

# The Human Milk Microbiome – What’s Normal, and Possible Factors Mediating Variability

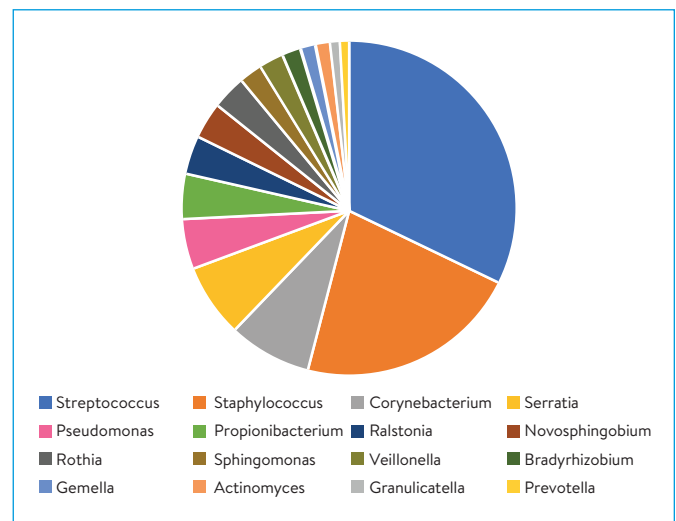
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Human milk is inarguably the only food designed by Mother Nature intended solely for the nourishment of humans. As such, carefully characterizing human milk composition is not only critical to understanding optimal nutrition during infancy, but likely also to understanding optimal nutrition throughout the human lifespan.

Since the advent of “germ theory” in the mid-19<sup>th</sup> century (and likely before), milk produced by healthy women has been assumed to be sterile. Indeed, the presence of culturable bacteria in milk has long been considered a sign of breast infection or sample contamination. A classic example of this belief can be gleaned from a study conducted some years ago by Wyatt and Mata<sup>1</sup> designed to analyze milk produced by impoverished Guatemalan women. The authors concluded, “The presence of Enterobacteriaceae in human colostrum and milk reflects the low levels of personal hygiene and environmental sanitation in the population studied.” It now appears that these bacteria were likely neither a consequence of poor sanitation nor environmental contamination. Instead, they were likely ubiquitously present in milk produced by these at-risk women living in a difficult environment. Even the researchers behind the now-famous Human Microbiome Project did not consider including milk as a biological specimen of sufficient interest in terms of identifying its microbial content.<sup>2,3</sup>

However, using both culture-dependent and culture-independent (molecular) methods, myriad independent investigators have now identified and sometimes quantified a rich and diverse microbial community in human milk.<sup>4-8</sup> As illustrated in Figure 1, the most abundant genera found in milk tend to be *Streptococcus* and *Staphylococcus*, followed by *Corynebacterium*, *Serratia*, *Pseudomonas*, and *Propionibacterium*.<sup>9</sup> As such, attention has shifted from a focus on the pathogenicity of microbes in milk to understanding variation in milk’s microbial profiles with the eventual hope of delineating the sources and health implications of these microbes to both mothers and infants.

For instance, several investigators have explored whether milk’s bacterial profiles shift over the postpartum period. Results are mixed in this regard, but generally indicate that changes are minor if any.<sup>7,10,11</sup> Delivery mode has also been examined, again with mixed findings. For instance, Cabrera-Rubio and



**Fig 1. Relative abundance of bacterial genera identified in human milk samples.<sup>9</sup>**

Left to right, Highest abundance (%) to lowest abundance (%) of bacterial groups comprising  $\geq 0.5\%$  of total sequence reads in human milk samples (n=79)

**Source:** Williams JE et al. *J Hum Lact.* 2017;33(3):540-551. Adapted by permission of Authors.

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colleagues<sup>10</sup> reported decreased diversity in milk produced by women delivering via surgical intervention compared to those delivering vaginally. In addition, they reported that the microbiome of milk produced by women undergoing emergency Cesarean deliveries was comparable to that of women delivering vaginally; but milk produced by women having elective Cesarean deliveries was different in composition than that of the other two groups. Khodayar-Pardo and colleagues<sup>12</sup> found greater amounts of bacteria in women delivering via Cesarean. Conversely, Urbaniak and coworkers<sup>13</sup> found no differences in the milk microbiome associated with delivery mode. Kumar and colleagues<sup>14</sup> also found differences related to delivery mode, but this was modified by the woman’s country of origin. Clearly, additional studies will need to better understand the potential of delivery mode (and all that goes with it, like antibiotic use) to influence the microbes in a woman’s milk.

