9.
46. Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. Microbiota is essential for social development in the mouse. Mol Psychiatry 2014; 19: 146 – 8.
47. Sherman MP, Zaghouani H, Niklas V. Gut microbiota, the immune system, and diet influence the neonatal gut-brain axis. Pediatr Res 2015; 77: 127 – 35.
48. de Steenhuijsen Piters WA, Sanders EA, Bogaert D. The role of the local microbial ecosystem in respiratory health and disease. Philos Trans R Soc Lond B Biol Sci 2015; 370.
49. Lal CV, Ambalavanan N. Cellular and humoral biomarkers of bronchopulmonary dysplasia. Early Hum Dev 2017; 105: 35 – 9.
50. Gallacher DJ, Kotecha S. Respiratory microbiome of new-born infants. Front Pediatr 2016; 4: 10.
51. Imamura T, Sato M, Go H, Ogasawara K, Kanai Y, Maeda H, et al. The microbiome of the lower respiratory tract in premature infants with and without severe bronchopulmonary dysplasia. Am J Perinatol 2017; 34: 80 – 7.
52. Lohmann P, Luna RA, Hollister EB, Devaraj S, Mistretta TA, Welty SE, et al. The airway microbiome of intubated premature infants: characteristics and changes that predict the development of bronchopulmonary dysplasia. Pediatr Res 2014; 76: 294 – 301.
53. Patel AL, Mutlu EA, Sun Y, Koenig L, Green S, Jakubowicz A, et al. Longitudinal survey of microbiota in hospitalized preterm very-low-birth-weight infants. J Pediatr Gastroenterol Nutr 2016; 62: 292 – 303.
54. van Schaik W. The human gut resistome. Philos Trans R Soc Lond B Biol Sci 2015; 370: 20140087.

55. Gibson MK, Crofts TS, Dantas G. Antibiotics and the developing infant gut microbiota and resistome. Curr Opin Microbiol 2015; 27: 51 – 6.
56. Thatrimontrichai A, Rujeerapaiboon N, Janjindamai W, Dissaneevate S, Maneenil G, Kritsaneepaiboon S, et al. Outcomes and risk factors of ventilator-associated pneumonia in neonates. World J Pediatr 2017. Doi: 10.1007/s12519-017-0010-0
57. Thatrimontrichai A, Apisarnthanarak A, Chanvitan P, Janjindamai W, Dissaneevate S, Maneenil G. Risk factors and outcomes of carbapenem-resistant *Acinetobacter baumannii* bacteremia in neonatal intensive care unit: a case-case-control study. Pediatr Infect Dis J 2013; 32: 140 – 5.
58. Thatrimontrichai A, Chanvitan P, Janjindamai W, Dissaneevate S, Jefferies A, Shah V. Trends in neonatal sepsis in a neonatal intensive care unit in Thailand before and after construction of a new facility. Asian Biomed 2014; 8: 771 – 8.
59. Thatrimontrichai A, Techato C, Dissaneevate S, Janjindamai W, Maneenil G, Kritsaneepaiboon S, et al. Risk factors and outcomes of carbapenem-resistant *Acinetobacter baumannii* ventilator-associated pneumonia in the neonate: a case-case-control study. J Infect Chemother 2016; 22: 444 – 9.
60. Khow-ean U. Probiotics. Songkla Med J 2006; 24: 315 – 23.
61. Patel R, DuPont HL. New approaches for bacteriotherapy: prebiotics, new-generation probiotics, and synbiotics. Clin Infect Dis 2015; 60 (Suppl 2): S108 – 21.
62. Thatrimontrichai A, Janjindamai W. Safety of superfortification of human milk for preterm. Asian Biomed 2011; 5: 825 – 30.
63. Thatrimontrichai A, Janjindamai W. Postprandial osmolality of gastric contents in very low-birth-weight infants fed expressed breast milk with additives. Southeast Asian J Trop Med Public Health 2009; 40: 1080 – 6.
64. Dilli D, Aydin B, Fettah ND, Ozyazici E, Beken S, Zenciroglu A, et al. The ProPre-Save study: effects of probiotics and prebiotics alone or combined on necrotizing enterocolitis in very ow birth weight infants. J Pediatr 2015; 166: 545 – 51.
65. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 2014: CD005496.
66. Aceti A, Gori D, Barone G, Callegari ML, Di Mauro A, Fantini MP, et al. Probiotics for prevention of necrotizing enterocolitis in preterm infants: systematic review and meta-analysis. Ital J Pediatr 2015; 41: 89.
67. Lau CS, Chamberlain RS. Probiotic administration can prevent necrotizing enterocolitis in preterm infants: a meta-analysis. J Pediatr Surg 2015; 50: 1405 – 12.
68. Olsen R, Greisen G, Schroder M, Brok J. Prophylactic probiotics for preterm infants: a systematic review and meta-analysis of observational studies. Neonatology 2016; 109: 105 – 12.
69. Rao SC, Athalye-Jape GK, Deshpande GC, Simmer KN, Patole SK. Probiotic supplementation and late-onset sepsis in preterm infants: a meta-analysis. Pediatrics 2016; 137: e20153684.
70. Costeloe K, Hardy P, Juszczak E, Wilks M, Millar MR, Probiotics in Preterm Infants Study Collaborative Group. Bifidobacterium breve BBG-001 in very preterm infants: a randomised controlled phase 3 trial. Lancet 2016; 387: 649 – 60.
71. Hays S, Jacquot A, Gauthier H, Kempf C, Beissel A, Pidoux O, et al. Probiotics and growth in preterm infants: a randomized controlled trial, PREMAPRO study. Clin Nutr 2016; 35: 802 – 11.
72. Partty A, Luoto R, Kalliomaki M, Salminen S, Isolauri E. Effects of early prebiotic and probiotic supplementation on development of gut microbiota and fussing and crying in preterm infants: a randomized, double-blind, placebo-controlled trial. J Pediatr 2013; 163: 1272 – 7.
73. Srinivasjois R, Rao S, Patole S. Prebiotic supplementation in preterm neonates: updated systematic review and meta-analysis of randomised controlled trials. Clin Nutr 2013; 32: 958 – 65.
74. Mastromarino P, Capobianco D, Campagna G, Laforgia N, Drimaco P, Dileone A, et al. Correlation between lactoferrin and beneficial microbiota in breast milk and infant's feces. Biometals 2014; 27: 1077 – 86.
75. Pammi M, Abrams SA. Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 2015: CD007137.
76. Quigley M, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev 2014; 4: CD002971.
77. Walker WA, Iyengar RS. Breast milk, microbiota, and intestinal immune homeostasis. Pediatr Res 2015; 77: 220 – 8.