



# The Human Breast Milk Microbiome: Establishment and Resilience of Microbiota over the Mother–Infant Relationship

Saqib Hassan, Ishfaq Hassan Mir, Meenatchi Ramu, Ayushi Rambia, Chinnasamy Thirunavukkarasu, George Seghal Kiran, Pallaval Veera Bramhachari, and Joseph Selvin

## Abstract

Human milk provides a continuous supply of good bacteria to the infant's gut, which contributes to the maturation of the digestive and immunological systems in the developing infant. Nonetheless, the origin of bacterial populations in milk is unknown, and they have been suggested to come from maternal skin, the infant's mouth, and/or endogenously from the maternal digestive tract via a

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S. Hassan

American Society for Microbiology, Washington, DC, USA

Department of Biotechnology, School of Bio and Chemical Engineering, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

I. H. Mir · C. Thirunavukkarasu

Department of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, Puducherry, India

M. Ramu

Department of Biotechnology, Faculty of Science and Humanities, SRM Institute of Science and Technology, Chengalpattu, Tamil Nadu, India

A. Rambia · J. Selvin (✉)

Department of Microbiology, School of Life Sciences, Pondicherry University, Puducherry, India

G. S. Kiran

Department of Food Science and Technology, School of Life Sciences, Pondicherry University, Puducherry, India

P. V. Bramhachari

Department of Biosciences and Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

mechanism involving immune cells. Understanding the composition, roles, and assembly of the human milk microbiota has significant consequences not only for the development of the infant gut microbiota but also for breast health, as dysbiosis in milk bacteria can cause mastitis. Furthermore, host, microbial, medical, and environmental factors may influence the composition of the human milk microbiome, potentially affecting the mother–infant relationship.

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**Keywords**

Breast milk microbiome · Human digestive tract · Mastitis · Gut microbiome

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## 9.1 Introduction

Since the Human Microbiome Project came into being in 2007, it has been breaking our stereotypical understanding that the human body is more or less sterile and only a few sites harbor many microorganisms. As opposed to the belief that microbes only reside in the skin, upper respiratory tract, gut, and vaginal canal, microorganisms are also said to be found in human milk (McGuire and McGuire 2017). The earliest evidence of microbes in human milk was reported in the study on bacteria in the colostrum and milk of Guatemalan Indian Women by Wyatt and Mata. The presence of Enterobacteriaceae suggested poor environmental sanitation and personal hygiene among the studied population (McGuire and McGuire 2017; Wyatt and Mata 1969). This gave the impression that microbes in the milk contaminated it, mainly due to underlying causes. But now there is evidence that microorganisms are an innate part of human breast milk (HBM) (Stinson et al. 2021). It is also believed that the microbes in human milk contribute to the early colonization of the infant's gut and thus provide the necessary genes and antigens eminent for the growth and development of the newborn (McGuire and McGuire 2017).

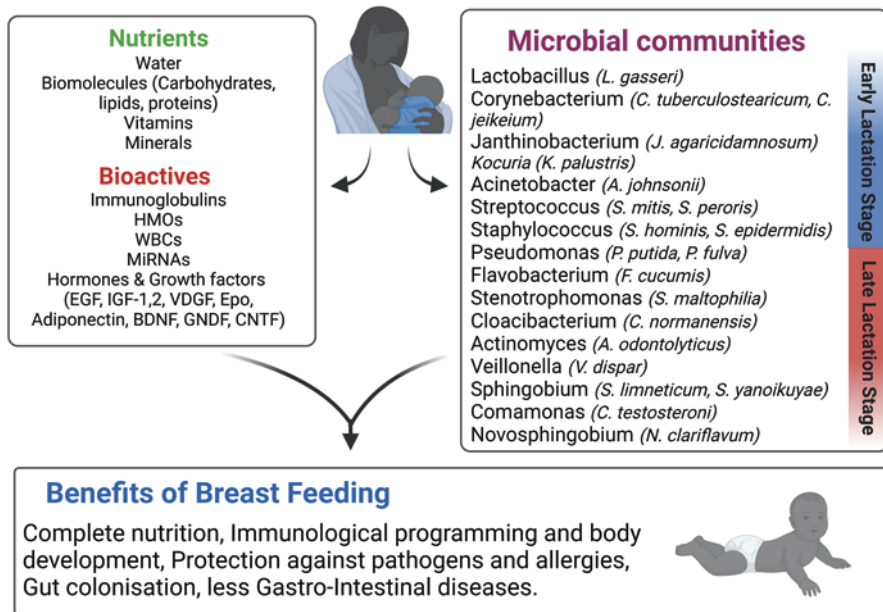
Several genera of bacteria, viruses, archaea, and microeukaryotes have been reported to form the Human Milk Microbiome (HMM) (Stinson et al. 2021). Among these microbes, Firmicutes form the largest proportion, followed by Proteobacteria, Bacteroidetes, and Actinobacteria as the major classes (Kim and Yi 2020). A total of 329 genera have been detected with *Streptococcus*, *Staphylococcus*, *Bacteroides*, *Acinetobacter*, Enterobacteriaceae(f), Ruminococcaceae(f), *Bifidobacterium*, *Prevotella*, Clostridiales(o), *Corynebacterium*, *Akkermansia*, *Lactobacillus*, *Pseudomonas*, *Dialister*, *Stenotrophomonas*, *Blautia*, *Sphingomonas*, *Haemophilus*, *Neisseria*, Lachnospiraceae (f), *Rothia*, and *Faecalibacterium* in the order of their abundance (Kim and Yi 2020). The presence of these microbes in the HBM microbiome is mostly attributed to maternal skin and infant oral cavity during lactation, as well as the maternal gastrointestinal tract (Stinson et al. 2021). The variability and abundance of the microbes mentioned above depend on maternal age, health, lactation duration, mode of delivery, and geographical location (McGuire and McGuire 2017).

