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# Breastfeeding and Gut Microbiota

*Bitra Najafian and Mohammad Hossein Khosravi*

## Abstract

Human breast milk (HBM) not only is a source of nutrition for infants but also contains a variety of biologically active components and bacterial species. These molecules and bacteria guide both intestinal microbiota and infantile immune system. Recently published studies have found several vital roles for gut microbiota including effects on the individual's personality, decreased predisposition to the diseases, and a variety of other health-related consequences such as possible therapeutic effects or preventing role. In this chapter the components of human breast milk and its effect on shaping the human gut microbiota have been reviewed.

**Keywords:** breastfeeding, gut microbiota, breast milk, microbiome

## 1. Introduction

Today, there is no doubt that human breast milk is the most beneficial source of nutrition for infants which is the result of several years of research and evaluation. Beside known nutrients such as proteins and carbohydrates, human breast milk contains a wide range of biologically active components and microbiota [1]. Previous researches have shown that mother's intestinal microbiome is transferred to her infant through breast milk. This relationship between mother and infant plays the key role in forming a healthy intestinal microbiome which is responsible for protecting against diarrheal and respiratory illnesses as well as asthma, obesity, diabetes, atopy, and other inflammatory diseases such as inflammatory bowel diseases (IBD) [2, 3]. The presence of bacteria in human breast milk not only improves the infantile health but also promotes mother's health by a variety of mechanisms such as preventing mastitis [4]. Human milk oligosaccharides (HMOs) have the main role in developing the intestinal microbiota [1]. Their synthesis is determined by maternal genotype.

Transferring immunity from mother to infant is started from the intrauterine life and is continued by breastfeeding. Breast milk includes antibodies and immunities targeting the mother's gut and airway microbes with which her infant is likely to encounter during the very first months of birth [5]. Recently conducted researches have revealed that breast milk directly modulates the development of immune system in breastfed infants as well as provides passive protection [6].

Colostrum is the most enriched part of the breast milk of immune factors which provides an appropriate immune response when the infant is at risk of exposure to new microbes [7]. Hormones, cytokines, growth factors, chemokines, and immunoglobulins are among the bioactive factors that are transferred to infant via breastfeeding [7, 8]. In this chapter a comprehensive review has been done on the role of breastfeeding and breast milk ingredients in forming infant's intestinal microbiota.

## 2. Microbial components of breast milk

Before year 2000, human breast milk (HBM) was considered to be sterile. Martin et al. mentioned the presence of commensal and probiotic bacteria in HBM [9]. *Lactobacillus fermentum* and *Lactobacillus gasseri* were more commonly found in breast milk samples using culture-dependent techniques [10]. Today, lactic acid bacterial strains with proven probiotic activities are referred to as probiotics [11]. Every milliliter of breast milk contains about 1000 colony-forming units of various bacterial species [12, 13]. An estimation reveals that infants receive about 800,000 bacteria from breast milk each day [14]. At birth and during delivery, infants receive a notable dose of microbes through different mechanisms such as vaginal flora which is followed by the first breastfeeding [15]. So, breast milk is the second important source of microbiota seeding in the infant's intestines [16, 17]. This has been proven by several previous epidemiologic studies in which the researchers have reported fundamental differences in gut microbiota between breastfed and formula-fed infants [18, 19]. Previous conducted researches have revealed that infantile stool and breast milk have some microbial strains, such as *Enterococcus*, *Staphylococcus*, *Bifidobacterium*, and *Lactobacillus*, in common [20, 21]. It also has been reported that more daily breastfeeding is attributed to more similarity between mother's milk and infant stool microbiome [17].

Newly developed methods, such as next generation sequencing, have augmented our knowledge regarding microbial composition of human breast milk. *Staphylococcus* and *Streptococcus* have been reported to be the most common microbiota families in the human's breast milk. Other families including *Bifidobacterium*, *Lactobacillus*, and *Enterobacteriaceae* family members are placed in the following ranks [22, 23]. Colostrum contains a more various number of bacterial species than do transitional and mature milk.

So far, we have no idea where the mother's milk microbiota exactly comes from; however a number of theories have come up. The first one considers that retrograde flow of breast milk from the infant's mouth to the areola and commensal skin area contaminates the milk with maternal skin flora; however, the presence of anaerobic species does not justify the commensal contamination [24–26]. On the other hand, *Streptococcus* which is abundant in salivary flora is also common in human breast milk microbiome, supporting the retrograde flow theory [27]. The theory of retrograde flow was first mentioned by Ramsay and colleagues where they used ultrasound technology to study the huge transmission of breast milk from the infant's mouth to the mammary gland ducts [25]. Another theory holds this belief that breast milk microbiota are originated from mother's intestinal flora, where they migrate via blood and lymphatic circulation to the mammary glands [27, 28]. Studying pregnant and lactating mice revealed that both aerobic and anaerobic organisms are translocated from gut to the mesenteric lymph nodes and mammary glands, subsequently [28]. In addition, another research team found that there are similar bacterial signatures in breast milk, lymph nodes, and dendritic cells (DC) of lactating mice [29]. Hormonal changes in late pregnancy and increased permeability of the intestinal endothelium are considered to have a supporting role for translocation of intestinal bacteria to mammary glands [27]. Hence, the origin of breast milk microbiota should be more investigated.

