# The Microbiome: From the Beginning to the End

*by Rajeev Aurora, PhD & Thomas Sanford, MD*

**All data strongly suggest that the microbiota have a significant impact early in life on neural/cognitive development, and on metabolism setting the health trajectory to develop, for instance obesity, cardiovascular disease, and other comorbidities of aging.**

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## Abstract

**The human microbiota, a community of microorganisms in our bodies, is crucial for our health. This paper explores its development from birth through old age, highlighting some of the unique roles at key life stages—infancy, adulthood, and in the elderly years. Understanding the significant health impacts and consequences of changes in the microbiota offers insights for both the public and clinicians.**

## Introduction

The human microbiome has been intensely studied over the last two decades, leading to some surprising findings. We have previously written on the topic summarizing known mechanisms by which microbes influence health in this journal.[1] Here, we highlight recent findings which show that at each age, the microbiota contributes uniquely to our health. Our bodies are protected by barriers such as the skin, as well as mucosa that lines the mouth, airway, and the intestinal tracts. Each anatomical site has a defined community of bacterial and fungal species. The collection of ("omes") genomes that are encoded by the microbiota is called the microbiome. The gut microbiome is the largest by mass, most diverse, and most well studied. The gut microbiome is affected by age, sex, and geography. In addition, it is influenced by diet, whether we live in urban or rural setting, ethnicity, socioeconomic status, and other aspects including medications and presence of household pets.[2-5] Rather than focusing on the variation across populations, here we summarize recent data that reveals how the microbiome develops and effects our health (Figure 1). We provide examples of functions encoded by the bacterial genomes and how gain or loss of these functions impacts health.

## Bacterial Nomenclature

A short paragraph on nomenclature may be helpful here. In the context of microbiology and clinical sciences, taxon refers to the groups or categories into which organisms are classified based on shared characteristics and genetic similarities. This classification system, known as taxonomy, is fundamental in understanding the diversity and relationships among different organisms, including bacteria, viruses, fungi, and other microorganisms commonly encountered in clinical settings. Taxa are arranged hierarchically, ranging from broad categories like kingdoms and phyla to more

