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REVIEW

Human behavior, not race or geography, is the strongest predictor of microbial succession in the gut bacteriome of infants

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ABSTRACT

Colonization of the gastrointestinal tract with microorganisms during infancy represents a critical control point for shaping life-long immune-mediated disease susceptibility. Abnormal colonization or an imbalance of microbes, termed dysbiosis, is implicated in several diseases. Consequently, recent research has aimed at understanding ways to manipulate a dysbiotic microbiome during infancy to resemble a normal, healthy microbiome. However, one of the fundamental issues in microbiome research is characterizing what a "normal" infant microbiome is based on geography, ethnicity and cultural variations. This review provides a comprehensive account of what is currently known about the infant microbiome from a global context. In general, this review shows that the influence of cultural variations in feeding practices, delivery modes and hygiene are the biggest contributors to microbial variability. Despite geography or race, all humans have similar microbial succession during infancy.

Introduction

The symbiotic relationship between mammals and the trillions of microorganisms that reside in their gastro-intestinal tract plays a critical role in host health and disease susceptibility. Collectively termed the gut microbiome, these organisms and their associated phenomes educate the immune system, protect against infectious diseases and contribute to host nutrient absorption. At parturition, a newborn's immune system is not completely developed and requires signals from the gut microbiome trigger maturation.[1] In the absence of microbial exposures, germ-free (sterile) mice show extensive immune and intestinal morphological defects.[2] Reconstitution of germ-free mice with bacterial communities can restore many of these immunological abnormalities suggesting that if we can change our microbiome, we can alter host physiological deficiencies. However, appropriate induction of immune maturation in the gut extends beyond the mere presence of bacteria, and requires microbes, which have coevolved with the host.[3] Therefore, the question becomes can we alter the gut microbiome and if so does time of induction matter? The microbial

corrections may be age dependent and it has been hypothesized changes must occur within a certain time-frame during infancy to ensure appropriate intestinal immune development.[4] In humans, current evidence indicates that microbial diversity increases in a stepwise manner during the first few years of life and stabilizes to an adultlike microbiome at approximately 3 y of age.[5] Infants with an abnormal temporal progression of microbes during these first 3 y could be predisposed to negative health outcomes due to improper mucosal immune maturation as a result of dysbiosis. Consequently, there has been an increase in research aimed at defining the successive colonization stages of gut bacteria in infants responsible for maturation of a healthy host immune system.

While generalizing a stepwise progression in infant colonization may be a logical first step, this may not actually be meaningful across geographic locations given that microbes have coevolved with various human cultures and behaviors. As early human ancestors settled in various geographic locations, their gut microbial communities adapted to these new environments to optimize nutrient absorption from available food sources. Consistent

with this notion, substantial divergences in adult gut microbial structures have been found in different populations of adults.[6] Hunter-gatherer populations, such as the Hadza tribesmen of Tanzania, are characterized by high *Prevotella* spp. and *Treponema* spp., whereas urban populations are enriched in *Bacteroides* spp., *Bifidobacteria* spp. and Firmicutes. These divergences are often attributed to dietary habits whereby foraging populations consume diets consisting of fiber-rich foods, and urban populations consume more processed foods rich in simple sugars, emulsifiers and oxidizable fats. While *Bifidobacteria* spp. are often cited as health-promoting bacteria, lower abundances of these species in Hadza populations do not appear to negatively impact Hadza health.[7] Instead, it appears that the Hadza people coevolved with their gut bacterial communities to allow for effective nutrient extraction from cellulose and fiber-rich foods, increasing their overall fitness and survival.[8]

Given the apparent geographic and genetic impact on the adult microbiota,[9] one key question is whether lifestyle, geographic distance, sex and gender, cultural ethnicity and overall social economic development impacts the acquisition of gut microbes in infants. A number of reviews have been published summarizing the infant colonization process, many presuming that geography does a play a role in the infant bacteriome given variations seen in literature, but a comprehensive account of variations in the infant microbiome based on geography with consideration of cultural behaviors is long overdue. In this review, we describe the global range of profiles that constitute a healthy, full-term infant gut bacteriome, the bacterial community within the gut microbiome, and highlight what is currently known across 53 different countries (Table S1). We conclude that cultural variations in feeding practices, delivery modes and hygiene drive the succession of the infant bacteriome given there is more variation from person to person in any population than there is from breastfed vaginally birthed full-term infants from any ethnic group around the world. This means that human behavior is the biggest predictor of bacterial colonization in the infant gut (summarized in Figure 1). While we cannot change our own genotype easily, we can control our behavior and therefore understanding how to alter the gut bacteriome toward a healthy community may be one of the biggest contributions to promoting self-care this century.
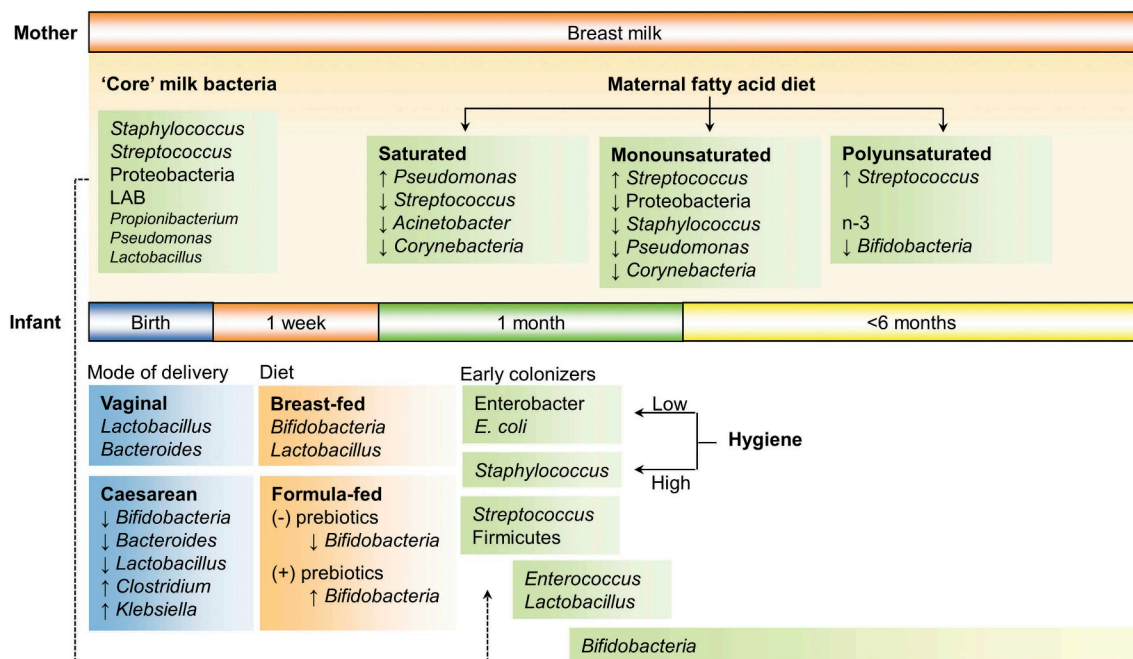
Figure 1. Human behavior and bacterial colonization in infants. Several features impact the establishment of bacterial communities in the infant gastrointestinal tract. Two of the most influential human behaviors that has affected infant gut colonization, globally are mode of delivery and diet. More subtle impacts may be driven by diet-driven changes to breast milk and hygiene practices.

Despite geography and race, interventions of birth mode reduce *Bifidobacterium*, *Bacteroides* and *Lactobacillus* spp. as primary colonizers in the infant gut

Until recently, the intrauterine environment was believed to be sterile. The consensus that the fetus is maintained in a sterile environment was established in the early 1980s when culture-based techniques failed to cultivate bacteria from amniotic fluid and meconium samples.[10] However, recent studies using newer molecular techniques based on DNA methodology have questioned the validity of previous culture-based findings and report complex bacterial communities.[11] Still, others maintain that any DNA detected using DNA-based methods may represent contaminants.[12] Whether infants are colonized prior to birth or not remains unclear, most agree that mode of birth, vaginal or Cesarean section (C-section), impacts infant gut microbial communities. Numerous epidemiological studies illustrate that infants delivered by C-sections have increased risk of allergic rhinitis, type I diabetes, obesity, asthma and other immune deficiencies.[13] This is concerning given the escalated frequency of non-medical C-section deliveries in recent years. China, similar to several developing nations, has seen C-sections rates increase from 5% in 1970s to more than 46.2% as their economy grows stronger.[14] Given that increasing popularity of elective C-section deliveries parallels a rise in prevalence of non-communicable diseases, a contributing factor in this association may be attributable to differences in pioneering bacteria between vaginally birthed and C-section delivered infants.

When infants are vaginally birthed, they are inoculated with their mothers' microbiota as they pass through the birth canal. This idea is supported through studies in both developed and developing countries. In Venezuela, vaginally delivered neonates contain established vaginal bacterial taxa like *Lactobacillus, Prevotella, Atopobium* or *Sneathia* spp.[15] These infants have communities more similar to their own mother than to other vaginally delivered infants suggesting that vaginal communities unique to each mother are vertically transmitted to their infants. As expected, C-sectioned babies in this cohort lacked bacteria from mothers' vaginal microbiome and were instead dominated by communities

found on the skin surface.[16] Interestingly, C-sectioned babies were no more similar to their own mothers' skin bacterial communities than to others despite the fact that adult skin microbes are highly personalized. Similar cases of the maternal vaginal microbes colonizing the neonatal infant gut have been reported in other developing countries like Estonia[17] and Puerto Rico[18] and the same holds true in developed countries. More than 70% of the Swedish infants vaginally birthed are colonized by bacterial species found in their mothers' stool, whereas only 41% of matching spp. were detected in C-sectioned infants.[19] Given the vertical transmission between mothers and vaginally delivered infants, as well as the environmental impacts on C-sectioned infants, one would expect to see geographic divergences in infants born in developed and developing countries where adult and environmental microbial communities differ. However, current reports do not support this theory and instead, general trends can be found between C-sectioned and vaginally birthed infants, regardless of geographic location or even genetics.

