REVIEW
Microenvironment & Microecology Research 2022;4(2):7. https://doi.org/10.53388/MMR2022007

Metagenomics and microbiome of infant: old and recent instincts

Tejswini Sontakke[1], Ashwini Biradar[2], Prashant Dixit[2], Dinesh Nalage[3*]

[1]Department of Zoology, MGV's, MPH Mahila College, Malegaon 423105, India. [2]Department of Microbiology, Dr. B. A. M. University, Sub Campus Osmanabad, Osmanabad 413501, India. [3]Department of Molecular Biology, SRL Limited, Mumbai 400062, India.

[*]**Corresponding to:** Dinesh Nalage, Department of Molecular Biology, SRL Limited, Prime Square, 1-5 floors, Near Gaiwadi Industrial Estate, S.V.Road, Goregaon (W), Mumbai 400062 (MH), India. E-mail: dineshnalage@gmail.com.

Competing interests
The authors declare no conflicts of interest.

Abbreviations
NGS, next-generation sequencing; ITS, internal transcribed spacer; C-section, Caesarean section.

Citation
Sontakke T, Biradar A, Dixit P, Nalage D. Metagenomics and Microbiome of Infant: Old and Recent Instincts. *Microenviron Microecol Res*. 2022;4(2):7. doi: 10.53388/MMR2022007.

Executive editor: Na Liu.
Received: 12 April 2022; **Accepted:** 18 May 2022; **Available online:** 23 May 2022.

Abstract
The human microbiota is made up of trillions of bacteria that live in the human being, whereas the microbiome is made up of the microbiota's genes and gene products. Bacteria have long been thought of as pathogens that eventually lead to human illness. Bacteria are increasingly recognized as generally beneficial commensal organisms and hence crucial to proper and healthy human development, thanks to breakthroughs in both cultivation-based approaches and the advent of metagenomics. This relatively new field of medical study has given more information on illnesses such as inflammatory bowel disease, obesity, metabolic and atopic disorders. However, there is a lot we don't know about the complexities of microbe-microbe and microbe-host interactions. Future work targeted at resolving crucial concerns about the early formation of the microbiome, in addition, what determines its dysbiosis, will most likely lead to long-term mitigation of health. In this article, review the research on prenatal and newborn microbiome modulations, the role of maternal and environmental variables on forming the newborn microbiome, and future issues and directions in the exciting new field of metagenomic medicine.

Keywords: metagenomics; microbiome; interactions; health; bacteria; organism

Highlights

In this article, we show reviewed metagenomics and microbiome importance for health, we also explain old and new methods of metagenomics and microbiome. This review, in first part, we explained what the predominant microbiota in gut, which type of microorganism was obtained in gut. In second part, we explained older and current method of metagenomics, also changes of data and out puts from both the methods. In third part, this review gives information related to main drivers of the human intestinal flora as well as microbial colonization, which carries very important role in the health development.

Background

The development of genomics, largely guided by the human genome project, has led to the metagenomics or application of modern genome techniques for studying communities of microorganisms directly in their regular habitat [1]. This expanding area of study has emerged critical advancement in entire genome investigation, have extraordinarily encouraged the investigation of the human microbiome.

The microbiome represents all the bacteria that live in or on the host. It has been called "the forgotten organ", with significant weight, genetic content, cellular content and metabolic activity [2]. It is estimated that 100,000 billion organisms, more than ten times the total number of cells in the human body, constitute the intestinal microbiome alone [3]. For the human, the interaction between the gut and its microbiome is a complicated risk-benefit relationship. These bacteria have a substantial impact on intestinal health and gut function in a normal host. The human microbiome is the finest example of a microbial community, which is defined as "the complete microbial life that dwells in or on man's body." It is an element that has far-reaching metabolic, nutritional, and immunological effects on the host, and as a consequence has sparked a huge interest in biomedical research.

The microbiome develops within a host from birth to death and continually adapts to maintain a homeostatic balance with the host's immune system. Continuous human postpartum microbiome expansion is determined by host factors such as adaptive and innate immune systems, and by external influences such as diet, disease, drug and toxin load [4, 5]. The study of the gut microbiome begins in the womb and continues throughout life, as well as its impact on health promotion and disease development.

Variations in microbial colonization and intestinal immune function in healthy neonates full-time and very low birth weight compared to preterm infant (birth weight 1,500 g) peers, and implications on illness development when the microbiome is disturbed (dysbiosis).

Predominant microbiota in infant gut

The classical measuring pattern colonization

A number of thoroughly completed culture experiments [6-10] defined the baby gut microbiome in the 1970s and 1980s. Classical research has found that the baby gut microbiota is less complicated and has a bigger proportion of optional microorganisms than the adult microbiome. For contrast, a significant study based on adult microbiota culture is provided [11]. Note however that the latter study bacterial population counts per gram of faeces dry dust instead of wet stool. This results in higher values of about one-log unit.

