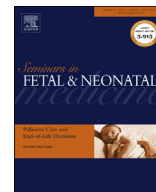




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## Review

## The human milk microbiome and factors influencing its composition and activity

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## S U M M A R Y

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Beyond its nutritional aspects, human milk contains several bioactive compounds, such as microbes, oligosaccharides, and other substances, which are involved in host–microbe interactions and have a key role in infant health. New techniques have increased our understanding of milk microbiota composition, but few data on the activity of bioactive compounds and their biological role in infants are available. Whereas the human milk microbiome may be influenced by specific factors – including genetics, maternal health and nutrition, mode of delivery, breastfeeding, lactation stage, and geographic location – the impact of these factors on the infant microbiome is not yet known. This article gives an overview of milk microbiota composition and activity, including factors influencing microbial composition and their potential biological relevance on infants' future health.

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## 1. Introduction

Recent reports have revealed the importance of our gut microbiome for optimal health. Accumulating evidence highlights the potential role of microbes in metabolic, immunological, and microbial programming [1]. Microbes are among the most important environmental factors providing specific signals to guide immune system development and maturation [2,3]. Shifts in microbiota composition and activity appear to be related to adverse human health outcomes [4]. The maternal microbial environment impacts the newborn's immune development and, consequently, the infant's health both early and later in life. The maternal microbiota is now recognized as a significant determinant of the maternally transferred factors that impact the child's health [5,6]. Outcomes can be affected by specific perinatal factors that also alter infant microbiome development. For example, excessive use of antibiotics, unbalanced diet, increasing incidence of caesarean section deliveries, unnecessarily stringent hygiene, and continuous stress influence the maternal microbiome. Alterations and disturbances in microbiota composition along with a reduction in microbial

diversity or richness have been described as strong risk factors for the development of lifestyle diseases, such as allergies, diabetes, obesity, and metabolic syndrome, irritable bowel syndrome and other inflammatory-related problems [4,7]. The maternal microbiota and infant diet play a key role in infant growth, adequate microbial colonization, immune system maturation, and metabolic development. Then, the infant stepwise microbial colonization process will have an impact on metabolic and immunological response, and these in turn may have an impact on programming of health later in life [3,8]. This review describes the impact of the maternal microbiota on infant health through breastfeeding, and provides a broad overview of milk microbiota composition and activity, along with the factors influencing microbial composition and their potential biological relevance.

## 2. Human breast milk: more than infant food

Human milk provides complete nutrition for the infant. Human milk's energy, nutrients, and bioactive components directly influence the development of newborn infants. Among its bioactive factors, HM contains several immune substances, such as immunoglobulins, cytokines and chemokines, growth factors, hormones, and antibodies. It also contains other nonspecific compounds, such as specific peptides, lactoferrin and other whey proteins,

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oligosaccharides, and a large number of bacteria [9]. All these components are transferred to the infant through breastfeeding.

Human milk is the most relevant postnatal element for the metabolic and immunological programming of the infant's health [10,11]. Breastfeeding shapes the infant immune system development and is needed for adequate gut function and immune homeostasis maintenance [12]. Breast-fed infants have been shown to have a reduced risk of necrotizing enterocolitis and diarrhea, allergy and asthma, inflammatory bowel disease, diabetes, and obesity, among other problems [13]. In addition, differences in gut microbiota composition and activity between exclusively breast- and formula-fed infants have been widely reported [14–18]. Breast milk is also a postnatal microbial link as its high diversity of microbes drives the infant's microbial colonization [19–27]. It has been reported that specific maternal gut microbial strains belonging to *Bifidobacterium* and *Staphylococcus* spp. are transmitted to infants [28–31]. These data suggest a unique link between family members as specific strains are present in each mother–infant pair. The findings also suggest that transfer of aberrant microbiota from the mother is possible. Altogether, it is important to define the milk microbiota composition and factors that may be transferrable as commensal bacteria in human milk. For example, transmission of lactic acid bacteria and bifidobacteria from the breastfeeding mother may form a natural protective mechanism that improves development of the infant's gut microbiota for later resilience and reduction in the risk of diarrheal and other dysbiosis-related problems. Improved knowledge in this area may suggest novel means of modulating the maternal and infant microbiota in order to potentially reduce the risk of specific microbiota-associated diseases (Fig. 1).