In general, reports on C-section delivered infants show lower abundances of *Lactobacillus* spp. when compared to vaginally delivered infants. This has been shown in developing countries such as Venezuela[15] and India,[20] as well as developed countries such as Greece,[21] Finland[22] and the United Kingdom.[23] Alongside *Lactobacillus* spp., *Bifidobacteria* spp. are often reported as decreased or having delayed colonization in C-sectioned infants. *Bifidobacteria* spp. are commonly cited as one of the major components of the infant intestinal microbiome. While the abundance of *Bifidobacteria* spp. is often attributed to diet, mode of delivery also appears to have an effect on early colonization. Mother-to-infant transmission of *Bifidobacteria longum* subsp. *longum* was first reported in vaginally delivered infants in Belgium.[24] A follow-up study from Japan[25] expanded on this finding and found that 92% of vaginally delivered infants had at least one monophyletic *Bifidobacteria* strain with their mother, whereas no monophyletic strains were identified in C-sectioned infants. Reduced transmission of *Bifidobacteria* spp. from mothers undergoing C-section procedures has been shown to decrease or delay *Bifidobacteria* spp. colonization in infants in several studies conducted in

developed countries such as Finland,[22,26] Sweden,[27] Greece,[21] Italy,[28,29] France[30] and the Netherlands[31] as well as in developing countries such as Singapore[32] and India.[20,33] However, there are some exceptions reported in the literature. For example, Fallani et al. report no difference in the relative proportion of *Bifidobacteria* spp. by delivery mode (both were approximately 40% of the total), in infants born in Sweden, Scotland, Germany, Italy and Spain.[34] Instead they found C-section delivery was associated with lower *Bacteroides* spp. and higher *Streptococcus* spp. and *Clostridium coccoides*. This reported decrease in *Bacteroides* spp. in infants delivered by C-section is another common finding. Like *Bifidobacteria* spp., *Bacteroides* spp. have become a major focus of human gut microbiology. Many studies have found that *Bacteroides* spp. are less abundant in C-sectioned infants in developed countries such as Sweden,[19,27,35] France,[30] the UK,[23,36] Canada,[37] Finland,[22] the Netherland[31] as well as in developing countries such as India[20] and Brazil.[38] Collectively, these studies report a global decreased or delayed presence of bacteria such as *Bifidobacterium, Lactobacillus* and *Bacteroides* spp. in C-sectioned infants, due to the lack of exposure to mothers' vaginal microbes during the birthing process.

In leu of vaginal bacteria, neonates delivered by C-section are colonized by potentially pathogenic bacteria such as *Clostridium* and *Klebsiella* spp. A comparative study of five European countries including Sweden, Scotland, Germany, Italy and Spain found higher *Clostridium* spp. in C-sectioned infants compared to vaginally delivered infants.[34] Using culture-based methods, a separate study comparing the fecal microbiota of infants from Sweden, the United Kingdom and Italy similarly found higher *Clostridium* and *Klebsiella* spp.[36] Independent European studies in the Netherlands,[31,39] Sweden[27] and Finland[22] likewise report increased *Clostridium* spp. colonization rates in C-sectioned infants. Therefore, in developed countries, it appears that *Bifidobacterium* spp. and *Bacteroides* spp. are largely replaced by *Clostridium* spp. and *Klebsiella* spp. There are, however, some exceptions reported in the literature. For example, a fluorescent in situ hybridization (FISH)-based study in Finland found that while *Bifidobacteria* spp. were 1,300-fold lower in C-section delivered infants at 1 month of age, there were no differences in *Clostridium, Bacteroides* and

Lactobacillus spp. between vaginally and Cesarean-born infants.[26] Instead, C-section deliveries resulted in a threefold lower total bacterial cell number in infants when compared to those born by vaginal deliveries. Nevertheless, the health impacts of higher *Clostridium* spp. and *Klebsiella* spp. reported in a majority of studies should be considered. In the United States, Goedert et al. found higher levels of certain *Clostridium* spp. in adults who had been born by C-section when compared with adults who had been born vaginally.[40] This suggests that differences in initial communities may lead to life-long differences in the microbial communities. It is therefore an auspicious finding that exposure of newborns delivered by C-section to maternal vaginal fluids at birth can at least partially restore *Lactobacillus* spp. and *Bacteroides* spp.[18] However, this "vaginal seeding" does not alleviate other factors accompanying C-section procedures that may drive microbial phenotypes such as labor and antibiotic administration.

While a global reduction in *Bifidobacteria* spp., *Bacteroides* spp. and to a lesser extent, *Lactobacillus* spp. in infants delivered by C-sections are replaced with potentially pathogenic bacteria such as *Clostridium* spp., and *Klebsiella* spp. in developed countries, the trend in developing countries remains to be fully elucidated due to the paucity of research and conflicting results. For example, a 16S rRNA cloning and sequencing study from India followed trends established in Europe, whereby C-sectioned infants were susceptible to colonization of potentially pathogenic bacteria from the environment, while vaginally delivered infants acquired their initial microbes from their mothers. In this study, 12 breastfed, C-section delivered infants had a predominance of *Clostridium* spp., *Citrobacter* spp. and *E. coli* at 1 week (65% of the clone library) and that the vaginally delivered infants had a predominance of *Acinetobacter, Bifidobacteria* and *Staphylococcus* spp.[33] In stark contrast, a study in Brazil conducted on 13 C-sectioned and 46 vaginally delivered infants reported a decrease in the abundances of *Clostridium* spp. in the transitional stool of C-sectioned infants.[38] In Venezuela, the lack of vaginal exposure resulted in early establishment of communities originating from the birth environment and skin surfaces.[15] As a result, C-sectioned infants had a higher relative abundance of

Staphylococcus, Corynebacterium and *Propioniba cterium* spp. These discrepancies could be due to differences in methodologies, feeding practices, sampling age or place of delivery.[39] Future studies are needed in to better understand the impact mode of birth has on pioneering bacteria in developing countries.

Diet is a strong predictor of infant microbial colonization

Alongside delivery mode, feeding practices can profoundly alter the trajectory of acquisition of the gut microbiota in infants. In fact, feeding practices can partially compensate for microbial differences between infants delivered vaginally or by C-section.[23] There are two main nutritional sources available for infants: breast milk and formula. While breastfeeding is an unequaled way to ensure infant health and survival, there are several social and economic factors that influence the prevalence and duration of breastfeeding. For example, in Japan, breastfeeding has been a strong practice for centuries and Buddhist teachings promote extended breastfeeding in children to the age of 6.[41] In stark contrast, Atlanta in the United States issued a law banning public breastfeeding for children over the age of two, citing public indecency. Similarly, in Ukraine, breastfeeding is viewed as socially unacceptable. Globally these social restrictions, religious viewpoints and cultural restraints have reduced breastfeeding practices. Data collected by UNICEF and the World Health Organization show that no country in the world fully meets the recommended standards for breastfeeding. In fact, only 23 countries were shown to have exclusive breastfeeding rates above 60% in the first 5 months postpartum.[42] Instead of lactation, mothers elect to feed their baby using infant formulas. Disparities between the microbiome of breastfed and formula-fed infants are frequently cited in the literature. However, far fewer studies report differences in the infant microbiome based on breast milk bacterial and nutritional composition. As mentioned above, adult dietary habits vary by geographic region. It is possible that alterations in dietary intake during lactation may influence the composition of mothers' breast milk, ultimately leading to altered colonization events in suckling infants. However, region-specific factors influencing breast milk properties are largely unexplored. The following discussion is organized into two main sections. First, the impact of geographic location on breast milk composition will be explored. Second, we will compare the impact of formula feeding and breast milk on the infant microbiome in various countries.

Global dietary habits alter breast milk lipid profile with limited impacts on the milk bacteria

Human breast milk is a complete source of nutrients and bioactive substrates required for optimal development and health in nursing infants. Human milk is composed of macronutrients such as protein, fats and carbohydrates, vitamins, minerals, digestive enzymes, immune cells and even bacteria. Theoretically, geography, ethnicity and cultural variations could alter any of these components. However, here we focus on the geographic impacts on breast milk macronutrients and bacteria, which are more frequently reported in the literature.

While there is considerable temporal and interpersonal variability in breast milk,[43] maternal nutritional status has little to no effect on the total amount of macronutrients (protein, carbohydrate, fat) in breast milk. Even if women consume less than the recommended dietary allowance, suckling infants will receive the needed macronutrients at the expense of maternal stores. In the Gambia, for example, the dietary mean intake is around 1,700 kcal/d.[44] Despite this, the mean energy content of mother's milk is maintained at 72 kcal/100 ml, which is comparable to well-nourished mother's milk.[45] It is not surprising then, that the macronutrient composition of human milk is remarkably conserved across populations.[46] However, while the total macronutrient status is relatively stable, the vitamin/micronutrient content as well as biochemical makeup of macronutrients does vary with the mother's nutrient intake. For example, veganism can result in vitamin B12 deficiency in the infant.[47] Similarly, types of fatty acids like saturated, monounsaturated and polyunsaturated can vary due to mother's diet.[48] Given the geographic variations in diet, fatty acid proportions in breast milk should vary by country. Indeed, Kumar et al.[49] show that breast milk fatty acid profiles differ between Europe (Spain and Finland),

Africa (South Africa) and Asia (China). In this study, it was reported that the highest variability in milk samples was observed in the omega-6 polyunsaturated fatty acids, which were increased in both the triacylglycerides and phospholipids of Chinese milk samples, likely due to the high consumption of soy products in this country. A recent study in Denmark and the United States similarly shows national differences in milk fat content.[43] These geographic variations in breast milk fat profiles are attributed to differences in diet and may affect colonization events in breastfed infants. In support of this notion, in nonhuman primates, it was shown that maternal high-fat diet (36% fat from animal fat and corn oil) consumed during gestation and lactation alters the composition of their offspring's microbiome for up to 1 y, irrespective of the infants' post-weaning diet.[50] A follow-up study in the United States sought to understand if this finding could be recapitulated in a human cohort.[51] To approach this, mother–infant dyads were separated into groups based on mother's dietary fat intake during gestation. Culture-independent 16 S rRNA gene sequencing was used to characterize the neonates gut microbiome at the time of delivery. The authors found that the neonatal microbiome significantly clustered according to maternal diet, whereby *Bacteroides* spp. were depleted in infants exposed to a maternal high-fat diet in pregnancy. The authors concluded that maternal high-fat diet consumed during gestation can alter their offspring's microbiome. However, this publication has several limitations with the major one being that maternal dietary intake of fat was estimated from a dietary screener questionnaire. The fat content in breast milk was never quantified and as a result, it is not possible to tell which fatty acids were altered. Given that the type of fatty acids in a high-fat diet uniquely alter the gut bacteriome,[52] data from "high-fat" studies are hard to interpret without quantifying fatty acid profiles. Regardless, this evidence suggests that geographic- or diet-related factors could drive differences in fatty acid profiles in breast milk. However, more studies investigating the relationship between maternal dietary fat intake and associated changes to the milk lipid profiles are needed.

In addition to fats, it has been suggested that human milk oligosaccharides vary geographically.[53] Human milk oligosaccharides represent the third largest portion of human milk following lactose and lipids.[54] In a comparison of 109 human milk samples collected 1 month post-partum from Australia, Japan, the USA, Norway and South Africa[55] it was shown that milk metabolites, such as human milk oligosaccharides, lactose and other milk sugars cluster by country of origin. Since many of these milk metabolites can be consumed by infant gut microbiota, ethnic differences in human milk metabolites may have a strong bearing on the bacterial community composition in infants or even, in the breast milk itself.

