Title: Got bacteria? The astounding, yet not-so-surprising, microbiome of human milk

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Abstract

Contrary to long-held dogma, human milk is not sterile. Instead, it provides infants a rich source of diverse bacteria, particularly microbes belonging to the *Staphylococcus*, *Streptococcus*, and *Pseudomonas* genera. Very little is known about factors that influence variation in the milk microbiome among women and populations, although time postpartum, delivery mode, and maternal factors such as diet and antibiotic use might be important. The origins of the bacteria in milk are thought to include the maternal gastrointestinal tract (via an entero-mammary pathway) and through bacterial exposure of the breast during nursing. Currently, almost nothing is known about whether variation in microbe consumption by the infant via human milk and that of the mammary gland, itself, impacts short- and/or long-term infant and maternal health although several studies suggest this is likely. We urge the clinical and public health communities to be patient, however, in order to allow human milk and lactation researchers to first understand what constitutes 'normal' in terms of the milk microbiome (as well as factors that impact microbial community structure) prior to jumping the gun to investigate if and how this important source of microbes impacts maternal and infant health.

Introduction Since the completion of the Human Microbiome Project (HMP), much has been learned about the complex microbial communities that live in and on the human body. Indeed, even prior to its commencement in 2007, the core team involved in this NIH-funded project posited that, considering the estimated 10-fold greater abundance of microbial than human cells, humans should actually consider themselves ‘supraorganisms’ that benefit not only from our own evolutionary history but also from that of the rapidly evolving microbes with which we live [1]. Although this oft-cited 10:1 ratio probably represents an over-approximation [2], the core HMP team was prophetic in urging all involved to exhibit patience while scientists worked to define ‘normal’ before determining the impact of the microbiota on disease predisposition and pathogenesis.

The initial idea for the HMP is often attributed to Relman and Falkow [3] who called for a “second human genome project: A large-scale genomic sequence survey of the four major microbial niches within the human body: the mouth, intestinal tract, vagina and skin.” Little did they know that they were missing what might be one of the most important niches for bacterial inhabitation within the human-microbe supraorganism: the lactating mammary gland. Indeed, as we have previously argued [4], human milk is likely mother nature’s prototypical probiotic food – safely supplying a cocktail of environmentally-determined microbes and their associated genes and antigens to the newborn infant during a critical period of growth and development. How did these world-class researchers (as well as those who worked to design the HMP) miss this important detail?

The answer to this question lies in the paradigm shift that has taken place over the last decade concerning the sterility (or lack, thereof) of human milk. Indeed, although there have

47 existed reports of viable bacteria in milk produced by healthy women for over 4 decades [e.g.,
48 5-9], because of long-standing dogma that blood-derived human fluids are sterile, the origin of
49 these bacteria was thought to be from the skin or other environmental sources. A classic
50 example of the influence of prevailing dogma on interpretation of results was a study
51 conducted Wyatt and Mata [8] designed to analyze milk produced by poor Guatemalan women;
52 they concluded, “The presence of Enterobacteriaceae in human colostrum and milk reflects the
53 low levels of personal hygiene and environmental sanitation in the population studied.” It now
54 appears that these bacteria are neither a consequence of poor sanitation nor environmental
55 contaminations, but instead are ubiquitously present in milk produced by healthy breastfeeding
56 women.

57 Indeed, most human lactation experts now concur that milk is not sterile. This
58 astounding, yet not-so-surprising, sea-change in thought should transform how we think about
59 human milk. Here, we briefly describe what we know about the milk microbiome as well as a
60 litany of research needed to answer the endless list of unrequited questions.

61 ***Global variation in dominant bacterial taxa*** Although many excellent studies reporting
62 the presence of viable bacteria using culture-dependent methods have been published [e.g.,
63 10-12], in all of these studies the researchers were limited by which bacterial taxa they were
64 prepared to identify – often driven by specific interests of a research group. As such, we will
65 generally focus here on describing what has been published using high-throughput methods to
66 characterize the overall bacterial communities in human milk. The first such study to use these
67 methods was conducted by our research group [13] which analyzed microbial community
68 structure of milk produced by 16 healthy women living in the United States. Using