HBM microbiome is quintessential for infants because it is the best source of nutrition and certain immune components such as secretory antibodies, immune cells, antimicrobial proteins, cytokines, and human milk oligosaccharides (Kim and Yi 2020). All of these ensure proper growth and development of the infant and protection against several lifestyle disorders such as obesity, diabetes (both type 1 and 2), asthma, and cardiovascular diseases (Fitzstevens et al. 2017; Stinson et al. 2021). Therefore, a detailed study of the HBM microbiome can help improve formula-based infant food and replicate the microbiome from healthy mothers to ensure proper growth and development of neonates and infants.

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## 9.2 Human Breast Milk (HBM) Microbiome and Its Importance

The HBM microbe is said to be composed of bacteria, viruses, archaea, and microeukaryotes. Their presence benefits the growing infant or may indicate signs of diseases and certain disorders (Fitzstevens et al. 2017; Stinson et al. 2021). HBM microbiome is responsible for the baby's gut colonization. Still, they may also have several other crucial roles, including affecting the maturation of the mucosal immune system, defending against infections, and assisting with digestion and nutritional absorption (Jeurink et al. 2013). HBM bacteria, in brief: bolster the gut immune system's homeostasis: Early microbial exposure is crucial to offer antigenic cues encouraging intestinal immune system maturation and improving intestinal homeostasis (Gensollen et al. 2016). By supporting a change from the predominate intrauterine T helper cell 2 immunological milieu to a TH1/TH2 balanced response and by inducing regulatory T cell development, the HBM microbiota specifically may enhance intestinal immune homeostasis. Additionally, a metagenomics analysis of the HBM microbiome revealed immunomodulatory DNA motifs that could aid in reducing excessive inflammatory reactions to bacterial colonization by enhancing intestinal functions. As HBM microbiome contains oligosaccharides indigestible by an infant's intestine, molecular analysis revealed that BM bacteria are metabolically active in producing short-chain fatty acids (SCFA). It has been proposed that this characteristic favors the proliferation of helpful bacteria against harmful taxa (Ward et al. 2013). Several BM isolates, including *Lactobacillus rhamnosus* and *crispatus*, were shown to inhibit the growth of pathogenic microorganisms in vitro studies. Other isolates had effects on an enteropathogenic *Salmonella enterica* strain that were both in vitro and in a mouse model that was bacteriostatic and/or bactericidal (Hirai et al. 2002). Breastfed infants also exhibit increased capacity for carbohydrate, amino acid, and nitrogen metabolism, cobalamin synthesis, membrane transport, oxidative stress, and human behavior and emotion (Lazar et al. 2019; Stinson et al. 2021; Valdes et al. 2018; Valles-Colomer et al. 2019) (Fig. 9.1).