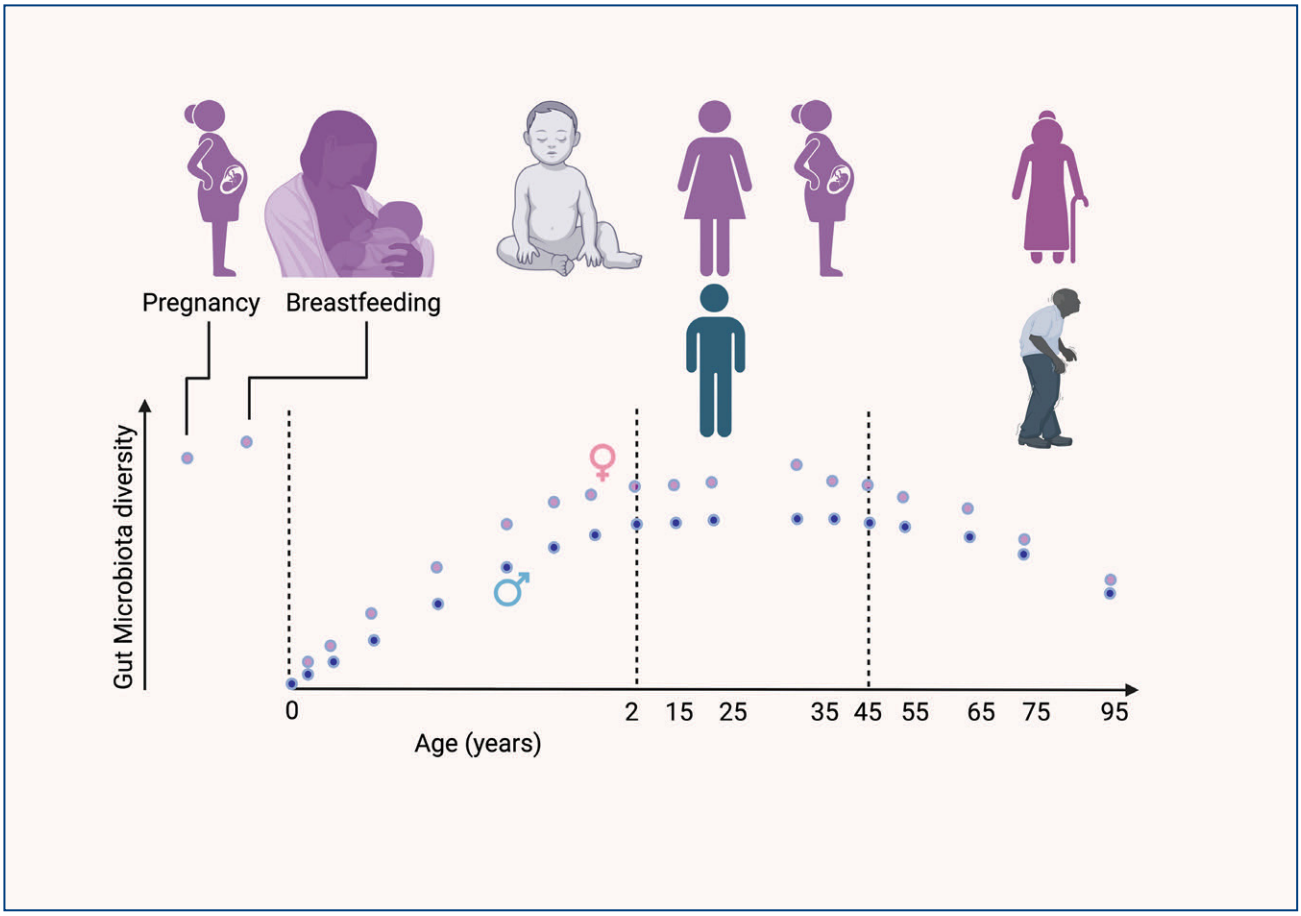


**Figure 1.** Changes in gut microbiome diversity across age: Multiple recent studies show that the gut microbiome is established by age of two years. A majority of the gut microbiota is inherited from the mother via breastmilk, that remains stable into adulthood. The diversity and abundance of the taxa vary with sex, geography, pets, hygiene, and urban vs. rural environments. Antibiotic use also has a significant impact. As individuals age, the microbiota starts to change, coinciding with reproductive senescence (menopause and loss of testosterone). Diet, medications, and lifestyle influences the rate of change that impacts human physiology. The microbiota has both a negative and positive influence of risk diseases, strongly suggesting that early events may set the health trajectory during aging.
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specific ones such as genus and species. For example, in the human microbiome, identifying taxa at various levels provides an understanding of the composition and functions of the communities present in different parts of the body. While clinically, bacterial classification uses gram staining positivity and growth in selective media (e.g., Bergey's classification), molecular techniques such as 16S ribosomal RNA sequencing has given rise to classification based on class, order, family, and genus. Sequencing of amplicons identifies orthogonal taxonomic units (OTU) that correspond approximately to species level of taxonomy. In this review, we use taxa to specify organisms, typically at the genus level.

## Microbiota Effect on Traits

A central tenet of biology is that all traits or phenotypes (P) arise from the interaction between the genetic material (G) inherited from the parents, and the environment (E), often written in shorthand as P = G x E. The microbiome is inherited and is also a consequence of the environment. As it is inherited the microbiome has been referred to as the second genome. Further, as it is both inherited and influenced by environment, the microbiome has a strong influence on development in early life. It has been shown to impact metabolism, education of the immune system, and cognitive development.[6,7] One reason to focus on early events is because they set the

foundation and determine the trajectory, or arc, of health and disease risk with age.