An interesting observation by West et al.[56] in the 1970s suggested that breast milk may not be sterile. Today, numerous studies reported a highly diverse microbial community in human milk.[57–60] Bacteria in human milk may constitute one of the main sources of gut bacteria in breastfed infants. Although it was originally thought that bacteria in breast milk are acquired from skin or fecal contamination, several recent studies support an entero-mammary pathway involving dendritic cells, macrophages and microfold cells. This controversial pathway is thought to allow for vertical transfer of bacteria from the maternal intestinal tract, to breast milk and finally to the infant's gut. In support of this, Qutaishat et al. show transmission of *Salmonella enterica* subspecies *enterica* serovar Typhimurium DT104 from maternal gut to maternal mammary gland.[61] Similarly, a study in Syria showed that identical random amplified polymorphic DNA (RAPD) genotypes of *L. fermentum, L. plantarum. L brevis, Enterococcus faecium, E. faecalis* and *Pediococcus pentosaceus* were isolated from mother's stool, breast milk and corresponding infant stool.[62] *Bifidobacteria* and *Staphylococcus* spp. have also been shown to transfer to infants.[63,64] For example, one study investigating the potential mother-to-child transmission of *Staphylococcus, Lactobacillus and Bifidobacteria* spp. in 20 Spanish participants found the same bacterial strains in 11, 9 and 3 mother-infant pairs, respectively.[63] Therefore, regional differences in mother's gut microbial communities may impact the types of bacteria transferred from the maternal gut to their breast milk. Moreover, since maternal diet influences the biochemical makeup of macronutrients in human milk, geographic variations in

dietary intake during lactation may indirectly select for bacterial species capable of utilizing various substrates in milk.

The idea that individual variations in breast milk bacteria may be influenced by geographic or dietary factors is supported by two independent studies. The first compared mature breast milk samples collected at 1 month of lactation from 20 participants in Europe (Spain and Finland), Asia (China) and Africa (South Africa) using the Illumina MiSeq sequencing platform.[49] This study found that milk samples collected from South Africa had a predominance of Proteobacteria whereas samples from Spain, China and Finland were all enriched with Firmicutes. At the genus level, *Pseudomonas* spp. were prevalent in the milk samples collected from South Africa, followed by *Staphylococcus* and *Streptococcus* spp. whereas breast milk collected from Spain and Finland both had a predominance of *Staphylococcus* spp. followed by *Streptococcus* spp. In China, *Streptococcus* spp. predominated followed by *Staphylococcus* spp. In general, milk samples collected from South Africa had higher abundances of Proteobacteria when compared with the other countries, which could be due to high Enterobacteriaceae and Pseudomonadaceae found in South African and Spanish milk samples. Spanish samples had a high relative abundance of *Propionibacterium* and *Pseudomonas* spp. compared with the other countries. Finnish milk samples had the lowest relative abundance of Proteobacteria and Chinese samples had the highest Actinobacteria. Other noteworthy findings include the fact that *Enterococcaceae* spp. were found in breast milk collected from every geographic region except China. In contrast, *Bifidobacteria* spp. were only found in samples collected from South Africa and *Lactobacillus* spp. were only found in samples collected from Finland. When milk bacteria profiles were combined with fatty acid profiles, it was found that fatty acids profiles associated with differences in the milk bacteria.[49] In all samples, monounsaturated fatty acids found in milk triacylglycerides were negatively associated with Proteobacteria. Monounsaturated fatty acids in triacylglycerides were also inversely correlated with abundances of *Pseudomonas* and *Staphylococcus* spp. Both monounsaturated fatty acids and polyunsaturated fatty acids in triacylglycerides positively associated with *Streptococcus* spp. whereas saturated

fatty acids were negatively correlated with relative abundances of both *Streptococcus* and *Acinetobacter* spp. In contrast, saturated fatty acids found in both milk triacylglycerides and phospholipids were positively associated with *Pseudomonas* spp and n-3 polyunsaturated fatty acid concentrations in milk phospholipids were negatively correlated with *Bifidobacteria* spp. Taken together, this study supports the notion that geographic variations in breast milk lipid profiles align with differences in milk bacterial composition.

The second cross-cultural study supporting the notion that individual variations in breast milk bacteria may be influenced by geographic location compared milk samples collected from different regions in East Asia.[65] In this study, milk samples were compared using 454 pyrosequencing of 16 S rRNA genes between Taiwan and six-regions of mainland China. The relative abundance of *Pseudomonadaceae* was found to be lower in samples from Taiwan (n = 31) compared to East (n = 34), Central (n = 24) and Northeast (n = 17) China. Similarly, North (n = 11) and Southwest (n = 9) China had lower relative abundance of *Pseudomonadaceae* than Central China. Finally, the relative abundance of *Staphylococcaceae* was higher in samples collected from Taiwan than East and Central China. Despite these notable differences, however, there was a high degree of overlap in the principal component analysis comparing the whole milk bacteriomes. Moreover, it seems that as with most natural assemblages in ecology,[66] few species comprise the majority of the individuals in breast milk from both cross-cultural studies. This 'core' set of bacteria is consistently reported in independent studies worldwide.

Assessment of bacterial diversity in breast milk using culture-dependent and independent approaches shows that *Streptococcus* and *Staphylococcus* spp. are almost always among the most abundant genera in breast milk, irrespective of geographic or cultural variables. This includes findings reported from the United States,[57] Canada,[67] Switzerland,[60,68] Spain,[59,69,70] Slovenia,[71] Mozambique,[72] Taiwan[65] and China.[65,73] Other prevalent bacteria include lactic acid bacteria such as *Lactobacillus*, *Weissella* and *Leuconostoc* spp., certain Proteobacteria such as *Pseudomonas* spp. and certain Actinobacteria such as *Propionibacterium* and *Corynebacterium*, globally. While these frequently reported taxa appear to make up a common 'core'

breast milk microbiome worldwide, data support a continuous gradient of these genera associated with interindividual variation.

High-interindividual variability in breast milk bacterial communities is commonly reported within a single population.[57,59,70,73,74] For example, pyrosequencing of breast milk collected from 16 women in the United States found that in one subject, *Staphylococcus* spp. were the first or second most predominant genera accounting for 22-59% relative abundance over a four-week period whereas in another subject, *Staphylococcus* spp. only represented <5% of bacteria.[57] Serial samples collected from a single individual tended to be more similar when compared to other individuals; however, while some participants had relatively stable milk bacterial communities over time, others had bacterial genera shifts. This high-interindividual variability within a single population makes it difficult to draw geographic conclusions from independent or underpowered studies. For instance, two independent studies in Spain assessing the bacterial communities in mature breast milk using qPCR found varying results. One found that the median count (log n° copies/ml) of *Lactobacillus* spp. (4.31) were highest in breast milk collected from 13 healthy women, followed by *Enterococcus* (3.95), *Streptococcus* (3.58) *Staphylococcus* (3.28) and *Bifidobacteria* spp (2.45).[75] All bacteria were found at 100% prevalence except for *Staphylococcus* spp. which were only in 77% of samples collected. A second study found a predominance of *Streptococcus* (4.5 mean $\log_{10}$ copies/ml, 100% prevalence), followed by *Lactobacillus* (3.74, 100%), *Bifidobacteria* (3.56, 100%), *Staphylococcus* (3.55, 100%), *Clostridium* cluster XIVa-XIVb (3.32, 96%); *Enterococcus* (2.56, 76%), and *Bacteroides* spp. (2.02, 40%).[69] Discrepancies between the abundance and prevalence of milk bacteria in these two studies within the same country could be attributed to methodical differences but the extraction kit and several of the primers used in this study were similar. Instead, differences between these studies may be due to high interindividual variability. While it is well recognized that low statistical power increases the probability of type II errors, paradoxically, low statistical power also increases the likelihood of finding a false positive.[76] Therefore, the geographic differences reported in the two cross-cultural studies

above may be falsely positive and instead, all breast milk samples contain a gradient of 'core' bacteria which varies between individuals.

The mechanisms driving high interindividual variation in breast milk remain poorly understood, but is presumably promoted by diet, genetics or possibly even mode of delivery. In Finland, higher *Bifidobacteria* spp. were reported in breast milk of women who delivered by vaginally at 6 months lactation when compared to mothers who delivered by C-section.[58] In contrast, *Streptococcus* spp. were higher in the breast milk of mothers who delivered by C-section. In Spain,[59] *Staphylococcus* spp. were marginally (P = .052) higher in the milk samples of women who delivered by C-section (n = 4). However, mode of delivery had no impact on milk samples collected in Canada[67] or China.[73]

Collectively these studies show that the influence of geographic location and culture does not greatly influence the 'core' bacterial composition of human breast milk; however, variations in dietary habits or mode of delivery may shift the relative abundance of bacteria to favor one 'core' genera over another. The biological impact of this breast milk gradient on the establishment of the infant microbiome remains unclear. However, as the presence of these 'core' bacterial groups in human breast milk are among the first colonizers in the infant gut, it stands to reason that variations in breast milk bacteria would alter the colonization process in infants. Future large-scale studies examining geographic variations in milk bacteria and corresponding bacteria in infants may begin to address this notion.

Formula feeding alters normal microbial colonization patterns in the infant gut

While mothers are increasingly tasked to work and rear children simultaneously, as well as lack societal and economic support, one of the most influential human behavior that has negatively affected infant gut health is formula feeding. Infant formula is intended to be an effective substitute for breast milk and while mostly not true, is advertised to be formulated to mimic the composition of breast milk. Supplemental feeding with formulas is reported in over 80% of the infants in developing countries including Venezuela, Gabon,

Chad, Somalia, Tunisia, Yemen, Romania and Belarus.[42] The low prevalence of exclusive breast-feeding, especially in countries in Africa, coincides with the highest rates of infant malnutrition in the world.[77] The complexities of the breast milk are not fully appreciated so while formulas are intended to mimic the properties of breast milk, epidemiologic studies consistently show that formula-fed infants are more susceptible to enteric infections.[78] Differences in health outcomes between formula and breastfed children may be due to differences in early colonization events. Indeed, many reports have shown that breastfed and formula-fed infants have distinct gut colonization patterns, but these results are not without contradictions in literature.

One notable difference between breast milk and formula is the absence of bacteria. Supplementing infant formulas with bacteria is currently not recommended by The Codex Alimentarius Commission[79] due to limited evidence of their clinical efficacy. Nevertheless, worldwide infant formulas supplemented with probiotics are commonly used. Even though Staphylococcus spp., Streptococcus spp. and Proteobacteria predominate breast milk globally, the typical probiotic strains included in formulas are Bifidobacterium and Lactobacillus spp. The supplementation is proposed to be necessary because formula-fed infants have lower abundances of Bifidobacteria spp. when compared to breastfed infants.[80] This has been reported in developed countries such as the Netherlands,[81,82] the United Kingdom,[83] Sweden,[19] Greece[84] and Japan.[80] Since Bifidobacteria spp. are associated with proxies for desirable health outcomes, it is thought that the lower abundances of these species in formula-fed infants may result in improper mucosal immune maturation. Indeed, administration of Bifidobacteria breve and oligosaccharides in C57BL/6 J male mouse pups enhanced gut immune and endocrine development in suckling mice.[85] Therefore, investigating ways to increase proportions of Bifidobacteria spp. in formula-fed infants has become an active area of research. The increased proportion of Bifidobacterium spp. in breastfed infants could, in theory, be due to bifidobacteria in human breast milk. However, while breast milk has been shown to harbor several bifidobacterial species such as B. longum, B. breve and B. bifidum, most milk samples show low or absent Bifidobacteria spp. For example, in Japan, it was shown that no bifidobacteria were detectable in breast milk collected before delivery or colostrum, but could be detected 1-week post-partum or later.[64] Similarly, in Spain, bifidobacteria were only isolated from 8 out of 23 milk samples collected between 3 and 7 d postpartum.[86] Therefore, it is not likely that the abundance of Bifidobacteria spp. in breastfed infants is due to breast milk bacterial colonies. Nevertheless, some groups have tried to increase Bifidobacteria spp. in formula-fed infants by seeding formula with Bifidobacteria spp. In a double-blind, randomized, placebo-controlled study of newborn infants in Germany, formula containing 10^7 colony forming units/g of B. bifidum, B. breve, B. longum, and B. longum subsp. infantis did not result in long-term colonization of Bifidobacterium strains in the formula-fed infant gut.[87] Another study sought to understand if probiotic supplements could ameliorate the effects of antibiotic use or C-section deliveries in Finland.[88] In this study, both mothers and infants were given a multispecies probiotic, consisting of B. breve, Propionibacterium freundenreichii subsp. shermanii, L. rhamnosus, and L. rhamnosus GG. The results showed that probiotic supplementation increased Bifidobacteria spp. in breastfed, C-sectioned infants, but not in formula-fed infants. Based on the study design, however, it is not possible to determine if these effects were due to infant or maternal probiotic supplementation. An observational study corroborates these findings and shows maternal and infant supplementation with probiotics associated with higher abundances of Bifidobacteria spp. at 1 week of age.[89] However, an inverse correlation between probiotic supplements during infancy and mucosal-associated illness in toddlers was reported. Given this, probiotic supplementation during infancy should be approached cautiously until rigorous controlled follow-up studies determining safety and efficacy have occurred.

