A Review of the Source and Function of Microbiota in Breast Milk

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Abstract

Breast milk contains a rich microbiota composed of viable skin and non-skin bacteria. The extent of the breast milk microbiota diversity has been revealed through new culture-independent studies using microbial DNA signatures. However, the extent to which the breast milk microbiota are transferred from mother to infant and the function of these breast milk microbiota for the infant are only partially understood. Here, we appraise hypotheses regarding the formation of breast milk microbiota, including retrograde infant-to-mother transfer and enteromammary trafficking, and we review current knowledge of mechanisms determining the extent of breast milk microbiota transfer from mother to infant. We highlight known functions of constituents in the breast milk microbiota—to enhance immunity, liberate nutrients, synergize with breast milk oligosaccharides to enhance intestinal barrier function, and strengthen a functional gut–brain axis. We also consider the pathophysiology of maternal mastitis with respect to a dysbiosis or abnormal shift in the breast milk microbiota. In conclusion, through a complex, highly evolved process in the early stages of discovery, mothers transfer the breast milk microbiota to their infants to impact infant growth and development.

Keywords

- ▶ enteromammary trafficking
- ▶ immune function
- ▶ mastitis

Breast milk provides optimal nutrition for infants and reduces their risk of infectious diseases. In addition, breast milk is a vehicle for transmission of bacteria and viruses from mother to infant. However, the factors dictating the composition of the breast milk microbiota and the function of the breast milk microbiota for the mammary organ and infant remain unclear. Breast milk typically contains both skin microbiota and what are typically considered enteric organisms. Proposed theories for the microbiota composition of breast milk include retrograde flow from the infant's oral cavity, transfer of organisms from maternal skin, and movement of microbiota from the maternal enteric tract to the mammary gland. Here, we review mechanisms for transfer of microbiota to breast milk, their potential function for infants, as well as dysbiosis of the breast milk microbiome related to maternal disease.

Until recently, our knowledge of the ecology of human-associated microbes was based primarily on bacterial culture. However, culture-based methods may be limited by our ability to optimize growth conditions. As such, fastidious and low-abundance organisms may not be identified using culture methods. As many as 60% of organisms detected using molecular techniques will not grow in standard bacterial culture media.^{1,2} While detection using molecular techniques has broadened our understanding of microbiota, many sequences cannot be classified, suggesting an expansive microbial world.

Within the human body, microbial communities assemble that are specific to location but not isolated from one another.³ For example, skin-associated microbiota are rich in gram-positive organisms such as *Staphylococcus* spp. and

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Propionibacterium spp.^{4,5} In comparison, the adult enteric microbiota are more diverse and generally dominated by anaerobic organisms such as *Bacteroides* and *Prevotella*.⁶ Using culture and molecular techniques, breast milk contains organisms typically identified as both skin- and enteric-associated organisms such as *Staphylococcus*, *Streptococcus*, *Escherichia*, *Enterococcus*, *Veillonella*, *Prevotella*, *Pseudomonas*, and *Clostridia*^{7–12} (► **Table 1**). Several associated factors influence the composition of the breast milk microbiota, including maternal health and mode of delivery (► **Table 2**). Furthermore, the composition is dynamic, changing from high diversity including typical skin- and enteric-type organisms in colostrum to less diverse flora with greater infant oral and skin microbiota as lactation progresses (► **Fig. 1**).

Models that are not mutually exclusive have been proposed for how breast milk contains viable diverse microbiota. Microbiota typically associated with the skin may be transferred to breast milk. Molecular approaches have been employed to genetically type gram-positive organisms from the maternal skin, breast milk, and her infant to demonstrate the commonality of specific strains in the dyad, thus suggesting that skin bacteria may be transferred in breast milk or through the process of breastfeeding from mother to infant.^{13,14} In studies examining microbiota in breast milk, before expressing milk for examination, mothers were instructed to perform special cleansing of the breast skin surface.^{10–12} Even after cleaning the periareolar area, these breast milk samples contain viable skin- and enteric-associated microbiota. In practice, mothers do not cleanse their breast before breastfeeding. In addition, during breastfeeding, the nipple and surrounding areolar region are in the infant's mouth introducing maternal skin-associated bacteria to the infant's oral cavity and enteric tract.¹⁵