**Fig. 9.1** Benefits of human milk microbiome for infants

### 9.3 Bacteria

Several genera of bacteria have been identified in the HBM through culture-dependent and culture-independent methods. The core genera primarily include *Staphylococcus*, *Streptococcus*, and *Pseudomonas*. These are found universally regardless of the lactating mothers' demographics, geographical location, or health status (Kim and Yi 2020; McGuire and McGuire 2017; Stinson et al. 2021). The most abundant genera found are *Staphylococcus*, *Streptococcus*, and *Propionibacterium*; *Bifidobacterium*, *Veillonella*, *Rothia*, and *Lactobacillus* are found in lower abundance (Stinson et al. 2021). *Corynebacterium*, *Ralstonia*, *Acinetobacter*, *Acidovorax*, *Pseudomonas*, *Bacteroides*, *Clostridium*, *Escherichia/Shigella*, *Gemella*, and *Enterococcus* are some of the other commonly found bacterial genera in the HBM (McGuire and McGuire 2017; Stinson et al. 2021).

Some of these microbes' early colonization of the infant's gut can help in short- and long-term health outcomes (Fitzstevens et al. 2017). Although found in low abundance, *Bifidobacterium* is the first to colonize the infant's gut and help utilize the glycans found in the HBM (Stinson et al. 2021). *Lactobacillus* in the gut provides resilience and reduction in the risk of diarrheal and other dysbiosis-related problems (Gomez-Gallego et al. 2016). Together these two bacteria help activate immunoglobulin A producing plasma cells in the neonatal gut (Khodayar-Pardo et al. 2014). Different bacteria also aid in decreasing the risk of respiratory tract infections, atopic dermatitis, asthma, obesity, type 1 and 2 diabetes, necrotizing

enterocolitis, gastroenteritis, and inflammatory bowel disease (Fitzstevens et al. 2017; Gomez-Gallego et al. 2016).

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## 9.4 Viruses

Members of certain viral families have been reported in infants up to 4 days of age. Bacteriophages are the most abundant, while certain eukaryotic viruses are the least. Members of Siphoviridae, Myoviridae, and Podoviridae families have also been reported in both HBM and breastfed infant stool (Stinson et al. 2021). Bacteriophages, in particular, help maintain the balance between different bacterial communities at different points in the early developmental age. Non-phage viruses from Papillomaviridae, Retroviridae, and Herpesviridae have also been reported (Stinson et al. 2021). Regarding the recent challenge of SARS-CoV-2 (COVID-19) in BM, there is no evidence of virus transmission during lactation (Zhu et al. 2021).

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## 9.5 Archaea

Of the several studies on HBM samples, only two have reported the presence of archaeal DNA. The species identified were *Haloarcula marismortui*, *Halorhabdus utahensis*, and *Halomicrobium mukohataei* (Jiménez et al. 2015; Stinson et al. 2021). Although the presence of halophilic archaea is questionable in the HBM, a protective function has been attributed to their presence (Stinson et al. 2021).

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## 9.6 Microeukaryotes

A few fungal species have also been reported in low diversity and biomass in the HBM, forming the HM mycobiome. These are generally members of *Malassezia*, *Davidiella*, *Ascomycota*, *Basidiomycota*, *Candida*, and *Saccharomyces* (Boix-Amorós et al. 2019; Jiménez et al. 2015; Stinson et al. 2021). Protozoal parasites, including *Giardia intestinalis* and *Toxoplasma gondii*, have also been reported (Jiménez et al. 2015; Stinson et al. 2021). However, the clinical significance of these fungal and protozoal species has not been reported yet.

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## 9.7 Origin of Milk Microbiome

Now that there is a consensus on the presence of microbes in the HBM, the next step is to elucidate their source in the HBM. Several theories and evidence suggest two possible routes for the entry of microbes into the HBM. First is the entero-mammary pathway, which involves the translocation of gut microbiota to the mother's mammary glands (McGuire and McGuire 2017; Moossavi et al. 2019). The presence of certain gut microbiome anaerobes such as *Veillonella*, *Bacteroides*,

*Parabacteroides*, *Clostridium*, *Collinsella*, *Faecalibacterium*, *Coprococcus*, and *Blautia* in the HBM indicate their entry via the lymphatic system (Stinson et al. 2021). This has been supported by the presence of bacteria in the mesenteric lymph nodes of pregnant mice as opposed to nonpregnant mice, indicating an increase in bacterial translocation during gestation (Perez et al. 2007; Stinson et al. 2021). This has been further confirmed by the increase in lymphatics in the mammary tissues during the lactation period (Hitchcock et al. 2020; Schenkman et al. 1985; Stinson et al. 2021).