Maternal nutritional status has also been studied as a possible mediator of the human milk microbiome. Again, like time postpartum and delivery mode, findings are inconsistent. For instance, Cabrera-Rubio and colleagues<sup>7</sup> reported that milk from obese mothers tended to contain more bacteria (although it was less diverse) than that produced by healthy-weight women. Higher maternal body mass index (BMI) was also related to greater relative abundances of *Lactobacillus* and *Staphylococcus* in colostrum and mature milk, respectively, and lower relative abundance of *Bifidobacterium* in milk produced at 6 months. Davé and coworkers<sup>15</sup> also found that prepregnancy BMI was inversely correlated with *Streptococcus* ( $r = -0.67$ ), and positively associated with microbial diversity ( $r = 0.77$ ) in milk produced by Mexican-American women ( $P < 0.05$  for both). These data, however, are difficult to assess in terms of causality because BMI is completely confounded by chronic maternal nutrient intake (both energy-yielding and non-energy-yielding nutrients).

Recent studies from our group, however, have found that usual maternal intake of many dietary components is associated with variation in the milk microbiome.<sup>9</sup> For instance, relative abundance of Firmicutes is highest in milk produced by women consuming the highest levels of energy-yielding macronutrients; higher relative abundance of Firmicutes in milk is also related to increased maternal consumption of long-chain, polyunsaturated, omega-3 fatty acids. Relationships between milk microbes and maternal consumption of amino acids, vitamins, and minerals were also noted. Whether these relationships are causal in nature, occur due to shifts in the mother’s gastrointestinal microbiome, or are due to direct effects of maternal diet on variation in milk nutrient content will require further study, including randomized controlled intervention trials.

Aside from variation in maternal nutritional status, several studies have provided convincing evidence that bacteria found in local fermented foods and probiotic supplements can be coordinately found in milk produced by women consuming the food or supplement.<sup>16,17</sup> Additional controlled intervention trials will be needed to understand how maternal nutritional status and dietary intake influence (or do not affect) the milk microbiome. Included in these studies should be analysis of the milk for micronutrient and macronutrient content, and analysis of the maternal fecal microbiome.

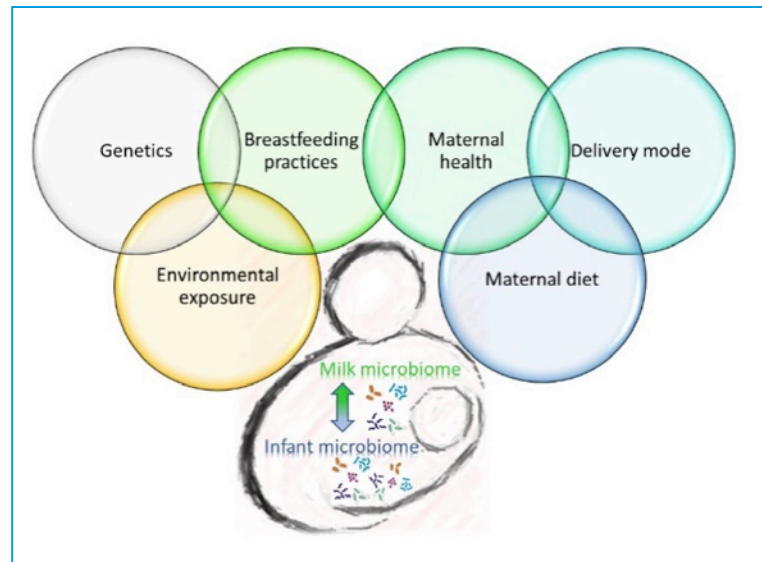
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A limited number of studies have also investigated the possibility that milk constituents, other than microbes, might be related to its microbial community structure. For instance, we have shown that the concentration of human milk oligosaccharides is positively associated with relative abundance of *Staphylococcus* and that this relationship is likely causal in nature.<sup>9,18</sup> Variation in immune cell profiles in milk is also associated with variation in milk microbial communities,<sup>9</sup> and this appears to be unique to each woman. Again, whether these relationships are causal in nature, and if so their directionality, will need further longitudinal study.

Regardless of its source, emerging research provides solid evidence supporting considerable variability in the human milk microbiome around the world.<sup>14</sup> We hypothesize that these differences are likely due to a complex interplay among maternal genetic variation, cultural and behavioral differences, environmental conditions, and pathogen risk (Fig 2). Indeed, it is likely that human milk has been “designed” over the millennia to fit the unique nutritional and immunologic needs of infants living in a particular culture and location, a process we refer to as *eco-homeorhesis*.<sup>19</sup> If this is true, then there likely is no ‘one-size-fits-all’ construct when it comes to the milk microbiome and even optimal infant nutrition around the globe.

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**Fig 2. Factors influencing variability in the human milk microbiome.**

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