A better approach to increasing Bifidobacteria spp. in formula-fed infants is to provide appropriate conditions to promote the growth and productivity of Bifidobacteria spp. It is thought that the higher proportions of Bifidobacteria spp. in breastfed infants is due to selective pressures exerted by available dietary substrates. Bifidobacteria spp. have been shown to effectively metabolize human milk

oligosaccharides through the bifidus pathway,[90] giving them a competitive advantage over other bacterial species in the infant gut. There are three broad classes of human milk oligosaccharides, each containing various types of oligosaccharides, including neutral fucosylated, neutral nonfucosylated and acidic molecules.[91] Certain types of these human milk oligosaccharides are utilized by various *Bifidobacteria* spp. more than others. For example, *B. infantis* strains are capable of metabolizing all types of milk oligosaccharides including sialylated and fucosylated molecules, whereas *B. breve* targets complex fucosylated molecules. In this sense, different human milk oligosaccharides give selective advantages to different bacterial species. The actual structures of human milk oligosaccharides are not easy to elucidate, and large-scale synthesis of these molecules is challenging. As a result, oligosaccharides in formulas do not effectively mimic human milk oligosaccharides, and other taxa which can effectively degrade these carbohydrates dominate. Therefore, differences in *Bifidobacteria* spp. between formula and breastfed infants can be attributable to differences in available oligosaccharides between the two nutrient sources.

Despite this, not all studies show decreased *Bifidobacteria* spp. in formula-fed infants. For instance, a study comparing *Bifidobacteria, Escherichia* and *Clostridium* spp. between 50 breastfed and 50 formula-fed infants in the Netherlands found that bifidobacterial counts were comparable using real-time PCR.[92] A culture-dependent study in Sweden similarly found no differences between breastfed and formula-fed infants and reported that in both groups, *Bifidobacteria* spp. occurred in less than half of the samples collected.[93] A pyrosequencing study in Europe likewise revealed a close relationship in the fecal microbial composition of breastfed and formula-fed infants born in Italy, Spain and Ireland.[94] The authors concluded that bifidobacterial abundances may not be influenced by diet alone. In this vein, the age of infants should also be considered when comparing profiles between breastfed and formula-fed infants. A comprehensive study in Sweden reported no differences between exclusive breast-feeding or mixed feeding on the newborn microbiome.[19] However, these infants diverged overtime and the gut microbiome in the infants exposed to formula "matured"

quicker than the microbiome in those exclusively breastfed. Namely, at 4 months of age, formula-fed infants had elevated *C. difficile, Granulicatella adiancens, Citrobacter* spp. *E. cloaceae* and *Bilophila wadsworthia* spp.,[19] whereas exclusively breastfed infants had increased abundances of *L. johnsonii/L. gasseri, L. paracaseil/L. casei* and *B. longum*. Developing countries show similar results. In Brazil, it was reported that *Bifidobacteria* spp. predominated the infant gut regardless of diet[95] and in India, it was reported that supplemental feeding associated with a reduction in Enterobacteriaceae.[20] Discrepancies between these findings could be attributed to differences in methodology, age, geographic location or mode of birth; however, a more likely explanation is differences in the formulas themselves. Formulas are available in various forms including powder, liquid or ready-to-feed, and are manufactured using various bases including cow-milk, goat-milk, soy, hypoallergenic, rice-milk and many more.[96] While a detailed comparison between formulas extends beyond the scope of this review, it is important to acknowledge that formulas from a single manufacturer, in a single country will vary depending on the formula type purchased. This holds true in both developed countries like Italy,[97] and developing countries like Brazil.[98] Therefore, different formulas purchased within a single population may result in different microbial colonization patterns due to variations in product composition.

Interestingly, studies performed in both developed and developing countries show that the addition of a prebiotic compound to formula, such as galactooligosaccharides or fructooligosaccharides,[99] can increase bifidobacteria proportions in formula-fed infants. In support of this, one study in Japan found that all major *Bifidobacterial* spp. were higher in mixed-fed infants compared to exclusively breastfed infants.[100] A separate study in the United Kingdom also found that prebiotic-fed infants have more *Bifidobacteria* spp. than breastfed or non-prebiotic, formula-fed infants.[101] These findings were recapitulated in developing countries such as Estonia where it was shown that the highest proportion of *Bifidobacteria* spp. were in infants receiving prebiotic formula.[102] Similar results were seen in Indonesian infants fed probiotic/prebiotic formula.[103] Collectively, this data shows that formula-fed infants often have lower bifidobacteria in

their gut because many formulas do not adequately mimic the nutrient composition of breast milk. Formulas that are fortified with prebiotics can increase *Bifidobacteria* spp. counts reducing the variability between breastfed and formula-fed infants. This is applicable to infants in both developing and developed countries. Therefore, improving the oligosaccharides in formula to better mimic breast milk may be the better strategy to increase *Bifidobacteria* spp. in formula-fed infants. Collectively, these reports show that niche dynamics, or the competition for nutritional resources essential for growth, give rise to patterns in the abundance of species in the infant gut. Any geographic differences reported in formula-fed infants is likely because of the nutritional content of product consumed and is not likely attributable to genetic, cultural or environmental differences.

Hygiene practices impact the ecological succession of bacterial communities in infants

Early colonization studies characterized the infant microbiome using carefully performed, culture-based techniques. These early studies led to a widely accepted classical colonization dogma whereby Firmicutes, aerobic and facultative anaerobic bacteria such as enterobacteria are the first intestinal colonizers in infants. As the oxygen levels are depleted from the infant intestinal tract, strict anaerobes such as *Bifidobacteria* spp., *Bacteroides* spp., eubacteria and clostridia spp. gain a competitive advantage and predominate. However, recently a shift in this pattern has been observed whereby initial colonization with enterobacter and *E. coli* are diminished and colonization rates of *Staphylococcus* spp. are high.[36,104] This shift may be a result of stricter hygiene in developed countries.[104] Using this logic, developing countries which have not imposed these hygiene measures should, theoretically, have higher *E. coli* and other Enterobacteriaceae than developed countries, matching classical studies. This notion is supported by next-generation sequencing studies (Figure 2; Table S2) as well as culture-dependent studies. For instance, a culture-dependent study comparing fecal bacteria between Ethiopian and Swedish neonates found that the dominating spp. among the

Swedish infants were *S. epidermidis* and *Bacteroides* spp.,[105] whereas *Bifidobacteria* spp. and *E. coli* were only found in a minority of infants between 0 and 2 weeks of age. In contrast, the 'classical' aerobic dominance of *E. coli* and *Enterococci* spp. was common in Ethiopian neonates. These differences disappeared by 2 weeks of age. Similarly, a study on 10 neonates from Brazil[106] reported a predominance by *Escherichia* and *Clostridium* spp., while *Staphylococcus* and *Bacteroides* spp. were only identified at low rates. The abundance of *Escherichia* spp. remained high throughout the first year of life,[107] whereas *Staphylococcus* spp. was absent, and *Bacteroides* spp. was low from the third week onwards. In a separate comparison between Pakistani and Swedish infants, it was likewise found that infants from the developing country were more rapidly colonized with enterobacteria[108] and had a predominance of *E. coli*. However, it should be noted that the Swedish infants were all breastfed and vaginally birthed whereas the Pakistani infants often started breastfeeding late and incompletely and were delivered both by vaginal and C-section. Still, other studies from Pakistan,[109] Ethiopia,[105] Brazil,[95] Singapore,[32] and Guatemala[110] all similarly report 'classical' colonization patterns with high enterobacteria observed. While colonization of enterobacteria such as *E. coli* is thought to signify fecal contamination, Adlerberth et al.[109] found that the high enterobacteria in Pakistani infants were likely derived from the environment as fewer than 50% of the *Escherichia* strains matched the mothers but could be found in the immediate home environment. Similarly, vertical transmission of *E. coli* was uncommon in infants born in hospitals in Japan.[111] Therefore, the birth environment may play a role in the initial colonization of enterobacteria in infants.