pyrosequencing of the DNA encoding the V1-V2 hypervariable regions of bacterial 16S rRNA, we found that the most abundant bacterial genera were *Staphylococcus*, *Streptococcus*, *Serratia*, *Pseudomonas*, and *Corynebacteria*. All milk samples contained a “core” of these genera along with *Ralstonia*, *Propionibacterium*, *Sphingomonas*, and *Bradyrhizobiaceae*; although, and importantly, inter-woman variability was much greater than intra-woman variability. Cabrera-Rubio and colleagues [14] used similar methods (but primers targeted to V1-V3) and found that *Leuconostoc*, *Weisella*, *Lactococcus*, and *Staphylococcus* were predominant in mature milk produced by women living in Finland; *Streptococcus* was more relatively abundant in colostrum. A direct comparison of these bacterial profiles to those of Hunt et al. [13] showed clear differences, but methodological variation may provide some explanation for this finding.

Work from Urbaniak and collaborators [15] utilizing the Ion Torrent platform and primers for the V6 region found that milk produced by healthy women living in Canada was dominated by *Actinobacter*, *Stenotrophomonas*, *Pseudomonas*, *Streptococcus*, and *Staphylococcus*. This same group later reported that milk produced by healthy Caucasian Canadian women was dominated by *Staphylococcus*, *Pseudomonas*, *Streptococcus*, and *Lactobacillus* [16], and Davé and coworkers [17] found that members of the genus *Streptococcus* dominated milk produced by Mexican-American women. Boix-Amorós et al. [18] reported that milk microbiota of Spanish women was dominated by *Staphylococcus*, *Pseudomonas*, *Streptococcus*, and *Acinetobacter*; importantly, they also reported a median bacterial load of 10^6 cells/mL, suggesting that exclusively breastfed infants consume 7-8 billion bacterial cells each day.

In summary, results from next-generation sequencing approaches suggest that human

milk is typically dominated by *Staphylococcus*, *Streptococcus*, and *Pseudomonas*. Differences in milk collection, DNA extraction and amplification, DNA sequencing, and bioinformatics may explain some of the differences in other bacteria reported thus far. Alternatively, genuine differences may exist in the bacterial communities of milk produced around the globe. Additional collaborative research using standardized methods will be needed to test this hypothesis.

Time postpartum, delivery mode and milk microbes Aside from simply attempting to characterize what is ‘normal’ in terms of the complex bacterial communities in milk, it is also important to understand factors related to their variation. A limited number of studies have investigated the potential relationship between time postpartum and the milk microbiome. These include research conducted by Cabrera-Rubio et al. [14] who provide evidence that there is a shift from *Weisella*, *Leuconostoc*, *Staphylococcus*, *Streptococcus*, and *Lactococcus* in colostrum to more typical inhabitants of the oral cavity (e.g., *Veillonella*, *Leptotrichia*, and *Prevotella*) in mature milk; no statistical analyses were conducted, however, making the data somewhat difficult to generalize. Khodayar-Pardo and colleagues [19] also reported an effect of time postpartum on the milk microbiome: *Bifidobacterium* and *Enterococcus* increased over lactation in milk produced by Spanish women. However, in a study involving Canadian women and utilizing more sophisticated statistical analyses, there was no evidence of an effect of time postpartum on milk microbial structure [16]. Soto and colleagues [20] also found no differences in the distribution of bacteria belonging to either *Lactobacillus* or *Bifidobacterium* genus in milk produced from 1 wk to 1 mo postpartum. Consequently, it is fair to conclude that we currently do not know if there is an important effect of time postpartum on the human milk microbiome.

Several investigators have also evaluated whether delivery mode is related to the milk microbiome. For instance, Cabrera-Rubio and colleagues [14] reported that milk produced by Finnish women who had undergone elective cesarean delivery appeared to contain different bacterial communities than those who had delivered vaginally or via emergency cesarean (e.g., relative abundances of *Leuconostoc* and *Weissella* were higher and *Acinetobacter* lower in the former than the latter). The authors speculated that these differences could not be attributed to the procedure itself, but instead to factors associated with absence of physiological stress or hormonal signals that could influence the microbial transmission to the breast. In a more recent study [21], the complex microbiome of milk produced at 1 mo postpartum was found to be different when produced by 6 Spanish women who had delivered vaginally compared to 4 who had delivered via cesarean. In addition, higher bacterial diversity and richness was found in milk samples from women delivering vaginally. Khodayar-Pardo and colleagues [19] also reported that *Bifidobacterium* was detected more frequently in milk produced by women having had vaginal deliveries. Conversely, Urbaniak et al. [16] did not find differences between milk bacterial communities based on delivery mode. They also found no effects of preterm vs. term birth and infant sex on milk microbiome. It is possible that these conflicting data may be due, at least in part, to differences in methods such as the particular primers used to amplify the bacterial DNA. In addition, differences in computational approaches may be important.