Second is the retrograde inoculation by the maternal skin and the infant's oral cavity (McGuire and McGuire 2017; Moossavi et al. 2019; Stinson et al. 2021). The presence of human skin commensals such as *S. epidermidis*, *S. hominis*, *S. haemolyticus*, *S. lugdunensis*, *Cutibacterium acnes*, and species of *Corynebacterium* in the HBM indicate their entry via the nipple into the mammary glands (Stinson et al. 2021). *S. epidermidis* has supported this in breastfed infants and its absence in formula-fed infants (Jiménez et al. 2008; Stinson et al. 2021). Reports have also suggested an exchange between the infant's oral and HBM microbiomes during the lactation period (Stinson et al. 2021). This has been supported by oral bacteria, such as *S. salivaris*, *S. mitis*, *Rothia mucilaginoso*, and *Gemella* spp., in the HBM of breastfed infants instead of bottlefed infants (Biagi et al. 2018; Stinson et al. 2021).

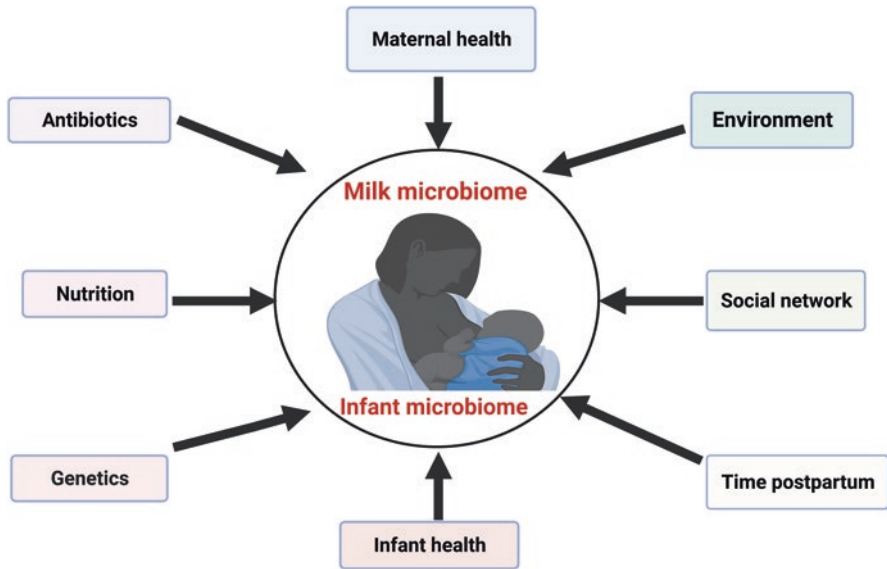
The next question in our understanding of the seeding of the HBM is whether this occurs due to constant influx during the gestation and lactation period or as a result of a permanent mammary gland microbiome. The low abundance of bacteria in the HBM compared to other mucosal surfaces in the body suggests the constant influx approach. This is further supported by the fact that mammary epithelium tissues are not specialized for mucus secretion (Stinson et al. 2021). Another argument suggests that although the mammary epithelial cells do not secrete mucus, they may act as a mucosal-like immune interface, thus allowing stratification and compartmentalization of resident microbes (Sakwinska and Bosco 2019; Stinson et al. 2021). This has been supported by the presence of *S. aureus* and lactic acid bacteria biofilms in the mammary epithelial cells in bovine models, favoring the permanent model of the mammary gland microbiome (Bouchard et al. 2015; Gomes et al. 2016; Stinson et al. 2021; Wallis et al. 2019).

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## 9.8 Potential Factors Influencing Milk Microbiome

Several factors influence the HBM microbiome at all stages of gestation as well as lactation (Fig. 9.2). These include the following:-

1. Maternal factors.
2. Breastfeeding factors.
3. Early life factors.
4. Infant factors.
5. Milk environment.



**Fig. 9.2** Factors affecting human milk microbiome

Maternal factors such as body mass index (BMI), gestational age, ethnicity, and diet tend to influence the composition of the HBM microbiome (Cabrera-Rubio et al. 2012; Moossavi et al. 2019). Mothers with high BMI tend to have a more homogenous composition of bacteria than those with normal BMI (Cabrera-Rubio et al. 2012). HBM from obese mothers shows a higher abundance of *Staphylococcus* and *Lactobacillus* while a lower abundance of *Bifidobacterium* (Cabrera-Rubio et al. 2012). Lower levels of *Bifidobacterium* in the milk may lead to improper seeding of the infant's gut, thereby affecting the child's overall health. Gestational age tends to influence the *Bifidobacterium* concentration in the HBM. Preterm mothers have a higher *Bifidobacterium* count in the colostrum and during lactation. At the same time, there is no significant correlation between gestational age and any other bacterial genera (Khodayar-Pardo et al. 2014). Ethnicity, as well as diet, also influences the HBM microbiome. Intake of prebiotics and probiotics in the diet tends to reflect in the milk microbiome and influence the human milk's lipid profile (Gomez-Gallego et al. 2016; McGuire and McGuire 2017). A calorie-intensive and nutrient-rich diet is also associated with a higher abundance of Firmicutes, indicating that diet influences the HBM microbiome (McGuire and McGuire 2017; Williams et al. 2017). Women from different ethnicities show differences in the composition of breast milk metabolites (Gay et al. 2018).