Classical studies preformed in the 1970s and 1980s ubiquitously report *E. coli* as a pioneering bacteria in the gastrointestinal tract newborn infants in both developed and developing countries.[109,110,112,113] However, as mentioned above, more recent studies in developed countries are trending away from this pattern and instead, report *Staphylococcus* spp. as the pioneering bacteria. *Staphylococcus* spp. were not previously been regarded as an intestinal commensal and were
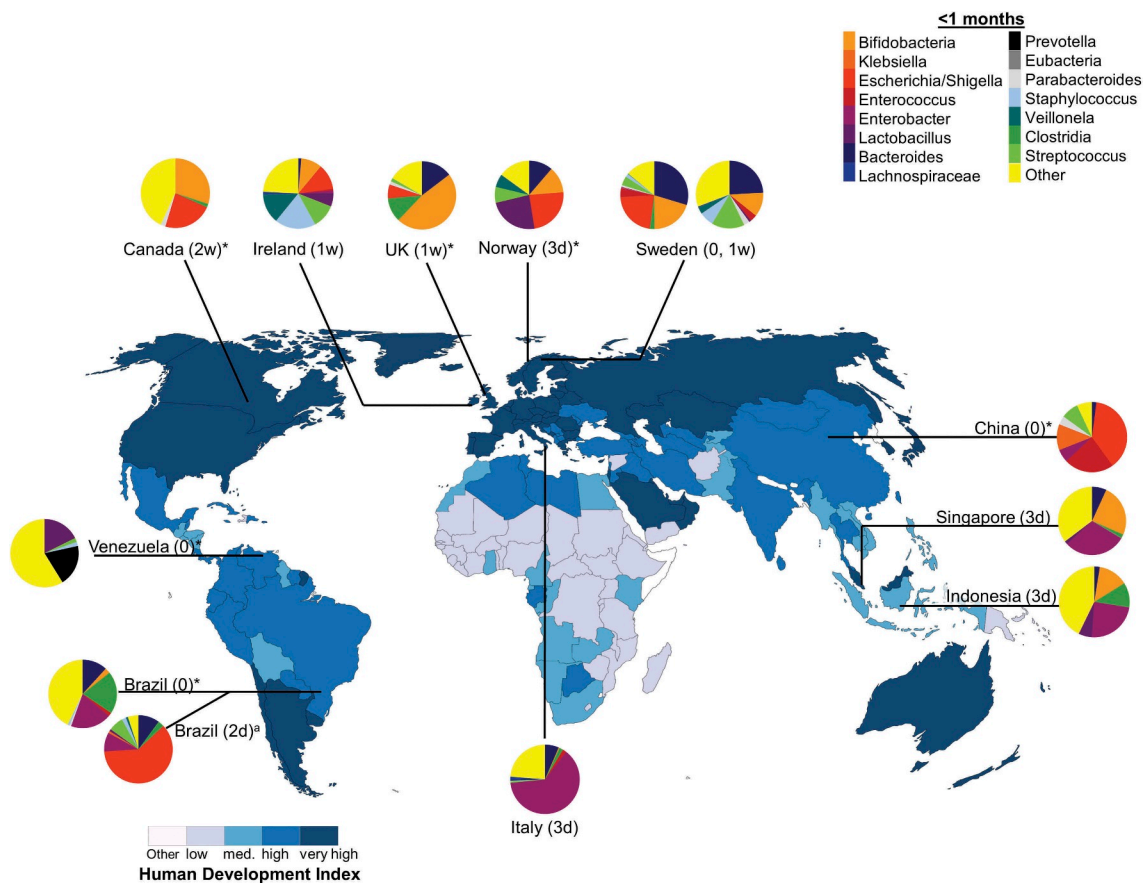
Figure 2. Neonate (<1 month) intestinal bacteria composition in diverse populations around the world. Pie chart color: bacterial taxa; color in map: human development index (http://hdr.undp.org/en/composite/HDI); *values were estimated from graph; [a] *Bifidobacteria* spp. were not detected using 16 S rRNA library technique; however, quantitative polymerase chain reaction detected *Bifidobacteria* spp. at 1 month which was the only tested timepoint; Country (age in d: days; w: weeks; 0: first stool).

infrequent in infant stool from Australia,[114] the United Kingdom[115] and Scotland[116] in the 1970s. However, in the 1980s and 1990s, *S. epidermidis* prevalence increased in industrialized countries such as Sweden[93,105] and *S. faecalis* predominated neonates in the United Kingdom.[112] A similar increase was described in France between 1975 and 1995.[117] Since the millennium, studies in Sweden have asserted that enterobacteria are not predominant and found that fewer than 50% of the infants were colonized with *Escherichia* at 1 week of age[104] and fewer than 61% at 2 months.[118] In contrast, *Staphylococci* spp. colonized 99% of the infants from d 3 postpartum onwards.[104] Another similar study showed staphylococci was positive in 73% of the infants by 2 months.[119] Geographic comparisons of infant microbiome between Göteborg, London and Rome similarly showed staphylococci as the earliest facultative anaerobic colonizers, predominating in nearly 100% of the

infants from d 3 postpartum onwards.[36] In this study, *E. coli* and other Enterobacteriaceae which were traditionally recognized as early colonizers, appeared late. Similarly, a well-controlled study in Norway[120] investigating exclusively breastfed, healthy, full-term infants who were not exposed to any medical interventions at d 4, 10, 30 and 120 found that while Enterobacteriaceae (*E. coli* and *Shigella*) were detected in over 70% of infants at 4 d post-partum, the prevalence of *Staphylococcus* was greater (90%). Collectively, these studies indicate that differences in hygiene between developed and developing countries impact initial colonization events in infants where enterobacteria are the pioneering bacteria in developing countries and *Staphylococcus* spp. predominate in developed countries.

While hygiene may impact initial colonization events, which would affect immune development, there are several exceptions to patterns described

above. A comparison of Pakistani and Dutch infants matched by age, sex, mode of deliver, birth weight and breastfeeding practices found no differences in diversity, richness or evenness, but reported higher Proteobacteria in Dutch infants including bacteria such as *Enterobacter* spp., *E. coli, Serratia* spp. and *Klebsiella* spp.,[121] which goes against the notion that developed countries have lower enterobacteria. Similarly in a study of 65–70 Nigerian neonates, rectal swabbed samples consisted mainly of coagulase-negative *Staphylococcus* (38.6%), followed by *S. aureus* (31.4%) and *E. coli* (30%) on the first day of life.[122] Coagulase-negative *Staphylococcus* remained the most frequently isolated bacteria at d 3, 9 and 14 d postpartum. Interestingly, this study reported an absence of *Actinobacteria* such as *Bifidobacteria* spp., which is consistent with previous findings in Nigeria published in 1982.[123] Therefore, not only does this publication contradict the notion that *Staphylococcus* spp. only predominate in developed countries but also challenges the dogma that *Bifidobacteria* spp. are the predominant anaerobe in healthy infants, globally.

While hygiene practices may impact early colonization events in infants, it is difficult to disentangle the impacts of hygiene in from other social and economic factors such as nutrition. As developing countries become urbanized, people's diets change. They move away from starch-rich staples to high sugars, fats and animal-based foods. This "nutrition transition,"[124] as opposed to improved hygiene, could account for the reported differences in breastfed infants. As discussed above, maternal dietary habits during lactation appear to shift the gradient of 'core' bacteria in breast milk. While there are currently too few studies to draw any definitive conclusions, we hypothesize that predominant microbial communities in breast milk would more effectively colonize the breastfed infants. In support of this, early colonization of *Staphylococcus* spp. in infants born in developed countries synergizes with increased *Staphylococcus* spp. in breast milk collected from Spain, China and Finland when compared with breast milk samples from South Africa, which were predominated by Proteobacteria.[49]

Collectively, ecological succession of microbial communities in infants start with pioneering bacteria adapted to an oxygen-rich environment, such as aerobes or facultative anaerobes. Hygiene and other social and economic factors associated with urbanization such as diet may contribute to taxonomic variations in these pioneer communities. Whether differences between these pioneering communities lead to functional health differences in infants remain to be determined.

Ecological succession of *Bifidobacteria* spp.

In general, the presence of early pioneering species such as Enterobacteriaceae, *Streptococcus*, and *Staphylococcus* spp. are succeeded by *Enterococcus* and *Lactobacillus* spp., which modify the infant gut habitat, generating an anaerobic environment that favors subsequent anaerobic colonizers. As mentioned above, niche dynamics in breastfed infants favor the predominance of *Bifidobacteria* spp. *Bifidobacteria* spp. have received a disproportionate amount of attention due to their reputed health benefits. It is thought that because *Bifidobacteria* spp. are a predominant component of the infant gut, they may be the main microbial candidate to influence the physiology/immunology of infants. As bifidobacteria are known producers of lactate and acetate in the gut, their abundance in breastfed infants may reduce risk of infections. In support of this, it has previously been shown that a low amount of acetate in infant stool is associated with infantile diarrhea and upper respiratory tract infections.[125] However, the association between *Bifidobacteria* spp. and reduced infant morbidity was not observed in Gambian and Malawian infants, whereas there was a positive association with higher *Prevotella* spp.[126] Nevertheless, identifying geographic differences in the succession of *Bifidobacteria* spp. in healthy infants may be useful in understanding global epidemiologic health differences.

While most researchers agree that *Bifidobacteria* spp. eventually predominates in breastfed infants, the rate of prevalence (Table 1), and overall abundance of *Bifidobacteria* spp. (Table 2) varies. For example, one study in Japan[127] reported that newborns were predominated by anaerobic bacteria such as *Streptococcus* (100%) and Enterobacteriaceae (100%) with low initial colonization rates (0%) of *Bifidobacteria* spp. Overtime *Bifidobacteria* spp. proliferated and was present in 100% of infants 3–7 d

Table 1. Frequency rates of bifidobacteria colonization by geographic region.

Study	Year	Country	n	% BF	Method	<1 w	1 w-1 m	1 m	2 m	3 m	4 m	5 m	6 m
HDI >0.80													
131[a]	2013	Australia	6 V, 4 C	50	16S rRNA	10	100	100	NA	NA	NA	NA	NA
114	1982	Australia	7	100	Culture	NA	NA	85.7	NA	NA	NA	NA	NA
137	1995	Belgium	34	41	Culture	NA	21	57	NA	NA	NA	NA	NA
						NA	NA	FF: 60	NA	NA	NA	NA	NA
						NA	NA	F: 20	NA	NA	NA	NA	NA
37	2013	Canada	18 V, 6 C	42	Illumina 16S rRNA; V5-V7	NA	NA	NA	NA	NA	75	NA	NA
150	2009	Czech Republic	22	100	Culture & FISH	NA	NA	NA	NA	64	NA	NA	NA
132	2000	Finland	34 V, 30 C	64	Culture	62	78	90	NA	NA	NA	NA	NA
21	2008	Greece	34 V	23, 10	Culture & PCR	23	NA	35	NA	NA	NA	NA	NA
101	2015	Ireland	11 V, 6 C	100	Culture & 16S rRNA-ITS + PFGE	47	NA	47	NA	NA	NA	NA	58
130	1992	Italy	12	100	Culture	20	NA	80[b]	NA	50	NA	NA	NA
36[a]	2007	Italy, Sweden, England	216 V, 108 C	23	Culture & PCR, collectively	63[b]	80[b]	90[b]	95[b]	NA	NA	NA	NA
151	2012	Japan	132	32	Reverse transcription qPCR	34	73	88	NA	97	NA	NA	98
100	2017	Japan	76	25	Reverse transcription qPCR	21	64	79	NA	97	NA	NA	99
127	1985	Japan	6 V	100	Culture	NA	100[b]	NA	NA	NA	NA	NA	NA
80	1983	Japan	5 V	100	Culture	40	100	100	NA	NA	NA	NA	NA
113	1984	Japan	35 V	100	Culture	NA	NA	100[b]	NA	NA	NA	NA	NA
152	2015	Korea, south	143	NS	Culture & T-RFLP	2.1	NA	NA	NA	NA	NA	NA	NA
31	2006	Netherlands	700	100	qPCR	NA	NA	99[b]	NA	NA	NA	NA	NA
92	2005	Netherlands	50	100	qPCR	NA	NA	100[b]	NA	NA	NA	NA	NA
120[a]	2011	Norway	85 V	100	16S rRNA clone libraries & PCR	80	NA	NA	NA	NA	NA	NA	NA
						78	NA	NA	NA	NA	NA	NA	NA
133	2018	Poland	79 V	100	Culture	73	NA	NA	NA	73	NA	NA	NA
153	2017	Slovenia	49 V, 9 C	67	qPCR	57	NA	94.7	NA	100	NA	NA	NA
27	2014	Sweden	109 V, 19 C	81	454 16S rRNA, V3-V4	91[b,c]	91[b]	NA	94[b]	NA	NA	NA	98[b]
						85[b,d]	88[b]	NA	92[b]	NA	NA	NA	97[b]
19	2015	Sweden	83 V	74.4, 68.8	Shotgun	62	NA	NA	NA	NA	80	NA	NA
129	1990	United Kingdom	15 V, 15 C	100	Culture & GC	27	NA	21	NA	NA	NA	NA	NA
138	2010	United States	1	100	Sanger sequencing	NA	NA	ND	NA	NA	NA	NA	NA
HDI <0.80													
106	2012	Brazil	9 V	100	16S rRNA & qPCR	NA	NA	100	NA	NA	NA	NA	NA
95	2017	Brazil	12	1 m: 100, 3 m: 75	16S rRNA qPCR	80	90	100	NA	100	NA	NA	NA
105[a]	1991	Ethiopia[e]	43 V, 17 C	NS	Culture	NA	25	35	NA	NA	NA	NA	NA
122	2016	Nigeria	65 V, 5 C	21.3	Culture	ND	ND	ND	NA	NA	NA	NA	NA

Whenever possible, exclusively breastfed, vaginally birthed data were reported.