Maternal health and milk microbes Maternal health variables may also potentially impact the milk microbiome. In the only controlled intervention trial designed to rigorously investigate whether maternal diet can impact the human milk microbiome, women with mastitis were randomly assigned to receive a supplement of human milk-derived *L. fermentum*

CECT5716, *L. salivarius* CECT5713, or standard antibiotic treatment [22]. Not only were the probiotic bacteria found in the milk after the treatment, but both treatments offered significant health benefits to the mothers over standard antibiotic treatment. This study provides unparalleled evidence that acute dietary consumption of certain bacteria can impact the human milk microbiome.

Long-term energy balance may also impact the milk microbiome, as illustrated by the findings of Cabrera-Rubio and colleagues [14] who reported that milk from obese mothers tends to contain more bacteria (although it is less diverse) than that from 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 mo. Davé and coworkers [17] 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 very 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). Indeed, we have shown that increased consumption of a more nutrient- and calorie-rich diet is positively associated with relative abundance of Firmicutes in the stools of lactating women [23]. No such relationship was found with prepregnancy or current BMI. Because maternal gastrointestinal (GI) microbes may impact the milk microbiome (see below), we urge the research community to look well beyond maternal body weight (or BMI) as a proxy for maternal nutritional status.

There is also limited evidence that maternal antibiotic use and other medical

interventions might impact the milk microbiota. In a study of women living in Germany or Austria, the concentration (CFU/mL) of lactobacilli- or bifidobacteria-positive samples was lower in milk produced by women who had received antibiotics during pregnancy or lactation [20]. Although these data could lead to the conclusion that antibiotic use *decreases* bacterial load in milk, the study by Arroyo et al. [22] discussed above found that mean bacterial counts in the milk produced by women receiving antibiotics were *higher* than that of milk produced by women taking probiotics. Thus, it is more likely that antibiotic use leads to a dysbiosis of the milk microbiome without necessarily decreasing the total bacteria present.

Finally, in a case report of a lactating woman undergoing chemotherapy for Hodgkin's lymphoma, Urbaniak and collaborators [24] found that members of the *Bifidobacterium*, *Eubacterium*, *Staphylococcus*, and *Cloacibacterium* genera decreased while those in the *Acinetobacter*, *Xanthomonadaceae* and *Stenotrophomonas* genera increased following treatment. Chemotherapy also decreased bacterial diversity in the milk.

In summary, there is ample evidence that individual bacterial taxa and overall microbial community structure might be modifiable by factors such as time postpartum, delivery mode, acute and chronic maternal diet, and medical interventions. However, available data are sparse and contradictory, needing to be replicated using controlled, longitudinal study designs with comparable milk collection, analysis, and computational approaches.

Viability and origin(s) of milk microbes Whether all of the bacterial DNA found in human milk represents that obtained from viable microbes is unknown, but a growing literature has shown at least some of it is. It is noteworthy that recent studies have tended to focus on bacterial taxa (e.g., members of the *Lactobacillus* genus) not previously studied because of their

generally nonpathogenic, and often health-promoting, characteristics related to antimicrobial and immunologic potential of milk-derived bacterial strains (mostly lactic acid bacteria) [11, 25-26]. Together, these and other studies have conclusively shown that much of the bacteria in milk with known growth cultures are indeed viable. Whether more fastidious, difficult-to-culture bacteria are similarly viable is an important topic for further research.