## Maternal Microbiomes Seed the Infant's Gut Microbiome at Birth

At birth, the infant's gut microbiome is primarily seeded by the maternal microbiome. During the passage through the birth canal, the baby is exposed to a diverse array of bacteria, marking the first significant microbial contact.[8] In the third trimester of pregnancy, the vaginal microbiome undergoes a transformation, increasing in microbial diversity and becoming rich in *Lactobacilli* species. These species are known to lower vaginal pH and enhance vaginal secretions, contributing to vaginal health. Concurrently, changes also occur in the maternal gut microbiome during pregnancy.

Infants born vaginally typically inherit a microbiome that reflects the maternal vaginal flora. This flora is abundant in *Lactobacillus*, *Prevotella*, and *Sneathia* species, which are instrumental in early gut colonization. These taxa are needed to digest and extract nutrient from milk. In contrast, infants delivered via Cesarean section are more likely to be colonized by skin and environmental microbes. This initial difference in microbial exposure can have enduring effects on the infant's health, potentially influencing their susceptibility to various conditions and diseases.[9] For instance, in premature infants admitted to neonatal intensive care units, prolonged exposure to antibiotics results in gut dysbiosis has been associated with adverse neonatal outcomes e.g., necrotizing enterocolitis a condition that is associated with high mortality and life-long morbidity in survivors.[10,11]

Interestingly, the composition of the vaginal microbiome varies based on factors such as ethnicity, maternal age, and parity.[12,13] Alterations from the normal vaginal microbiota, known as dysbiosis, have been linked with pregnancy complications, with preterm labor being the most extensively studied condition.[9] Thus, the mother's microbiome, encompassing vaginal, gut, and skin microbiota, serves as a critical source for the newborn's initial microbial colonization, setting the foundation for the development of the infant's own microbiome.

## Breastfeeding and Microbiome of the Infant

Exclusive human milk feeding for the first six months of life, with continued breastfeeding for one to two years of life or longer, remains the normative standard and is recommended by the American Academy of Pediatrics for infant feeding.[14,15] Mother's own milk is uniquely suited to infants, both in its nutritional composition and in the non-nutritive bioactive factors that promote survival and healthy development.[16] Milk is not only a nutrient source for the infant, but also contains oligosaccharides and lipids that promote growth of specific gut microbiota (prebiotics).[17-21] There are roughly 4,000 different species of (~$10^{14}$ cells) bacteria that inhabit the gut. Remarkably, mothers sense their baby's health and development and alter the composition of the milk within hours.[22] Saliva during suckling is allowed to flow back into the breast, and the interaction between the saliva and the milk is sensed to alter the breastmilk composition.[23-25]

Recent studies on breastmilk microbiome have shown that various bacterial species contain genes for bile salt hydrolases (BSHs).[26] The existence of BSHs in these bacteria is noteworthy. These enzymes split the peptide bond in bile acids, separating the amino acid group from the steroid core.[27] Human milk, with a fat content of 5%, requires bile salts for fat solubilization. These salts are synthesized by the infant's liver and are stored in the gall bladder. Normally, bile acids are effectively recycled through a process called enterohepatic recirculation. Both conjugated and unconjugated bile acids are absorbed throughout the gut passively and actively in the terminal ileum. BSH-produced bile acids and their metabolites act as ligands for the farnesoid X receptor (FXR), found in the liver. The metabolic derivatives of these reactions have diverse effects on human health.[28] Some lead to weight gain, fatty liver disease, and other metabolic disorders.[29] Other derivatives are implicated in cognitive and inflammatory diseases.[30,31]

The infant's gut microbiota is colonized from both breastmilk and the skin. As the communities that colonize the skin are distinct from those in breastmilk, the infant's fecal microbiome shows

that about 70% is derived from breastmilk and remainder comes from the areole.[32,33]

Next, we summarize evidence that the microbiome can have a positive and negative influence in commonly observed pathologies like atherosclerosis, metabolic diseases, and bone fracture healing in adults.