Abbreviations: % BF: percent exclusively breastfed; n: number of vaginally (V) and C-section (C) delivered infants; HDI: human developmental index score; FISH: Fluorescence in situ hybridization; qPCR: quantitative polymerase chain reaction; GC: gas chromatography; T-RFLP: Terminal restriction fragment length polymorphism; NA: not applicable; ND: none detected; FF: fortified formula; F: formula; m: month.

[a]Indicates values were estimated from available figures.
[b]Indicates *Bifidobacteria* spp. were predominant.
[c]Anthroposophic cohort.
[d]Non-anthroposophic cohort.
[e]21 infants receiving antibiotic treatment at the time of sampling.

postpartum. Other countries reporting low initial colonization rates of *Bifidobacteria* spp. include South Korea (2.1%),[128] Greece (23%),[21] Japan (21%),[100] Nigeria (0%),[122] the United Kingdom (27%),[129] Italy (20%)[130] and Australia (10%).[131] However, while rates of *Bifidobacteria* spp. colonization are low in these neonates, counts are often high (Table 2). For example, in Japan, despite only 34% of the samples being positive for *Bifidobacteria* spp., mean counts were 5.7 ± 1.7 $\log_{10}$ CFU/g wet stool, which was higher than the more prevalent counts of *Enterococcus* (5.0 ± 1.8 $\log_{10}$ CFU/g; 57%) and *Staphylococcus* spp. (5.4 ± 1.5 $\log_{10}$ CFU/g; 60%). Similar results were reported in the United

Table 2. Bifidobacteria abundance by geographic location.

						Counts (mean $\log_{10}$ CFU/g wet stool)							
Study	Year	Country	n	% BF	Method	<1 w	1 w-1 m	1 m	2 m	3 m	4 m	5 m	6 m
HDI >0.80													
114	1982	Australia	7	100	Culture	NA	NA	10.6[b]	NA	NA	NA	NA	NA
137	1995	Belgium	34	41	Culture	NA	8.51	BF: 8.61 FF: 8.10 F: 9.75	NA	NA	NA	NA	NA
150	2009	Czech Republic	22	100	Culture & FISH	NA	NA	NA	NA	NA	NA	NA	NA
132	2000	Finland	34 V, 30 C	64	Culture	10.1[b,c]	10.7[b,c]	10.8[b,c]	NA	10.14[b]	NA	NA	NA
154	2012	Finland	22	100[i]	FISH-FC & qPCR	NA	NA	NA	NA	NA	NA	NA	10.7[b]
154	2012	Germany	8	100[i]	FISH-FC & qPCR	NA	NA	NA	NA	NA	NA	NA	9.9[b]
21	2008	Greece	34 V, 48 C	23, 20, 12	Culture & PCR	7.70	NA	7.47	NA	NA	NA	NA	NA
130[a]	1992	Italy	12	100	Culture	8.9	NA	9[b]	NA	NA	NA	NA	NA
36[a]	2007	Italy, Sweden, England	216 V, 108 C	23[j]	Culture & PCR	NA	9.5[b]	9.6[b]	9.8[b]	9.8[b]	NA	NA	NA
151	2012	Japan	132	32	RT qPCR	5.7	8.2	9.0	NA	9.8[b]	NA	NA	10.3[b]
100	2017	Japan	76	25	RT qPCR	6.2	8.0	8.5	NA	9.3[b]	NA	NA	9.6[b]
127	1985	Japan	6 V	100	Culture	ND	10.4[b]	NA	NA	NA	NA	NA	NA
80	1983	Japan	5 V	100	Culture	5.0	9.7[b]	11.3[b]	NA	NA	NA	NA	NA
113	1984	Japan	35 V	100	Culture	NA	NA	10.74[b]	NA	NA	NA	NA	NA
152	2015	Korea, south	143	NS	Culture & T-RFLP	2.24	NA	NA	NA	NA	NA	NA	NA
31	2006	Netherlands	1032	68	qPCR	NA	NA	10.68[b,d]	NA	NA	NA	NA	NA
92	2005	Netherlands	50	100	qPCR	NA	NA	10.56[b,d]	NA	NA	NA	NA	NA
133	2018	Poland	79 V	100	Culture	9.14[d]	NA	NA	NA	8.32[d]	NA	NA	NA
153	2017	Slovenia	49 V, 9 C	67	qPCR	NA	NA	NA	NA	NA	5.89[d]	NA	NA
129	1990	United Kingdom	15 V, 15 C	100	culture of lacto and bifido +GC	10.24[b]	NA	9.48[b]	NA	NA	NA	NA	NA
HDI <0.80													
95	2017	Brazil	12	1 m: 100, 3 m: 75	16S rRNA NAqPCR	10.9	12.98[b]	11.68[b]	NA	11.98[b]	NA	NA	NA
102[a]	2017	Estonia	4 V, 5 C	NS[b]	Illumina MiSeq 16S rRNA, V1-V2 & qPCR	ND	NA	NA	NA	3.25[e]	NA	NA	1.69[f]
105[a]	1991	Ethiopia	43 V, 17 C	NS	Culture	NA	6.5[f]	6.4[b,f]	NA	NA	NA	NA	NA
155	2014	Bangladesh	8 V, 40 C	6 w: 79.2, 11 w: 60.4, 15 w:45.8	Illumina MiSeq 16S rRNA, V4 & T-RFLP, PCR	NA	NA	NA	NA	7.48[d]	NA	NA	NA
Relative % Abundance													
37	2013	Canada	18 V	42	Illumina 16S rRNA; V5-V7	NA	NA	NA	NA	NA	35.2[b,c]	NA	NA
102	2017	Estonia	4 V, 5 C	NS[b]	Illumina MiSeq 16S rRNA, V1-V2 & qPCR 100[i]	0	NA	NA	NA	NA	NA	NA	5
154	2012	Finland	22	100[i]	FISH-FC & qPCR	NA	NA	NA	NA	NA	NA	NA	68.1[b]
154	2012	Germany	8	100[i]	FISH-FC & qPCR	NA	NA	NA	NA	NA	NA	NA	58.3[b,g]
35	2014	Sweden	15 V	73	454 16S rRNA, V3-V4	NA	11.6	34.4[b]	NA	44.7[b]	NA	NA	32.1[b]

(Continued)

Table 2. (Continued).

Study	Year	Country	n	% BF	Method	Counts (mean log$_{10}$ CFU/g wet stool)							
						<1 w	1 w-1 m	1 m	2 m	3 m	4 m	5 m	6 m
27	2014	Sweden	109 V, 19 C	81	454 16S rRNA, V3-V4	A: 35^b NA: 26^b	39^b 36^b	NA	37^b 42^b	NA	NA	NA	45^b 33^b
19	2015	Sweden	83 V, 15 C	74.4, 68.8	Shotgun	20.2	NA	NA	NA	NA	NA	NA	NA
23^a	2017	United Kingdom	83 V, 74 V, 74 V, 24 V	100	Illumina 16S rRNA, V4-V5 region	NA	47.5^b	55.7^b	59^b	NA	42.2^b	NA	49.1^b
138	2010	United States	1	100	Sanger sequencing	NA	NA	ND	NA	NA	NA	NA	NA
156^a	2015	United States	39 V	70^b	Illumina 16S rRNA, V4	23^b	30^b	NA	27^b	NA	45^b	NA	NA
139	2017	United States	71 (mixed VC)	100	16S rRNA – V3-V5; 454 pyrosequencing	NA	NA	NA	NA	NA	NA	NA	9.9
HDI <0.80													
134	2017	Armenia	53 V	100	Illumina 16S rRNA, V4	NA	30^b	55^b	50^b	NA	NA	NA	NA
95	2017	Brazil	12	1 m: 100, 3 m: 75	16 S rRNA qPCR	10.9	12.06^b	11.68^b	NA	11.98^b	NA	NA	NA
135	2016	China	7 V, 7 C	NS	454 16S rRNA, V3-V5	0	NA	NA	2	NA	NA	NA	NA
139^a	2018	Ecuador	59 V, 11 C	89	Illumina MiSeq 16S rRNA	NA	NA	NA	50^b	50^b	NA	NA	NA
126	2017	Gambia	85	NS	Illumina 16S rRNA, V4	NA	NA	NA	NA	NA	NA	68.3	NA
134	2017	Georgia	17 V, 11 C	100	Illumina 16S rRNA, V4	NA	40^b	40^b	55^b	NA	NA	NA	NA
20^a	2013	India	73 V	1 w: 92,	qPCR	3^i	NA	43^h	NA	NA	NA	NA	NA
103	2013	Indonesia	72 V, 8 C / 67 V, 13 C	100 / 0	qPCR	NA / NA	BF: 45^{b,g} / FF: 45^{b,g}	NA	NA	NA	NA	NA	NA
155	2014	Bangladesh	8 V, 40 C	6 w: 79.2, 11 w: 60.4, 15 w:45.8	Illumina MiSeq 16S rRNA, V4 & T-RFLP, PCR	NA	NA	NA	NA	59.6^b	NA	NA	NA

Whenever possible, exclusively breastfed, vaginally birthed data were reported.

Abbreviations: %BF: percent exclusively breastfed; n: number of vaginally (V) and C-section (C) delivered infants; HDI: human development index score; FISH: Fluorescence in situ hybridization; qPCR: quantitative polymerase chain reaction; RT reverse transcription; GC: gas chromatography; T-RFLP: Terminal restriction fragment length polymorphism; NA: not applicable; ND: none detected; FF: fortified formula; F: formula; w: week; m: month.

[a] Indicates values were estimated from available figures.

[b] Indicates *Bifidobacteria* spp. were the most abundant taxa at this point.

[c] Expressed as median value.

[d] Log10 value calculated from mean copies/g feces.

[e] Values expressed as mean log10 mg of stool.

[f] 21 infants receiving antibiotic treatment at the time of sampling.

[g] Values expressed as mean percentage of bacteria compared to total bacteria analyzed.

[h] Values expressed as relative difference to 'universal' bacteria domain sequence.

[i] Value presented at d 1 postpartum, value increased 10-fold on d 4.