The origin of the milk microbiome is also an area of active debate, but a growing literature suggests that its genesis is likely complex [see 27 and 28 for more detailed reviews of this topic]. For instance, viable bacteria reside in mammary tissue of women who have never breastfed [29-31]. These findings strongly suggest that one source of bacteria for milk includes the mammary gland itself. Where these bacteria come from is not entirely known, but two likely sources are external (via the mammary ducts) and internal via an entero-mammary (GI tract to mammary tissue) pathways. During breastfeeding, there is substantial retrograde flow of milk in the mammary gland during suckling [32]. As such, anything that is present in the infant's mouth is likely to be present (at least for a short period of time) in the mammary ductal system. Indeed, both human milk and saliva are rich in *Streptococcus* spp. [33], suggesting the possibility that one might shape the other. Similarly, some bacterial taxa commonly isolated from skin (e.g., *Staphylococcus* and *Corynebacterium*) are also found in human milk [34]. However, correlation does not infer causality, and delineating the potential directionality of this relationship (if, indeed, a causal relationship exists) will require controlled intervention studies.

Studies also point to the possibility of selective translocation of bacteria from the mother's GI tract to the mammary glands. One of the first studies to carefully examine this was conducted by Perez and colleagues [35] who aseptically collected milk and peripheral blood

from healthy breastfeeding women and feces from their infants. They also studied bacterial translocation to extra-intestinal tissues in mice. Their results suggested that intestinally-derived bacteria might be transported to the lactating mammary gland within mononuclear cells. Using a caco-2 cell culture system, Langa and colleagues showed that 2 lactobacilli strains isolated from human milk can cross an epithelial cell layer via a dendritic cell-mediated mechanism – at least *in vitro* [28]. In addition, milk produced by mice fed a labeled strain of *Enterococcus faecium* during pregnancy was found to harbor this exact strain [28]. Thus, although additional work is needed, there is mounting evidence that the bacteria in human milk originate from a variety of sources. Creative intervention studies utilizing unique and/or labeled bacterial strains delivered orally to the mother and/or infant will be needed to untangle this complex web of potential origins of the milk microbiome.

Why should we care? Aside from representing a scientifically-relevant paradigm shift, the finding that human milk and its source, the mammary gland, are not sterile is probably important for the health of both mothers and their infants. For infants, variation in early colonization of the GI tract appears to be related to both short- and long-term health and risk of disease, such as necrotizing enterocolitis, celiac disease, inflammatory bowel disease, and inflammatory bowel syndrome [36-38]. Even childhood malnutrition and development of kwashiorkor has been associated with variation in early fecal microbiomes [39]. As such, understanding factors (such as breastfeeding and human milk) that are associated with early establishment of the infant's microbial niches may lend important insight as to nutrition-related diseases in childhood and beyond. In addition, microbial dysbiosis in the mammary gland has been strongly associated with inflammatory breast conditions such as mastitis [27] and cancer

[29-30], and breastfeeding women living in an HIV-endemic region and with HIV RNA in their milk have been found to have a different pattern of in their milk when compared to women without HIV RNA in their milk [25]. Consequently, exploration of what is ‘healthy’ in terms of a mammary microbiome might not only help identify women at risk for breast cancer or other infectious diseases, but might also provide new, innovative ways to help prevent and treat these conditions.

Next steps Undoubtedly, the research community is just beginning to chip away at the tip of the iceberg in terms of understanding the milk microbiome, its origins and sources of variation, and its implications for health and disease. Drawing heavily on work from the HMP during its early stages of development [1], here we propose a series of ‘next questions’ that urgently need to be answered in this regard.

- How stable and resilient is the milk microbiome over the course of a feed, a single day, a single lactation, and during the course of a lifespan?
- How similar are the milk microbiomes between members of a family, a community, and between communities living in different environments?
- Do delivery mode and other medical interventions (e.g., antibiotic use) or cultural practices (e.g., skin-to-skin contact, kangaroo care, bathing, pumping) impact the milk microbiome, and how durable are these effects over time? What about typical breastfeeding and childcare practices (e.g., feeding on demand, allo-parenting, daycare)?
- Does chronic and/or acute maternal nutritional status impact the milk microbiome? What about the potential impact of supplementary foods to the infant?

- Do all breastfeeding women share an identifiable ‘core’ milk microbiome, and if so, how is it acquired and transmitted?
- How does maternal genetics impact the milk microbiome, and how might this diversity impact microbial and host adaptations to markedly different lifestyles and physiological or pathophysiological states?