## Changes in the Microbiome From Two Years Old to Adulthood

Puberty is a critical milestone that is associated with multiple physiological changes driven by sex hormones during the transition towards adulthood. Therefore, this dynamic period could have significant impact on the gut microbiota. Several studies have examined the dynamics of the microbiome between the ages of two and 15 years of age. These studies show that by the age of five years there are clear differences in the microbiome between boys and girls. Surprisingly, puberty (i.e., sex hormones) does not appear to have a significant impact on the gut or oral microbiome.[34-36] The overwhelming conclusion from these studies is that by age two years the microbiome is established and then remains stable over time.[37-39]

As the microbiota is being established at this early age, broad-spectrum antibiotics can have a lasting impact on the disease risk in the infant and into adulthood. In addition to having concerns for developing drug resistance, the benefits of antibiotics should be carefully balanced against the risks on the infants' health. Probiotics are live bacteria that provide benefits. The use of appropriate probiotics should be considered post-antibiotic therapy, but clinical trials are needed to determine the use of standardized probiotics.

## Impact of the Adult Microbiome on Disease Risk

The adult human microbiome is a complex ecosystem, comprised of approximately 5,000 bacterial species. A meta-analysis representing 545 microbiomes collected from around the world showed that B*acteroides, Ruminococcus, Blautia, Clostridium*, and *Coprococcus* are prevalent in all human populations studied.[40] This research underscored the crucial role of the microbiome in maintaining systemic homeostasis, influencing a range of physiological processes including metabolism, epithelial barrier integrity, immune and inflammatory responses, neuroendocrine functions, and hematopoiesis. The gut microbiome functions akin to an autonomous organ within the host, orchestrating a bidirectional communication network with various body systems through neural, endocrine, immune, and metabolic pathways. While stable from the age of two years, the adult microbiome's composition can be modulated by dietary habits, cultural factors, use of prebiotics, probiotics, medications like proton pump inhibitors (PPIs) and antibiotics. Alterations leading to a detrimental imbalance in the microbiome, termed dysbiosis, have been implicated in various disease states.[41,42]

## Interplay Between Gut Microbiota and the Immune System

The gut microbiota has a complex relationship with the immune system that is multifaceted and impacts host physiology. The gastrointestinal tract, beyond its roles in digestion and nutrient absorption, harbors a diverse microbial community that engages in a symbiotic relationship with the host. Many gut bacteria, such as commensal *Escherichia coli* providing essential nutrients derived from metabolic byproducts in exchange for a habitat and steady nutrient supply. The immune system, particularly within the gut mucosa, is instrumental in distinguishing pathogenic microbes from commensals, maintaining a balance between immune tolerance and activation. Interestingly, the same *E. coli* strain can elicit distinct immune responses depending on their location within the body. T cells, for instance, demonstrate both tolerance to commensal *E. coli* in the gut and robust inflammatory responses to these bacteria in other tissues, such as the bladder via a contaminated catheter, or lungs post intubation. This suggests a sophisticated level of immune education by the microbiome that is both microbial species and tissue specific. This implies that the gut microbiome is pivotal in shaping the resident T cell memory population.[43-45]

## Gut-Bone Crosstalk in Osteoimmunology

Recent advancements in osteoimmunology have elucidated the intricate relationship between the gut microbiome and bone health. Notably, research in murine models has identified key pathways linking the microbiome to bone physiology, encompassing the immune system, metabolic byproducts, endocrine signals, and extracellular vesicles. The microbiome's influence on T cell differentiation, particularly in the context of bone healing, has garnered attention. The work of Dar et al. highlights the microbiome's role in bone repair processes.[46] They observed that gut microbiota influences the production of $T_H17$ cells in the intestines. Post-fracture, sphingosine-1-phosphate, a signal molecule released by bone tissue, attracts these $T_H17$ cells from the gut. These cells are crucial in promoting bone healing. Colonization of segmented filamentous bacteria (SFB) increased $T_H17$ cell populations in the gastrointestinal tract, correlated with increased $T_H17$ presence at fracture sites in the mouse model. This was further confirmed using a UV reporter mouse strain to track the migration of $T_H17$ cells from the gut to the healing bones, underscoring the gut microbiome's impact on bone healing. These findings suggest the gut microbiome's potential as a therapeutic target, especially considering how disruptions like antibiotic use could impact bone healing.[46,47]