### 3. Human breast-milk microbiome

Historically, human breast milk has been considered an almost sterile fluid, but this dogma has been revised over the years. Studies

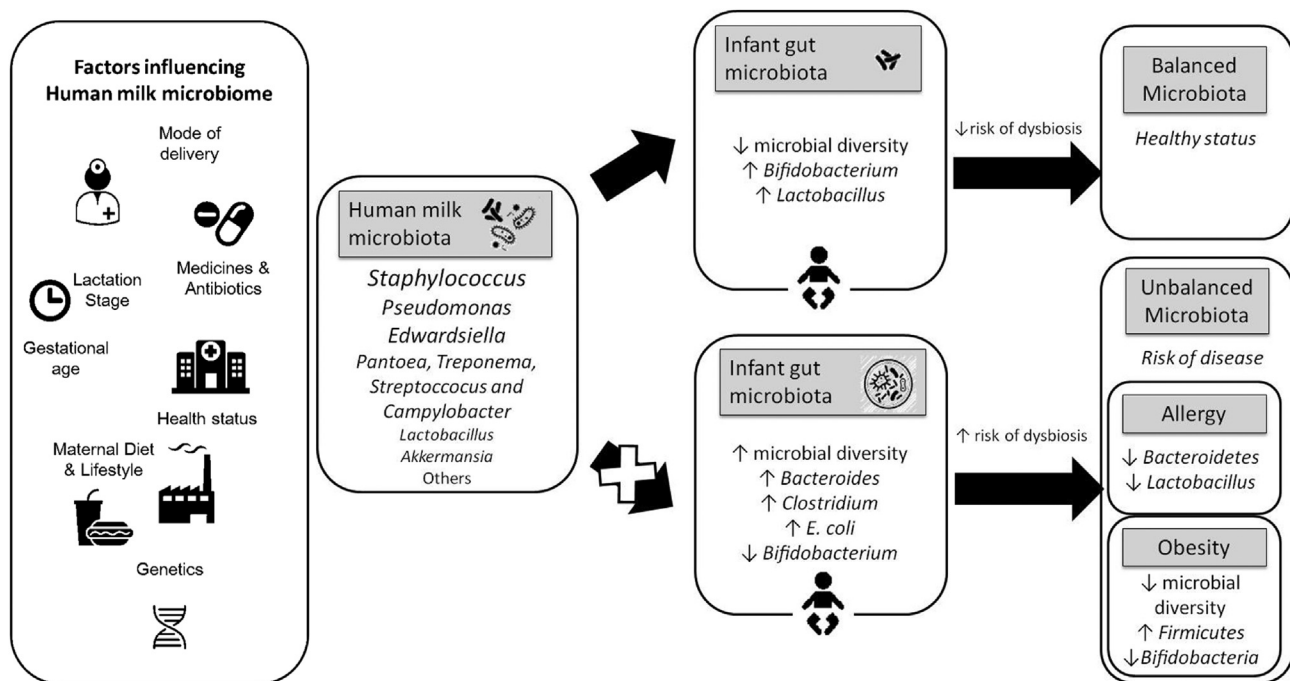
in primates have reported that rhesus monkey (*Macaca mulatta*) milk contains culturable strains of at least 19 species of bacteria belonging to at least five different genera [32,33]. Similar results have been described in other mammals [34].

Traditionally, the presence of microbes in human milk has been confirmed by use of culture-dependent techniques. Most of the bacteria isolated from milk belong to *Staphylococcus*, *Streptococcus*, and *Lactobacillus* and *Bifidobacterium* spp., latter two genera having several strains with a long record of use as probiotics [35]. It has been stated that “human milk constitutes one of the main sources of bacteria to the breastfed infant gut since an infant consuming approximately 800 mL/day of milk would ingest between  $1 \times 10^5$  and  $1 \times 10^7$  bacteria daily” [26,27]. Moreover, the biological role of these microbes on neonatal health at short and long-term has not yet been identified.

The origin of breast milk bacteria is currently not known, but their presence corresponds to a perinatal period which starts during the third trimester of pregnancy and continues through lactation [25]. Several reports have proposed that the human milk microbiota could derive from colonization from the mother's skin, the infant's oral cavity during suckling, or the mother's gut via the entero-mammary pathway [1,26,27,36]. However, a commensal microbiota has been reported in human breast tissue [37,38], suggesting that specific microbes inhabit the breast tissue and potentially colonize the milk ducts.

With the development and application of culture-independent techniques (most of them based on polymerase chain reaction techniques) and next-generation sequencing platforms, the presence of microbial DNA has been confirmed. These techniques have confirmed the existence of a rich and diverse breast-milk microbial community [20–27,35].

Human milk harbors a unique microbial ecosystem which differs from any other in humans. The milk microbiota is not linked with any mucosal or fecal samples, nor does it seem to be a subset of any other specific human sample [21]. The breast milk microbial



**Fig. 1.** Human milk microbiota composition and comparison between breastfeeding and formula-feeding microbiota. Metagenome analysis of human milk shows that human milk microbiota is mainly dominated by *Staphylococcus*, *Pseudomonas* and *Edwardsiella*, but other groups are also represented in minor amounts [41,42]. Breast-fed and formula-fed infants have different bacterial populations which seems to modulate the susceptibility of non-communicable diseases (NCDs) such as allergy and/or obesity during infancy and/or in adult life.

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