Not until we gain a basic understanding of what constitutes a ‘normal’ milk microbiome across healthy populations and how genetic, environmental, and behavioral factors impact its diversity and community structure will we ever be able to move into the next, critically important realm of correlating variation in milk microbiome to maternal and infant health and disease. Similar to what the core team leading the HMP recommended over a decade ago, we urge patience while scientists work to define ‘normal’ before examining the impact of the milk microbiota on disease predisposition and pathogenesis. This push for prudence is based, in large part, on the possibility that there might not exist a one-size-fits-all optimal milk microbiome. In other words, ‘normal’ might vary – a concept which we have referred to as eco-homoeorhesis [41]. Let’s not put the proverbial cart (investigating disease) before the essential horse (understanding health).

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References

1. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI: **The human microbiome project.** *Nature* 2007, 449:804-810.
2. Sender R, Fuchs S, Milo R: **Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans.** *Cell* 2016, **164**:337-340.
3. Relman DA, Falkow S: **The meaning and impact of the human genome sequence for microbiology.** *Trends Microbiol* 2001, **9**:206-208.
4. McGuire, MK, McGuire MA: **Human milk: mother nature's prototypical probiotic food?** *Adv Nutr* 2015, **6**:112-123.
5. Carroll L, Osman M, Davies DP, McNeish AS: **Bacteriology of raw breast milk.** *Lancet* 1979, **2**:1186.
6. Eidelman AI, Szilagyi G: **Patterns of bacterial colonization of human milk.** *Obstet Gynecol* 1979, **53**:550-552.
7. el-Mohandes AE, Schatz V, Keiser JF, Jackson BJ: **Bacterial contaminants of collected and frozen human milk used in an intensive care nursery.** *Am J Infect Control* 1993, **21**:226-230.
8. Wyatt RG, Mata LJ: **Bacteria in colostrum and milk of Guatemalan Indian women.** *J Trop Pediatr* 1969, **15**:159-162.
9. Thomsen AC, Hansen KB, Møller BR: **Leukocyte counts and microbiologic cultivation in the diagnosis of puerperal mastitis.** *Am J Obstet Gynecol* 1983, 146:938-941.
10. Martín R, Langa S, Reviriego C, Jiménez E, Marín ML, Xaus J, Fernández L, Rodríguez JM: **Human milk is a source of lactic acid bacteria for the infant gut.** *J Pediatr* 2003,

286 **143:754-758.**

287 11. Heikkilä MP, Saris PE: Inhibition of *Staphylococcus aureus* by the commensal bacteria of
288 human milk. *J Appl Microbiol* 2003, **95**:471-478.

289 12. Hunt KM, Preuss J, Nissan C, Davlin CA, Williams JE, Shafii B, Richardson AD, McGuire
290 MK, Bode L, McGuire MA: **Human milk oligosaccharides promote the growth of**
291 **staphylococci.** *Appl Environ Microbiol* 2012, **78**:4763-4770.

292 13. Hunt KM, Foster JA, Forney LJ, Schutte UME, Beck DL, Abdo Z, Fox LK, Williams JE,
293 McGuire MK, McGuire MA: **Characterization of the diversity and temporal stability of**
294 **bacterial communities in human milk.** *PLoS ONE* 2011, **6**:e21313.

295 14. Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A: **The human milk**
296 **microbiome changes over lactation and is shaped by maternal weight and mode of**
297 **delivery.** *Am J Clin Nutr* 2012, **96**:544-551.

298 15. Urbaniak C, Cummins J, Brackstone M, Macklaim JM, Gloor GB, Baban CK, Scott L,
299 O'Hanlon DM, Burton JP, Francis KP, Tangney M, Reid G: **Microbiota of human breast**
300 **tissue.** *Appl Environ Microbiol* 2014, **80**:3007-3014.

301 * In this epidemiologic study, the research team compared microbial communities in
302 mammary tissue collected from women with and without cancer in Canada and Ireland. All
303 samples were found to contain complex bacterial communities, even though some of the
304 women had never breastfed and none of the subjects had signs nor symptoms of infection.
305 There were differences in mammary microbiomes based on geographic site and cancer
306 history. Culture-dependent analyses confirmed viability of some bacteria.

307 16. Urbaniak C, Angelini M, Gloor GB, Reid G: **Human milk microbiota profiles in relation to**

308 **birthing method, gestation and infant gender.** *Microbiome* 2016, **4**:1.