Others have proposed that organisms travel in a retrograde fashion from the infant's oral cavity into the ductal tissue.¹⁰ Based on the physiology of infant suckling, there may be backward flow of breast milk from the infant's oral cavity through the nipple into the mammary gland.^{16,17} This mechanism may explain the presence of organisms that have been noted in the oral cavity of both neonates and breast milk such as *Gemella*, *Veillonella*, *Staphylococcus*, and *Streptococcus*.^{10,18} However, other organisms typically noted in the oral cavity of

Table 1 Bacteria commonly found in breast milk using culture and molecular techniques

Phyla	Genera
Firmicutes	<i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Veillonella</i> , <i>Gemella</i> , <i>Enterococcus</i> , <i>Clostridia</i> , <i>Bifidobacterium</i> , <i>Lactobacillus</i>
Actinobacteria	<i>Propionibacterium</i> , <i>Actinomyces</i> , <i>Corynebacterium</i>
Proteobacteria	<i>Pseudomonas</i> , <i>Sphingomonas</i> , <i>Serratia</i> , <i>Escherichia</i> , <i>Enterobacter</i> , <i>Ralstonia</i> , <i>Bradyrhizobium</i>
Bacteroidetes	<i>Prevotella</i>

Source: Data adapted from Hunt et al¹⁰; Thompson et al¹²; Perez et al¹⁴; Cabrera-Rubio et al¹¹; and Delgado et al.⁶¹

Table 2 Factors influencing breast milk microbiota community structure

Maternal factors	Postnatal factors
Obesity Atopy Diet Immunologic status	Mode of delivery Gestational age Maternal antibiotic use Stage of lactation

neonates such as *Actinomyces* have not been consistently found in breast milk. In addition, initial colostrum samples contain DNA signatures of bacteria before infants have breastfed.¹¹ Thus, though milk transfer from an infant's oral cavity may explain the presence of some organisms, it does not fully explain the composition of the breast milk microbiota.

Recently, an alternative model has been proposed to account for the presence of typically enteric organisms in breast milk. Evidence suggests that mucosal intestinal dendritic cells regularly engulf intestinal bacteria, which may subsequently be trafficked into the systemic circulation.¹⁹ In pregnant and lactating women, these leukocytes with intracellular bacteria may be trafficked to the mammary gland and secreted into breast milk. These organisms or parts of organism may directly seed the infant enteric tract or alter the community structure, providing the basis for a model of enteromammary trafficking (EMT).

In support of the EMT model, in three studies of mothers and their term infants, a subset of genomic signatures corresponding to *Bifidobacterium longum*, *Streptococcus thermophilus*, and *Bifidobacterium pseudocatenulatum* were common to maternal stool, maternal blood, breast milk, and infant stool samples.^{14,20,21} In terms of viruses, human immunodeficiency virus (HIV) in breast milk appears to be distinct based on phylogenetic classification from HIV found in the peripheral blood of infected mothers.²² Some have proposed that the strains of HIV found in breast milk may originate from gut-associated lymph tissue, with subsequent trafficking via infected lymphocytes to the mammary gland.²³ Because this process is dependent on maternal enteric microbiota, it may be affected by maternal diet, body habitus, immunological status, and geography.^{6,24}

Another potential mechanism for transfer of microbiota from mothers into breast milk involves spread from the mammary gland. In a murine animal model of cytomegalovirus (CMV), virus may remain quiescent in the mammary gland following primary infection.²⁵ The process of lactation is proposed to reactivate these viruses. Consistent with this

Figure 1 During the course of lactation, there is an overall decrease in bacterial diversity compared with colostrum samples. The composition of the microbiota shifts from skin- and enteric-associated organisms to infant oral and skin organisms.

idea, CMV has been detected in the breast milk of asymptomatic CMV seropositive women. Virolactia, the presence of live virus in breast milk, correlates with duration of lactation and peaks during weeks 3 to 4 of lactation. In addition, the shedding of CMV appears to be limited to breast milk.²⁶ Premature infants may be at risk of postnatal acquisition of CMV from breast milk due to decreased transplacental transmission of antibodies against CMV.²⁷ Because of the benefits of breast milk for premature infants, in most neonatal intensive care units, breast milk is frozen before administration, decreasing the inadvertent transmission of CMV via breast milk.²⁸ Recent data suggest the CMV shedding in mothers of premature infants, also may be influenced by local immune factors in the mammary gland.²⁹