A variety of maternal factors have effects on the diversity of mother's milk microbiota. Previously published studies have reported that mothers with vaginal delivery have more various microbial species in their breast milk than that of those who deliver by cesarean section [30, 31]. Also, it has been reported that there is no remarkable difference for milk bacterial concentration between different genders

or race groups as well as geographical regions [32, 33]. Various types of breast milk have different bacterial concentrations as the colostrum has lower concentrations than do transitional and mature milk. Results from comparative clinical studies have revealed that breast milk bacterial composition is affected by maternal health condition such as obesity, human immunodeficiency virus (HIV), and celiac disease [34, 35]. Also, it is expectable that maternal chemotherapy and antibiotic use decrease the microbial diversity in mother's milk [36, 37].

### 3. Mammary gland microbiome

Recently, many efforts have been made to determine the mammary gland bacteriome in different ways [38, 39]. Biopsies from different sites of the breast have approved the viability of bacteria by culture. Human breast tissue bacteriome was shown to be similar to those of the human breast milk where the *Proteobacteria* is the main phylum [40]. Also, nipple aspirate fluid (NAF) has been recently used to determine the breast ductal bacteriome. NAF, which is secreted regularly by breast duct endothelial cells, can be easily collected using a syringe connected to the suction cup applied with a negative pressure [41, 42].

## 4. Infant gut and what breast milk microbiota has to do

### 4.1 HBM microbiome as anti-infective

It is believed that breast milk microbiota reduces the incidence of bacterial infections through a variety of mechanisms in breastfed infants. Commensal bacteria modulate growth and replication of pathogen bacteria through their antimicrobial power or as a result of competitive exclusion; as the *Escherichia coli*, *Shigella*, or *Salmonella* strains are inhibited by lactobacilli isolated from human breast milk [43, 44]. In a randomized clinical trial, researchers prescribed breast milk lactobacilli to infants between 6 and 12 months of age which reduced the total incidence of infections [45]. There are a variety of studies which have assessed the antimicrobial activity of the intestine; however, more studies should be conducted for assessing antimicrobial specificities of human breast milk.

### 4.2 Immunomodulatory role

Animal studies have shown an important role for gut microbiota in increasing and modulating immune functions [46–48]. Lymphoid tissue development was shown to be altered in organs such as the spleen, lymph nodes, and thymus when there is a reduced number of microbiota in the animal intestines. The intestines without any germs have shown reduced numbers of IgA-producing cells, lamina propria CD4+ cells, and hypoplastic Peyer's patches [49]. Production of Th1 cytokines including Il-2, Il-12, and TNF-alpha by macrophages has been shown to be augmented by breast milk lactobacillus strains. Recently conducted studies have shown an improved immunologic and better Th1 response in breastfed infants that that of those fed by formula [50]. *Lactobacillus fermentum* and *Lactobacillus salivarius* have been reported as potent activators of natural killer cells and both innate and acquired immunity as a result of in vitro studies [51]. In addition, human milk metagenome has been shown to contain immunomodulatory DNA motifs which may help modulate exaggerated inflammatory responses

to bacterial infection [52]. Most of these regulatory effects were not found in non-milk-derived probiotic bacteria [51].

### 4.3 Anti-allergic role

A protective association has been discovered between human breast milk lactic acid bacteria and allergies. The main etiology of allergy has been described as the disturbance in regulation of immune system [53]. Previously published animal studies have revealed that probiotic bacteria originated from human breast milk, such as *Lactobacillus gasseri* and *Lactobacillus coryniformis*, have a modulatory role for immune response in cow milk protein sensitivity [54]. However, a conducted randomized clinical trial has shown that prescribing probiotics in the first 6 months of life does not reduce the risk of atopic eczema [55]. Other similar studies have reported that prescribing specific *Lactobacillus* and *Bifidobacterium* species to mothers has led to a reduced incidence of infant eczema in the first 2 years of life [56, 57]. This anti-allergy property of probiotics has been attributed to the downregulation in the production of Th2 cytokines by the hygiene hypothesis [54]. Another clinical study has reported that infants who have more *Klebsiella* species, as the dominant bacteria in their gut, have a higher chance for involving with Atopia, whereas the presence of *Viridans streptococci* in the gut microbiome has the contrary role [58].

### 4.4 Antitumor properties

However not many studies have been conducted for assessing antitumor properties of the gut microbiome; there are some points in the literature [59]. *Enterococcus faecalis* and *Staphylococcus hominis*, which are isolated from human breast milk, have shown some antitumor properties against a breast cancer cell line [60]. Another similar study has reported that a subspecies of *Lactococcus lactis* has shown therapeutic effect against colon cancer [61].

## 5. Conclusion

In this chapter we went through the definition and application of human breast milk microbiome and its role on building infant gut microbiome as well as infant's health and disease. Also mentioned is that this gut microbiome may play important roles as anti-infective, immunomodulatory, and anticancer properties. As the importance of breast milk microbiome is getting more notices, further studies should be conducted to assess it more and provide some ways for enriching mother's milk microbiome with beneficial bacteria.

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