309 ** In this study, milk was collected from 39 Canadian women and analyzed using 16S rRNA
310 technology. There was no evidence of differences based on delivery mode, gender of infant,
311 and gestation length. Dominant genera in the milk samples were *Staphylococcus*,
312 *Pseudomonas*, *Streptococcus*, and *Lactobacillus*. The authors postulate that “*there may be a*
313 *fail-safe mechanism whereby the mother is "ready" to pass along her bacterial imprint*
314 *irrespective of when and how the baby is born.*”

315 17. Davé V, Street K, Francis S, Bradman A, Riley L, Eskenazi B, Holland N: **Bacterial**
316 **microbiome of breast milk and child saliva from low-income Mexican-American**
317 **women and children.** *Pediatr Res* 2016, **79**:846–854.

318 18. Boix-Amorós A, Collado MC, Mira A: **Relationship between milk microbiota, bacterial**
319 **load, macronutrients, and human cells during lactation.** *Front Microbiol* 2016, **7**:492.

320 19. Khodayar-Pardo P, Mira-Pascual L, Collado MC, Martínez-Costa C: **Impact of lactation**
321 **stage, gestational age and mode of delivery on breast milk microbiota.** *J Perinatol*
322 2014, **34**:599-605.

323 ** In this longitudinal, observational study, milk was collected repeatedly from 32 healthy
324 women and analyzed using quantitative PCR. *Lactobacillus*, *Streptococcus*, and *Enterococcus*
325 were the predominant bacterial genera. Bifidobacteria were more abundant in milk samples
326 collected from women delivering term infants, compared to those produced by women
327 delivering prematurely. Higher bacterial counts were found in colostrum and transitional
328 milk produced by women undergoing Cesarean section deliveries.

329 20. Soto A, Martín V, Jiménez E, Mader I, Rodríguez JM, Fernández L: **Lactobacilli and**

bifidobacteria in human breast milk: influence of antibiotherapy and other host and clinical factors. *J Pediatr Gastroenterol Nutr* 2014, **59**:78-88.

21. Cabrera-Rubio R, Mira-Pascual L, Mira A, Collado MC: **Impact of mode of delivery on the milk microbiota composition of healthy women.** *J Dev Orig Health Dis* 2016, **7**:54-60.

22. Arroyo R, Martín V, Maldonado A, Jiménez E, Fernández L, Rodríguez J: **Treatment of infectious mastitis during lactation: antibiotics versus oral administration of Lactobacilli isolated from breast milk.** *Clin Infect Dis* 2010, **50**:1551-1558.

23. Carrothers JM, York MA, Brooker SL, Lackey KA, Williams JE, Shafii B, Price WJ, Settles ML, McGuire MA, McGuire MK: **Fecal microbial community structure is stable over time and related to variation in macronutrient and micronutrient intakes in lactating women.** *J Nutr* 2015, **145**:379-388.

24. Urbaniak C, McMillan A, Angelini M, Gloor GB, Sumarah M, Burton JP, Reid G: **Effect of chemotherapy on the microbiota and metabolome of human milk, a case report.** *Microbiome* 2014, **2**:24.

25. González R, Maldonado A, Martín V, Mandomando I, Fumadó V, Metzner KJ, Sacoó C, Fernández L, Macete E, Alonso PL, Rodríguez JM, Menendez C: **Breast milk and gut microbiota in African mothers and infants from an area of high HIV prevalence.** *PLoS ONE* 2013, **8**:e80299.

26. Martín R, Jiménez E, Olivares M, Marín ML, Fernández L, Xaus J, Rodríguez JM: **Lactobacillus salivarius CECT 5713, a potential probiotic strain isolated from infant feces and breast milk of a mother-child pair.** *Int J Food Microbiol* 2006, **112**:35-43.

27. Fernández L, Langa S, Martín V, Maldonado A, Jiménez E, Martín R, Rodríguez JM: **The**

human milk microbiota: origin and potential roles in health and disease. *Pharmacol Res* 2013, **69**:1-10.

28. Rodríguez JM: **The origin of human milk bacteria: is there a bacterial entero-mammary pathway during late pregnancy and lactation?** *Adv Nutr* 2014, **5**:779-784.