The gut microbiome has also been shown to contribute to osteoporosis. Das et al. analyzed the fecal microbiomes from 181 men and women over the age of 55 with either normal bone mineral density (BMD), osteopenia, or osteoporosis.[48,49] They corrected for several confounding factors such as dietary habits, prescribed drugs, leisure time, and physical activity using statistical models to partition the taxa and assign them to each of these factors. They identified six genera that were significantly altered in abundance in the osteoporosis or osteopenic groups compared with age- and gender-matched controls. Despite controlling for biological confounders like BMI, health status, diet, and medication, which explained 15-17% of the variance within the microbiota dataset, these taxa remained significantly associated with bone mineral density. The cohort with osteoporosis had lowest microbial diversity with lowest levels of these taxa. Unexpectedly, the cohort with osteopenia had the highest diversity and higher abundance the six taxa relative to the cohort with normal BMD. Consistent with prior studies, the study finds that proton pump inhibitors (PPI) had a large impact on reducing the diversity of microbiome taxa, presumably because it alters the gut pH and reduction of these taxa also affected BMD. Together, these studies indicate that either an increase or decrease in diversity impacts the immune response and bone health.

## Changes with Aging

Aging has been an area of intense study over the last five decades in hopes of understanding the biology and regulation of the process. Recently scientists have proposed twelve hallmarks of aging. Broadly, these hallmarks are interconnected among each other and include dysbiosis of the host microbiome. It is now recognized that aging is influenced by the interaction of the host and the gut microbiome. The microbiome itself has mechanistic pathways that have been associated with aging.

Recent research of individuals 65 years and older has revealed important connections between the composition of the gut microbiome and various health indicators, such as physical fitness, frailty, and dietary habits.[50] This research builds on earlier findings that demonstrated a change in the microbiome from adulthood into older age. This change is characterized by a decrease in microbiome diversity, lower levels of *Bifidobacterium*, and increased populations of bacteria such as *Clostridium, Lactobacillus, Enterobacteriaceae*, and *Enterococcus*. A significant 2021 study, examining the gut microbiomes and metabolic products of 9,000 people across three cohorts, found that microbiome diversity increases with age.[51] Despite the variations in microbiome composition, metabolic functions remained relatively consistent. The study highlighted two metabolites—tryptophan-derived indole and phenylacetylglutamine—which are linked to longer lifespans in mice and found in high concentrations in human centenarians. Notably,

this unique microbiome evolution was mainly seen in healthy individuals aged 80 and above, while it was less evident in their less healthy peers. These insights suggest an ongoing development and specialization of the gut microbiome in the context of healthy aging.[52-54]

Is it possible to slow aging by altering the gut microbiome? Metformin, a diabetes treatment used for over 60 years, offers an interesting case.[55] Not only does metformin extend life expectancy in humans and animals, but it also influences various aging-related factors, including the gut microbiome.[56] Its concentration in the gut is significantly higher than in the blood.[57] Metformin's impact on the gut microbiome includes inhibiting the growth of pathogenic bacteria in older individuals. This action helps reduce inflammation and counteract the immune system weakening caused by dysbiosis.[58,59]

## Conclusion

Summarizing, the gut microbiome changes dynamically in the first two years of life and then is highly stable until age 40 (Figure 1). The microbiome is inherited primarily from the mother in these first years. After the age of 45 years, the microbiome changes, which coincides with reproductive senescence. In infants the gut microbiomes vary by sex, ethnicity, and geography. All data strongly suggest that the microbiota have a significant impact early in life on neural/cognitive development, and on metabolism setting the health trajectory to develop, for instance obesity, cardiovascular disease, and other comorbidities of aging.

A great achievement of modern medicine has been to increase lifespans. Recognizing the key contributions of microbiome starting at infancy and its relationship to the hallmarks of aging may allow us to develop new interventions that reduce the comorbidities of aging.

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