[j] % Breastfed at 6 months .

Kingdom where only 27% of the infants were colonized but had high numbers (10.24 log$_{10}$ CFU/g wet stool).[129] Moreover, the low reported prevalence of *Bifidobacteria* spp. colonization in neonates is not consistent. For instance, in Finland, 62% of the infants were colonized with a mean of 10.1 log$_{10}$ CFU/g wet stool *Bifidobacterium* spp. 3 d postpartum.[132] This exceeded the prevalence of other bacteria tested including lactobacillus-like bacteria, *C. perfringens* and *B. fragilis*, and was the highest reported count. Similarly, studies from Poland (73%),[133] Sweden (76-91%),[19,27] Brazil (80%),[95] Norway (78–80)[120] all report high colonization rates of *Bifidobacteria* spp. in neonates. By 1-week postpartum, culture and PCR-based counts of *Bifidobacteria* spp. were the most numerous taxa in infants born in Finland,[132] Netherlands,[31] Italy, London, Sweden,[36] Indonesia,[103] Brazil,[95] Japan,[127] and Armenia and Georgia[134] despite not always being the highest % colonized (Tables 1 and 2).

Studies using 16 S sequencing approaches (Table 2) yield similar results. By 1 month, the majority of studies analyzed globally by 16 S sequencing methods report a predominance of *Bifidobacteria* spp. (Figure 3), which persists through 4-month postpartum (Figure 4). However, while the large majority of published studies show *Bifidobacteria* spp. predominate the infant gut by 1-month postpartum, a select few do not. For example, in vaginally delivered Chinese infants, Proteobacteria such as *Escherichia/Shigella* and *Klebsiella* spp. still predominated in 2-month-old infants.[135] A separate study using microarray assays and sequencing of cloned libraries of PCR-amplified small subunit ribosomal rDNA techniques in the United States found that *Bifidobacteria* spp. did not appear until several months after birth in 14 full-term infants and persisted only as a minority population.[136] Moreover, despite high relative abundances, several papers still report low prevalence (<60% colonized) including
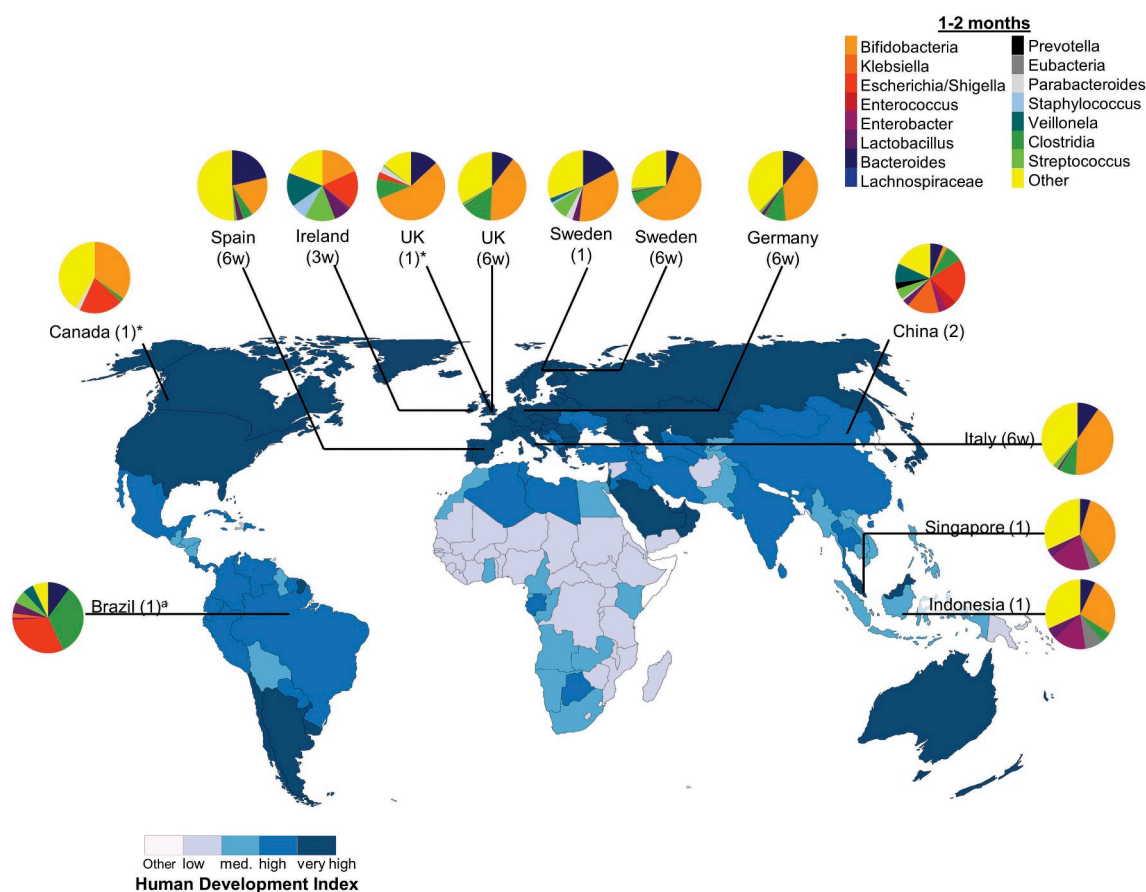
Figure 3. Intestinal bacteria composition in infants aged 1–2 months from diverse populations around the world. Pie chart color: bacterial taxa: human development index (http://hdr.undp.org/en/composite/HDI); *values were estimated from graph; [a] *Bifidobacteria* spp. were not detected using 16 S rRNA library technique; however, quantitative polymerase chain reaction detected *Bifidobacteria* spp. at 1 month which was the only tested timepoint; Country (age in months unless otherwise stated; w: weeks).
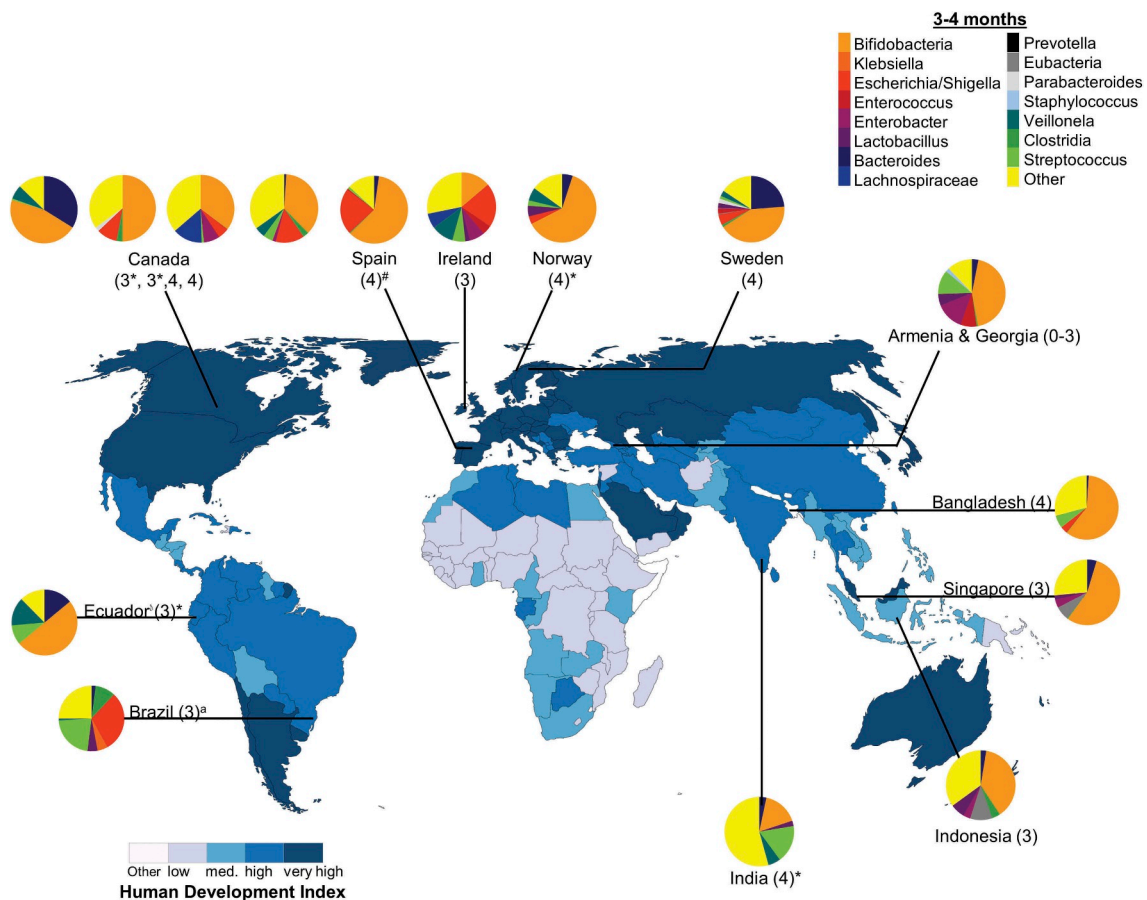
Figure 4. Intestinal bacteria composition in infants aged 3–4 months from diverse populations around the world. Pie chart color: bacterial taxa; color in map: human development index (http://hdr.undp.org/en/composite/HDI); *values were estimated from graph; #values were calculated from provided data; a Bifidobacteria spp. were not detected using 16 S rRNA library technique; however, quantitative polymerase chain reaction detected Bifidobacteria spp. at 1 month which was the only tested timepoint; Country (age in months).

papers from Belgium (57%),[137] Greece (35%),[21] Ireland (47%)[101] the United Kingdom (21%),[129] the United States (none detected [ND]),[138] Ethiopia (35%)[105] and Nigeria (ND).[122] While these studies show that Bifidobacteria spp. are often only present in a small fraction of infants or are not numerically dominant, some argue that the paucity of Bifidobacteria spp. in these studies may be a result of technical bias relating to media selectivity, DNA extraction protocols and PCR primers used.[94] Indeed, in Scotland, the prevalence of Bifidobacteria spp. largely depended on the method used.[83]

Collectively, these independent studies support the ecological succession from aerobic and facultative anaerobic pioneering bacteria to a community rich in species like Bifidobacteria spp. which can replace or reduce pioneering species abundance

through competition. The rate of this succession varies but does not appear coincide with any patterns based on geography.