Certain breastfeeding factors, such as whether the infant is breastfed or bottle-fed, or both and the lactation period influence the milk microbiome. Breastfed infants tend to have a more abundant microbiome than bottle-fed ones, especially that of *Bifidobacterium* (Stinson et al. 2021). It has also been found that colostrum has a more diverse microbiome than transition or mature milk (Cabrera-Rubio et al.



2012). The abundance of certain bacteria like *Bifidobacterium*, *Enterococcus* spp., *Staphylococcus*, *Streptococcus*, *Lactococcus*, *Veillonella*, *Leptotrichia*, and *Prevotella* has been reported to increase from colostrum to mature milk (Cabrera-Rubio et al. 2012; Khodayar-Pardo et al. 2014).

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## 9.9 Mode of Delivery

Women who give birth vaginally or through emergency and elective caesarian show significant differences in their milk microbiome. For vaginal delivery, the HBM microbe shows an increase in *Bifidobacterium*, *Leuconostocaceae*, *Lactobacillus* spp., while the elective caesarian shows an increase in *Carnobacteriaceae* (Cabrera-Rubio et al. 2012; Gómez-Gallego et al. 2018; Khodayar-Pardo et al. 2014). The microbiome is more or less similar for vaginal and emergency caesarian procedures. This indicates that the difference does not arise due to the difference in procedure but the lack of physiological stress and hormonal signals which mediate the seeding of the milk microbiome (Boix-Amorós et al. 2016). Lactating mothers on antibiotics or chemotherapy also show a reduced bacterial diversity in the HBM microbiome (McGuire and McGuire 2017). Since the infant's oral cavity also seeds the HBM microbiome, it gets influenced by the infant's sex, as hormonal differences between the males and females alter the gut microbiome (Markle et al. 2013; Moossavi et al. 2019). All of the above-discussed factors tend to influence the composition of the HBM in terms of lipid profile, HMOs, cytokine levels, creatine, and riboflavin. The composition also tends to differ depending upon the demographic settings of the mother (Gay et al. 2018; Gomez-Gallego et al. 2016).

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## 9.10 Global Variation in Dominant Bacterial Taxa

The HBM microbiome also varies depending on the geographic location of the lactating mothers. This global variation can be attributed to differences in diet, economic setting, and usual birth methods opted in the country. Several studies have shown that Caucasian Canadian women's milk microbiome predominantly contains *Staphylococcus*, *Pseudomonas*, *Streptococcus*, and *Lactobacillus*, while *Streptococcus* predominates in Mexican-American women (Davé et al. 2016; McGuire and McGuire 2017; Urbaniak et al. 2016). Another study showed that Spanish women's milk microbiome contained *Staphylococcus*, *Pseudomonas*, *Streptococcus*, and *Acinetobacter* (Boix-Amorós et al. 2019). The microbiome of women in the United States showed the presence of *Serratia* and *Corynebacteria*, while that of Finland showed the presence of *Leuconostoc*, *Weissella*, *Lactococcus*, and *Staphylococcus* (Cabrera-Rubio et al. 2012; McGuire and McGuire 2017). More studies regarding the Asian HBM microbiome are required to draw a better contrast.



## 9.11 Potential Factors to Modulate Milk Microbiota

Once we are clear with the source, difference, and importance of the HBM microbiome, it becomes evident to know how the milk microbiota can be modulated to mimic that of a healthy mother. Dietary interventions can prove helpful with the administration of probiotics and topical probiotics in improving the milk microbiome since the seeding route is both entero-mammary and retrograde (Stinson et al. 2021). The dairy industry predominantly uses phage therapy to modulate the bovine milk microbiome and protect bovine mothers from mastitis and other diseases. The same approach can also be used for the HBM microbiome (Dias et al. 2013; Porter et al. 2016; Stinson et al. 2021).