* This comprehensive review outlines what is currently known (and posited) about the origin of bacteria found in the mammary gland and milk. These origins include the infant's mouth and migration to the mammary glands via an endogenous cellular route.

29. Xuan C, Shamonki JM, Chung A, Dinome ML, Chung M, Sieling PA, Lee DJ: **Microbial dysbiosis is associated with human breast cancer.** *PLoS ONE* 2014, **9**:e83744.

30. Chan AA, Bashir M, Rivas MN, Duvall K, Sieling PA, Pieber TR, Vaishampayan PA, Love SM, Lee DJ: **Characterization of the microbiome of nipple aspirate fluid of breast cancer survivors.** *Sci Rep* 2016, **6**:28061.

31. Latuga MS, Stuebe A, Seed PC: **A review of the source and function of microbiota in breast milk.** *Semin Reprod Med* 2014, **32**:68-73.

32. Ramsay DT, Kent JC, Owens RA, Hartmann PE: **Ultrasound imaging of milk ejection in the breast of lactating women.** *Pediatrics* 2004, **113**:361-367.

33. Nasidze I, Li J, Quinque D, Tang K, Stoneking M. **Global diversity in the human salivary microbiome.** *Genome Res* 2009, **19**:636-643.

34. Gao Z, Perez-Perez GI, Chen Y, Blaser MJ: **Quantitation of major human cutaneous bacterial and fungal populations.** *J Clin Microbiol* 2010, **48**:3575-3581.

35. Perez PF, Doré J, Leclerc M, Levenez F, Benyacoub J, Serrant P, Segura-Roggero I, Schiffrin EJ, Donnet-Hughes A: **Bacterial imprinting of the neonatal immune system:**

374 **lessons from maternal cells?** *Pediatrics* 2007, **119**:e724-732.

375 36. Heida FH, van Zoonen AG, Hulscher JB, Te Kiefte BJ, Wessels R, Kooi EM, Bos AF,
376 Harmsen HJ, de Goffau MC: **A necrotizing enterocolitis-associated gut microbiota is**
377 **present in the meconium: results of a prospective study.** *Clin Infect Dis* 2016, **62**:863-
378 870.

379 37. Zhuang X, Xiong L, Li L, Li M, Chen MH: **Alterations of gut microbiota in patients with**
380 **irritable bowel syndrome: A systematic review and meta-analysis.** *J Gastroenterol*
381 *Hepatol* 2016, Jun 14. doi: 10.1111/jgh.13471.

382 38. D'Argenio V, Casaburi G, Precone V, Pagliuca C, Colicchio R, Sarnataro D, Discepolo V,
383 Kim SM, Russo I, Del Vecchio Blanco G, Horner DS, Chiara M, Pesole G, Salvatore P,
384 Monteleone G, Ciacci C, Caporaso GJ, Jabrì B, Salvatore F, Sacchetti L: **Metagenomics**
385 **reveals dysbiosis and a potentially pathogenic *N. flavescens* strain in duodenum of**
386 **adult celiac patients.** *Am J Gastroenterol* 2016, **111**:879-890.

387 39. Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, Kau AL, Rich SS,
388 Concannon P, Mychaleckyj JC, Liu J, Hout E, Li JV, Holmes E, Nicholson J, Knights D,
389 Ursell LK, Knight R, Gordon JI: **Gut microbiomes of Malawian twin pairs discordant for**
390 **kwashiorkor.** *Science* 2013, **339**:548-554.

391 40. McGuire MK, Meehan CL, Brooker S, Williams JE, Foster JA, McGuire MA: **An**
392 **evolutionary, biosocial perspective on variation in human milk microbes and**
393 **oligosaccharides: an example of eco-homeorhesis?** In *Prebiotics and Probiotics in*
394 *Human Milk*. Edited by McGuire MK, McGuire MA, Bode L. Elsevier; 2016 (expected).

395 * This monograph, based on scientific fundamentals and anthropologic paradigm, uses a

396 biosocial lens to relate how evolution and behavior may have, together, shaped variation in
397 global human milk microbiomes and oligosaccharide profiles to optimize health in a
398 particular location and culture. The term “eco-homeorhesis” is introduced and defined as a
399 shift in what would be considered a “normal” homeostatic range or profile to support
400 health in a particular ecological/behavioral niche.