Proposed Function of Microbiota in Human Breast Milk

The function of microbiota in breast milk may include enhanced immune development through microbial ligands, nutrient metabolism and absorption, improved intestinal barrier function, and stimulation of the gut-brain axis. All neonates have immature immune function, as evidenced by increased anti-inflammatory T regulatory cell populations in cord blood.³⁰ Within the CD4 positive T-cell population, there are T helper 2 (Th2) and T helper 1 (Th1) cells. Th1 cells produce interleukin (IL) 2, interferon, and tumor necrosis factor, all of which promote cytotoxic T-cell function. In comparison, Th2 cells secrete IL4, IL5, IL6, IL10, and IL21, which support humoral immunity.³¹ Infants have higher Th2:Th1 ratios compared with adults, suggesting an enhanced B-cell response and potential for allergic sensitization.³²

Feeding with human milk advances immune development in full-term infants: compared with formula-fed infants, breastfed infants demonstrate increased Th1 activity, higher proliferative T-cell response to tetanus toxoid,³³ and moderated CD4 counts using flow cytometry.³⁴ In a small study of preterm infants, infants fed breast milk also had lower B-cell counts than infants who were fed formula.³⁵ Because of the immaturity of the neonatal humoral response, infants facing infection rely on maternal antibodies and a robust cytotoxic Th1 response. Enhanced cytotoxic function in infants fed breast milk may be promoted by bacterial ligands in breast milk.³⁶ In support of this theory, in vitro stimulation of dendritic cells with lipopolysaccharide supported T-cell differentiation.³⁷ Animals raised in a germ-free (GF) environment had lasting impairments in their immunologic function.³⁸ The effect of breast milk on maturation of Th17 cells remains unclear.³⁹ Thus, microbiota in breast milk may stimulate maturation of cytotoxic Th1 cells and improve their ability to fight infection.

In the enteric tract, the microbiota contributes to nutrient metabolism and synthesis. "Enterotype" describes the collective functional digestive and nutritive capacity of the enteric microbiota. Enterotypes may be associated with diet, geography, or body habitus.^{24,40} While enterotypes have not yet been fully defined in human infants, there is evidence of a breastfed infant enterotype. In the feces of 8 breastfed infants

compared with 10 formula-fed infants, metagenomic analysis suggests an increase in carbohydrate metabolism, amino acid and nitrogen metabolism, and cobalamin synthesis.⁶ Similarly, the breast milk metagenome is enriched for nitrogen metabolism, membrane transport, and the oxidative stress response.⁴¹ In premature and term infants fed breast milk and formula, metagenomic analysis of stool samples has revealed an enhanced virulence potential with the presence of bacteriophage and genes encoding for type III and IV secretion systems.⁴²⁻⁴⁴ These data are corroborated with an animal model in which there is increased oxidative stress and a decreased production of proteins utilized in cell adhesion with formula feedings compared with breast milk feedings.⁴⁵

The breast milk microbiota also may be involved in enhancing intestinal barrier protection. Animal studies have demonstrated that enteric colonization is critical for upregulation of epithelial junctional complexes, stimulation of antimicrobial peptide defenses, and expression of key detoxifying enzymes such as alkaline phosphatase to mitigate overstimulation by bacterial lipopolysaccharide ligands.⁴⁶⁻⁴⁸ In an animal model, heat shock protein 70 (HSP70) in breast milk decreased bacterial translocation from the gut lumen.⁴⁹ It is possible that microbiota in breast milk may increase HSP70 levels in the intestinal lumen and contribute to epithelial barrier function in neonates.⁵⁰

Oligosaccharides in breast milk have a dynamic relationship with microbiota in breast milk and the enteric tract. Structurally, these are complex glycans found in human breast milk. Traditionally, oligosaccharides were thought to serve as a substrate for the growth of intestinal bacteria in the distal enteric tract.⁵¹ Recent data suggest a more complex relationship, through which oligosaccharides in breast milk are not consumed by microbiota but still alter the growth of microbiota.⁵² In a rat model, oligosaccharide levels were diminished in the small intestine and differentially secreted into urine, suggesting selective absorption of oligosaccharides possibly in concert with differing microbiota throughout the enteric tract.⁵³ In addition, oligosaccharides have independent immune function in neonates.⁵⁴ Ultimately, oligosaccharides may work synergistically with breast milk and enteric microbiota to strengthen barrier function.