The influence of geography on infant fecal microbiota does not supersede classical patterns of bacterial colonization

While many studies have reported on bacterial species in infants within one population, far fewer studies have attempted to compare the temporal progression of bacterial communities across cultural and geographic settings. A recent study compared the bacterial composition of healthy 3-month-old Ecuadorian infants with data previously collected in Canada[140] and found infants from both countries were dominated by Bifidobacteria spp., followed by

either *Bacteroides* or *Veillonella* spp. However, Canadian infants were found to have comparably higher abundances of *Bacteroides* spp., and lower abundances of *Veillonella* spp. Unfortunately in this study, methodical differences between the Canadian (CHILD) and Ecuadorian (ECUAVIDA) study make comparisons difficult and interpretations should be taken cautiously. A similar approach was used by Kuang et al.,[135] who compared the fecal microbial composition of 29 healthy, 2-month-old Chinese infants to studies previously published in Brazil, the United States, Sweden, Canada and Bangladesh. They reported marked differences among countries at the phylum level, which broadly separated into three clusters. All the infants from China and 70% of the infants from Brazil fell into a high Proteobacteria cluster. Additionally, 82% of the infants from the United States, 54% of the infants from Sweden and 79% of the infants from Canada contained a high prevalence of Actinobacteria, whereas 70% of the infants from Bangladesh and 33% of the infants from Sweden were highly abundant in Firmicutes. Using a PERMANOVA, they found geographic location accounted for 19.6% of variation in infant fecal microbiota, which was larger than age, delivery mode and feeding patterns and concluded that geographical factors have a strong impact on infant colonization. However, as they utilized data extracted from other studies there are limitations, such as differences in primers, DNA extraction methodology, hypervariable region amplified as well as the sequencing platforms. To eliminate methodological biases between independent studies, large cross-cultural studies are needed.

In an effort to approach this, one cross-cultural study comparing the 6-week fecal bacterial composition of infants born in Sweden, Scotland, Germany, Italy and Spain reported that geography had a high impact on levels of *Bifidobacteria* spp., *Bacteroides* spp., and enterobacteria.[34] In this study, infants born in Spain had higher proportions of *Bacteroides* spp. and enterobacteria and lower *Bifidobacteria* spp. compared with infants born in Sweden, Scotland, Germany and Italy. In contrast, infants in Sweden had higher proportions of *Bifidobacteria* spp. While the authors reported that Swedish and Italian bacterial communities did not cluster as a function of

feeding method, there were notable differences in breastfeeding rates between countries. For example, in Spain breastfeeding rates were 43.1% at the time of sample collection whereas in Sweden breastfeeding rates were 75.9% and Germany had rates of 45%. In a separate analysis, the authors reported that breastfed infants had higher proportions of *Bifidobacteria* spp. and lower proportions of *Bacteroides* spp., which corresponded with varying rates of breastfeeding between the countries. Regardless of geographic location and breastfeeding practices, all infants had a predominance of *Bifidobacteria* spp., followed by *Bacteroides* spp. then enterobacteria, supporting previous findings.[92,93,113,114] A separate study by Turroni et al.[94] similarly compared fecal samples of infants born in Italy, Ireland and Spain. In contrast to the geographic impacts reported by Fallani et al., this study found that infants from within a given population displayed widely dissimilar microbial patterns and concluded that infant gut colonization was not influenced by the geographic origin to which the infant was born. Another European study compared the infant microbial colonization process between Armenia and Georgia.[134] While these are neighboring countries, they possess distinct language and cultural differences. This study found that country of origin accounted for 8.4% of the total variation between samples, delivery mode accounted for 3.1% and mother's secretor status accounted for 1.6%. However, despite these factors, the overall patterns of colonization were similar between the two countries. In both countries, *B. breve* predominated. The major non-*Bifidobacteria* taxa included enterobacteria, streptococci, lactobacilli, enterococci, *Bacteroides* spp., *Clostridia* spp. and staphylococci, in agreement with other studies.

While differences between developed and developing countries have been reported, some groups propose that the influence of lifestyle is subtle compared with the variability between infants within a single population, at least until weaning. For example, Yatsunenko et al. show that major geographic differences between the fecal microbial communities of breastfed infants in rural Malawi, Venezuela (Amerindians) and metropolitan America do not appear until 3 y of age.[141] This

study reported three notable findings. The first was that bacterial diversity increased with age in all infants and that all infants diverged toward an adultlike bacterial community within a 3-y period. The second was that interpersonal variations are significantly greater between infants than between adults. The authors confirmed that this finding is robust to geography. Finally, they reported differences in the phylogenetic composition of fecal microbiota between infants living in different countries. Using shotgun and 16 S rRNA-V4 sequences it was found that infants from all three populations had a predominance (75 ± 20%) of *Bifidobacteria* spp. throughout their first year of life, which declined as the infants aged. There were several operational taxonomic units which could be used to distinguish between Malawian, Venezuelan and American infant microbiomes. In general, the American babies had an overrepresentation of *Prevotella* spp. compared to the Malawian and Venezuelan infants. While a total of 28 operational taxonomic units distinguished American infants from those born in Malawi and Venezuela, there were 21 operational taxonomic units discriminating between Malawian and Venezuelan infants. The Venezuelan infants were found to have higher Enterococcaceae compared to the Malawian. The authors concluded that a Western lifestyle appears to systematically affect the bacterial component of the intestinal microbiome, but the influence is subtle compared to the high degree of variability observed in infants within each population. Insights from independent studies corroborate these findings. Using 16 S rRNA gene-based microarrays, it was shown that there is considerable intra- and interpersonal variability in the fecal bacteria of 12 unrelated American children in the first year of life.[136] This significant level of inter-individual variation among unweaned infants has also been reported within infants born in Italy, Ireland and Spain[94] as well as Japan.[142] Similar to the results obtained by Yatsunenko et al., this variability decreased and converged toward an adultlike microbiome as the infants age. When looking at population-specific differences in the microbiome functions, it was observed that Malawian and Venezuelan infants had higher genes involved in riboflavin (vitamin B2) biosynthesis suggesting an adaptive response to vitamin availability. Similarly, genes involved in utilizing host glycans differed suggesting differences in the glycan content of breast milk. Both these findings are not surprising given that human milk vitamin content and oligosaccharides cluster by country of origin. What was somewhat surprising was that *Bifidobacteria* spp. may not be pivotal to biosynthetic pathways in infants and that these effects remained when all the *Bifidobacteria* spp. reads were excluded.

Cross-cultural comparisons between developing countries show similar trends to those reported in Europe. For example, a comparison of infants from Bangladesh, the Gambia, Kenya and Mali found that healthy infants in these countries followed the stereotypical[136,143] colonization process described in developed countries. During the first year of life, the "healthy" microbial community was characterized by low overall diversity and high proportions of facultative anaerobes and potentially pathogenic organisms such as *Escherichia/Shigella* spp.[144] As these infants were weaned, microbial diversity increased as did the abundance of *Prevotella* spp. While these observations broadly held true when stratifying by geographic location, some country-specific effects were observed. For example, infants in Bangladesh were characterized by lower proportions of *Prevotella* spp. and higher *Escherichia/Shigella* and *Streptococcus* spp. over the study period (up to 5 y of age).[144] A separate study comparing the microbiota colonization in two partially breastfed Asian populations, Singapore and Indonesia, likewise found a similar trend in bacterial colonization over the first year of life, despite differences in geography, cultural ethnicity and overall social economic development.[145] They reported that both populations had Enterobacteriaceae and *Bifidobacteria* spp. predominating the stool microbiota of infants under 3 months of age. However, the relative abundance of *Bifidobacteria* spp. was lower in the Indonesian group, who had higher relative abundances of *C. leptum* and *Atopobium* compared to the Singapore population.

Clinical implications and limitations

The aim of this review was to synthesize data across studies on the healthy infant gut colonization process, globally. The trajectory of the infant colonization process has lifelong clinical

implications as discussed previously.[146,147] The results from this review emphasize that human behaviors and not genetics are the largest predictor of microbial variability in infants and can be used as a guideline for behavioral changes. This includes increasing global breastfeeding rates by providing education about breastfeeding and reducing societal stigma. For mothers who are unable to breastfeed, it includes having access to formulas that truly mimic breast milk composition.[148] With respect to cesarian section deliveries, every effort should be made to reduce non-medical cesarian deliveries as there is strong evidence of overuse in all parts of the world.[149]

There are several limitations in comparing data extracted from independent studies. This includes differences in DNA extraction methodology, hypervariable region amplified, reference databases, primers and sequencing platforms. These differences in experimental protocols may produce variation that undermines subtle biological differences. Furthermore, there are inherent shortcomings of molecular approaches used in studies. For instance, differences in bacterial communities cannot be distinguished at the species level using operational taxonomic units.[150] Finally, we did not merge and statistically analyze the summarized data. Merging and analyzing from different studies is limited in practice. Current and future developments, such as amplicon sequence variants methods and standardization of procedures, will better allow for data to be compared across studies.[150] With these caveats stated, studies of the microbiota based on 16 S rRNA gene targets can, at least in principal, be compared to gain insight into broad evolutionary patterns.

Conclusion

Microbial communities in the infant gut exhibit orderly and predictable patterns of succession beginning with relatively few pioneering bacteria which develop through increasing complexity until they reach a relatively stable "climax" (adult) community. The trajectory of successional change appears to be influenced by human behaviors that might influence the availability of colonists. In newborn infants, the gastrointestinal site is oxygenated, and only aerobic and facultative anaerobic bacteria are capable of thriving in this habitat. The availability of initial colonists is dependent on delivery mode whereby vaginally delivered infants are exposed to maternal vaginal and fecal microbial communities and C-section delivered infants are exposed to skin and environmental microbes. These pioneering bacteria modify the habitat, generating an anaerobic environment which favors subsequent anaerobic colonizers. While site conditions allow anaerobes to colonize, nutrient availability from feeding practices predicts dominating species. Breast milk, despite maternal diet, increases Bifidobacteria in the infant gut likely due to their ability to metabolize human milk oligosaccharides, whereas the predominant anaerobes in formula-fed infants vary depending on the available dietary substrates in the formula used. In addition to delivery mode and feeding practices, environmental factors such as hygiene also impacts infant microbial colonization with hygiene resulting in higher *Staphylococcus* spp. and lower enterobacteria. Overall, however, independent studies investigating the gut microbiota at various points in postnatal development are limited either by low sample sizes, analytic methods used, or the scope of the population surveyed. While these studies provide important insights, large cross-cultural studies are needed given the variability between infants within a single population are higher than any other factor including geography, genetics and environment. Despite this, the general succession of microbial colonization is similar among vaginally birthed and exclusively breastfed infants globally. We conclude that human behavior including cultural variations in feeding practices, delivery modes and hygiene, over geography or race, are the largest predictors of microbial variability in the infant gut.

Methods

Literature search strategy

For this literature review, we searched Web of Science (1864–2019), PubMed (1966 to 2019), Google Scholar and hand searches for primary studies profiling the microbiome in infants aged 0 to 6 months. The search strategy was based on a clear and careful selection of key words including combinations of: (microbiome* OR bacteriome*

OR commensal* OR flora* OR microorganism* OR coloniz* OR colonis*) AND (neonatal* OR infant* OR baby*) AND [country] where we systematically searched for publications stating one of the 195 counties in the world today.

Study eligibility

The included primary studies had to report a healthy infant gut bacterial community. Healthy was defined as having no preexisting or diagnosed conditions such as allergic diseases, autoimmune diseases or known infections at the time of sample collection. The methods for assessing bacterial composition could include sequencing or culture-based methods. All levels of reported phylogeny were included. Studies were excluded if they did not report on the gastrointestinal microbiome, the full text was not available, the study population was not less than 6 months of age, or there was no means to quantify the findings.

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Authors' contribution

Critically evaluated the data: CQ. Wrote the paper: CQ, DLG

Competing Interests

The authors declare no conflicts of interest.

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