## 9.12 Role of Bacterial Extracellular Vesicles in HBM Microbiota (Their Involvement in the Vertical Transfer of Gut Microbiota)

The gastrointestinal tract of humans is housed in approximately 100 trillion microorganisms, which work in mutual harmony with the host to support the advancement of the host's defense mechanisms. The initial establishment of the gut microbiota throughout childhood has long-term consequences for human health. Several variables, including the type of delivery, antibiotic treatment, surroundings, and dietary exposure, are thought to trigger early infant gut colonization (Ojo-Okunola et al. 2018). Diet is vital in promoting the growth of the infant's immunity. The most significant driver of nourishment for newborns is human breast milk (HBM), which is recognized to include immunological factors such as immunoglobulins, cellular machinery of the immune system, antibacterial agents (like lactoferrin and lysozyme), cytokines, and other important factors (Kim and Yi 2020). Previously, microorganisms in HBM were considered a sign of harm or contamination, but multiple investigators have shown that HBM includes microbial species by employing culture-dependent strategies. It is understood that HBM includes many commensal microbes that may influence how the infant's gut colonizes (Murphy et al. 2017).

Extracellular vesicles (EVs) are membrane vesicles with a dimension of a few nanometers having a wide range of bioactive molecules within, including lipids, membrane proteins, cytoplasmic proteins, nucleic acids, and peptides found in the cytosol. Every microbe produces EVs, and this is a widely recognized fact. According to the properties of their membranes, microbes are subdivided into Gram-positive (G+) or Gram-negative (G-) bacteria. The EVs produced by G+ bacteria are typically known as bacterial membrane vesicles, while those produced by G-bacteria are known as outer membrane vesicles (Brown et al. 2015). Microbe-derived EVs in bodily fluids like blood, urine, or feces suggest that these microbes can influence host cellular machinery by triggering host receptors, releasing different biologically active chemicals, or fostering EVs into the cells of hosts.

In an investigation by Kim and Yi, the top categories of bacteria found in mammalian breast milk they included *Bacteroides*, *Acinetobacter*, and *Lactobacillus*, whereas *Streptococcus* and *Staphylococcus* predominated in microbial samples (Kim and Yi 2020). Such findings suggest that the microorganisms producing EVs have a variance and do not always correspond with the microorganisms found in human breast milk. Additionally, this research indicates that EVs may aid in the vertical transmission of commensal microbes from women to their offspring. In addition, because such vesicles are abundant in microRNAs, they may also affect children's mucosal defenses and boost the range of their intestinal microbiomes (Macia et al. 2019).

Furthermore, Wang et al. (2019) examined the exosomes derived from human milk by contrasting the milk of term pregnancies to that of preterm pregnancies. Researchers observed that peptide contents of preterm and term milk exosomes differed significantly. These results suggest a possible variation in the bacterial EV makeup of human milk, which may impact a child's future health-related consequences. Human breast milk contains exosomal miRNAs (exomiRs) encased within the exosomes. These exomiRs are transported from the human milk to the newborn via the alimentary canal and may be extremely important for the immunological functioning of the child. The development of thymic regulatory T cells (Tregs), which prevents Th2-mediated atopic sensitization and atopic effector responses, is another benefit of milk-derived miRNAs. Exosomes generated from human and bovine milk exhibit exceptionally high concentrations of immune-modulatory miRNAs (miR-155, miR-146a, and miR-21) that have been demonstrated to be linked to thymic Treg development (Mirza et al. 2019).

It is crucial to separate EVs to examine their biochemical components and/or explicitly investigate their function to obtain familiarity with the roles played by EVs found in milk. The prevailing isolation procedures for milk EVs have been modified from procedures created for EV isolation from plasma or conditioned cultured media. Most of these techniques encompass size exclusion chromatography (SEC), density gradient centrifugation, commercial precipitation kits, and ultracentrifugation (Hu et al. 2021).

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## 9.13 Conclusions and Future Perspectives

The "ideal" source of nutrition for infants is human milk. It is recommended to breastfeed, since it promotes healthy newborn development. Breastfeeding exposes the newborn to milk-associated bacteria, which may influence the newborn's microbiological, metabolic, and immunological health. Since this could alter the perception of the microbial BM ecosystem and have implications for infant health, more research is required to understand the connections between the microbial components of the HBM microbiome, including bacteria and fungi, archaea, and viruses. To improve our understanding of HBM microbiome regulation, it is essential to consider the complexity of BM and the variables that influence its composition.

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