In colonizing or transiting the infant enteric tract, the breast milk microbiota may have broader developmental consequences for the infant. Microbiota in breast milk may also establish a normal gut-brain axis. Animals raised in GF environments have decreased intestinal peristalsis that can be restored with the introduction of enteric microbiota from animals with conventional microbial exposure.^{55,56} In a comparison of GF, specific pathogen-free (SPF), and gnotobiotic animals, GF showed an exaggerated stress response compared with SPF mice. This response could be reversed with early exposure to *Bifidobacterium infantis*, an organism that has been identified in breast milk.^{14,57} Oral antibiotic administration to animals raised in an SPF environment alters enteric microbiota, upregulates brain-derived neurotrophic factor, and increases exploratory behavior.⁵⁸ Further work will elucidate the relationship between breast milk microbiota, the developing enteric microbiota, and the gut-brain axis.

Mastitis: A Case of Dysbiosis of Breast Milk Microbiota

Mastitis is defined as inflammation of the breast, with or without infection.⁵⁹ Although *Staphylococcus aureus* has traditionally been considered the primary cause of infectious mastitis, in recent studies, in the breast milk of healthy women. Kvist et al⁶⁰ compared bacterial species in milk samples from 192 women with a clinical diagnosis of mastitis versus 466 healthy controls. *S. aureus* was present in 45% of women with mastitis and 31% of healthy donors, and mean colony counts were identical in the two groups. Moreover, the authors found no correlation between colony counts and symptom severity among women with mastitis. These results suggest the presence of *S. aureus* in breast milk does not independently result in clinical mastitis.

Delgado et al⁶¹ have explored the role of coagulase-negative *Staphylococcus* spp. in mastitis. Using pulsed field gel electrophoresis genotyping to identify species present in the milk of women with mastitis symptoms, they found that *S. epidermidis* was present in 85% (17/20) of samples, compared with *S. aureus* in 40% (8/20) of samples.⁶¹ Milk samples were collected after cleansing the nipple and areola with soap and sterile water, before applying chlorhexidine, to minimize contamination with skin microbiota. They subsequently compared strains of *S. epidermidis* present in women with mastitis versus healthy controls. Women with clinical signs of infection were more likely to harbor strains of *S. epidermidis* with the *icaD* gene (33 vs. 11%, $p = 0.03$), which was correlated with biofilm production.⁶² Thus, virulence factors of *S. epidermidis* strains found in breast milk may contribute to the pathogenesis of mastitis.

Based on tentative evidence from animal studies and clinical trials, probiotic-like organisms may compete with putative mastitis pathogens, and administration of probiotics may prevent or ameliorate mastitis symptoms. In an in vitro bovine animal model, certain strains of *Lactobacillus* inhibited adhesion and internalization of *Staphylococcus* spp. by mammary epithelial cells.⁶³ *Lactobacilli* also produce anti-inflammatory and antimicrobial factors.⁶⁴ In clinical trials, oral administration of probiotics to women with mastitis appears to reduce mastitis symptoms, and orally administered strains can be detected in human milk.⁶⁵ Arroyo et al tested human milk-derived probiotic strains for treatment of mastitis in 352 women.⁶⁶ They reported a greater reduction in bacterial counts of *S. epidermidis*, *S. aureus*, and *S. mitis*, as well as greater reduction in pain, in the probiotic-treated group, compared with antibiotic-treated controls.

Of note, the methods reported in the published manuscript differ from ClinicalTrials.gov (NCT00716183) in several ways, including the types of probiotics and antibiotics used and the study allocation method, which is described on Clinical Trials as an open-label, nonrandomized trial and as a randomized, double-blinded trial in the published manuscript. Collectively, based on these results, clinical mastitis may result from virulent *S. epidermidis* strains in breast milk and may be moderated with probiotic therapy.

Conclusion

In summary, breast milk has a dynamic microbial ecology with a microbiota composed of skin- and enteric-associated bacteria and pathogenic viruses. These organisms are transferred from maternal and infant microbial communities into breast milk via multiple mechanisms, including transmission from skin, movement from infant oral cavity into the mammary gland during breastfeeding, EMT, and reactivation from mammary gland in the case of CMV and HIV. Microbiota in breast milk advance neonatal immune function, enhance nutrient metabolism, improve intestinal barrier function, and contribute to the development of the gut-brain neural axis. The pathogenesis of clinical mastitis may result from dysbiosis in the mammary gland with virulent strains of *Staphylococcus* spp. Administration of probiotics may reduce the symptoms of mastitis by restoring mammary gland and breast milk microbiota. With improved understanding of the impact of breast milk microbiota, it may be possible to manipulate these microbial communities to improve the health and development of mothers and their neonates.

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