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

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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 this 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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► immune function
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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.

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Propionibacterium spp. In comparison, the adult enteric microbiota are more diverse and generally dominated by anaerobic organisms such as Bacteroides and Prevotella. 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. Several associated factors influence the composition of the breast milk microbiota, including maternal health and mode of delivery. 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.

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 presence of skin bacteria may be transferred in breast milk or through the process of breastfeeding from mother to infant. In studies examining microbiota in breast milk, before expressing milk for examination, mothers were instructed to perform special cleansing of the breast skin surface. 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.

Others have proposed that organisms travel in a retrograde fashion from the infant's oral cavity into the ductal tissue. Based on the physiology of infant sucking, there may be backward flow of breast milk from the infant's oral cavity through the nipple into the mammary gland. 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. However, other organisms typically noted in the oral cavity of 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. 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. 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 shown to be maternal stool, maternal blood, breast milk, and infant stool samples. 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. 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. Because this process is dependent on maternal enteric microbiota, it may be affected by maternal diet, body habitus, immunological status, and geography.

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. In a murine animal model of cytomegalovirus (CMV), virus may remain quiescent in the mammary gland. The process of lactation is proposed to reactivate these viruses. Consistent with this

<table>
<thead>
<tr>
<th>Phyla</th>
<th>Genera</th>
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<tbody>
<tr>
<td>Firmicutes</td>
<td>Staphylococcus, Streptococcus, Veillonella, Gemella, Enterococcus, Clostridia, Bifidobacterium, Lactobacillus</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Propionibacterium, Actinomyces, Corynebacterium</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Pseudomonas, Sphingomonas, Serratia, Escherichia, Enterobacter, Ralstonia, Bradyrhizobium</td>
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<tr>
<td>Bacteroidetes</td>
<td>Prevotella</td>
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Source: Data adapted from Hunt et al; Thompson et al; Perez et al; Cabrera-Rubio et al; and Delgado et al.

Table 2 Factors influencing breast milk microbiota community structure

<table>
<thead>
<tr>
<th>Maternal factors</th>
<th>Postnatal factors</th>
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<tbody>
<tr>
<td>Obesity</td>
<td>Mode of delivery</td>
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<tr>
<td>Atopy</td>
<td>Gestational age</td>
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<tr>
<td>Diet</td>
<td>Maternal antibiotic use</td>
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<tr>
<td>Immunologic status</td>
<td>Stage of lactation</td>
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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 asymptom-
atic 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 trans-
mision of antibodies against CMV.27 Because of the benefits
of breast milk for premature infants, in most neonatal inten-
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 en-
hanced 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 moder-
ated 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 dif-
ferrntiation.37 Animals raised in a germ-free (GF) environ-
ment 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 collec-
tive functional digestive and nutritive capacity of the enteric
microbiota. Enterotypes may be associated with diet, geogra-
phy, or body habitus.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.41 Similarly,
the breast milk metagenome is enriched for nitrogen
metabolism, membrane transport, and the oxidative stress
response.42 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 bacte-
riophage and genes encoding for type III and IV secretion
systems.43–44 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 en-
hancing intestinal barrier protection. Animal studies have
demonstrated that enteric colonization is critical for upregu-
lation of epithelial junctional complexes, stimulation of anti-
microbial peptide defenses, and expression of key detoxifying
enzymes such as alkaline phosphatase to mitigate overstim-
ulation 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 relation-
ship 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 indepen-
dent immune function in neonates.54 Ultimately, oligosac-
charides 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 consequen-
ces 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, upre-
gulates brain-derived neurotrophic factor, and increases explor-
atory behavior.58 Further work will elucidate the relationship
between breast milk microbiota, the developing enteric micro-
biota, 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. Although *Staphylococcus aureus* has traditionally been considered the primary cause of infectious mastitis, in recent studies, in the breast milk of healthy women, Kwist et al 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 al 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. 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. 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. Lactobacilli also produce anti-inflammatory and antimicrobial factors. 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. Arroyo et al tested human milk–derived probiotic strains for treatment of mastitis in 352 women. 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.

References

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rus-specific immune responses and symptomatic postnatal cyto-


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