Facultative bacteria

Facultative bacteria predominate the initial flora in quantity, and oxygen in the newborn gut hinders the growth of anaerobes. Historically, *E. coli* and enterococci (*E. faecalis* and *E. faecium*) were recognized as the first baby invaders [7-9]. *E. coli* specializes in the colonization of the human gut and other animals. Population levels may exceed 1010 colony forming units (CFU)/g of faeces [9], compared with 106-108 CFU/g in adults. The other Enterobacteriaceae members, such as *Klebsiella* and *Enterobacter* are both present in nature (e.g. on plants and fruits) and in the gut microbiota. *Klebsiella* and *Enterobacter* is common in the intestinal flora of the newborn [12, 13], but not in adults [11], reflecting their temperament intestinal competitiveness versus *E. coli. Aeromonas*, *Pseudomonas* and *Acinetobacter* are other gram-negative aerobic/facultative anaerobic bacteria that can briefly colonize newborns during the first weeks of life [6, 8, 12], but is unusual in adults.

Alpha hemolytic streptococci, e.g. *S. intermedius*, *S. mitis* and *S. salivarius*, appear to colonize 20-30% of newborns in the first weeks of life. Group B streptococci, which is a common cause of neonatal sepsis colonize about 5% of newborns [14]. Some classical studies describe regular colonization by a low to moderate number of coagulase-negative staphylococci in neonates [9, 10], while colonization by *S. aureus* was rare [7, 9].

Yeasts

Few studies have studied colonization by yeast. Isolation reported rates were 0% in the first week, < 15% in first month and 50% at 4 months. Settled babies usually have $10^{3\text{-}5}$ yeast population levels CFU/g faeces [6, 7].

Anaerobic bacteria

As the bacterial populations of facultative anaerobes expand, they consume the oxygen and create an anaerobic environment. This enforce anaerobic bacteria growth. In the 1970s and 1980s, in the first week of life, *Clostridium*, *Bacteroides* and *Bifidobacterium* sp. were isolated from at least half of the researched infants in ≤ 10^{11} CFU/g faeces [7-9]. Species of bifidobacteria common to infants included *B. bifidum*, *B. catenulatum*, *B. breve*, *B. adolescentis*, *B. infantis* and *B. longum* [6, 15, 16], similar to adults. The belongs to Bacteroides, in both infants and adults, *B. fragilis* group (e.g. *B. fragilis*, *B. vulgatus* and *B. thetaiotamicron*) predominates. *C. Difficult* as well as *C. Perfringens*, belonging respectively to the clostridial clusters I and XI, appear to dominate young infants, followed by *C. Tertium* and *C. paraputrificum*, these belong tocluster I [6, 7, 11, 16]. *C. difficile* may, for unknown reasons, reach relatively high levels of population without causing symptoms in young infants.

Lactobacillus sp. colonization was uncommon in classical studies in the first year of life [6, 7], and it was suggested that these bacteria do not form stable infant populations. However, occasional studies reported higher rates of isolation [17].

Other anaerobes were often not targeted in these early studies, but *Veillonella* were found in 40–90 percent of neonates, < 50 percent of *Eubacterium*, and < 10 percent of *Ruminococcus* when investigated. On the other hand, *Peptostreptococci* appeared only after solids were introduced. *Fusobacteria* were found in < 5 percent of one month-old infants, which are extremely sensitive to oxygen and difficult to cultivate.

Comprehensive approaches for determination microbes

General characteristics

Despite the fact that microorganisms are pervasive and prolific, there is presently a lack of fundamental mechanistic knowledge of many of the critical functions of microorganisms play in nature, including those in the human body [18]. Until the current discovery of novel culture methods [19], only a very tiny proportion of the human gut microbiota has been isolated and investigated in pure culture [18]. Because it was assumed that, a large portion of intestinal microbiota had not been cultivated [20], independent culture approaches, such as

metaproteomic, metatranscriptomic, and metagenomics, were developed to uncover the activities, identities, and functional roles of previously uncultured gut microbiota microorganisms.

The 16S rRNA based microbial profile technique is based on universal primers for single or multiple hypervariable amplification 16S rRNA regions [21]. Readings from next-generation sequencing (NGS) platforms are processed using bioinformatic pipelines such as the popular Qiime software package [22] or Mothur [23], allowing the microbial composition of the examined environmental sample to be rebuilt. This technology also makes it simple to attribute identification to strangers in microbial communities by discriminating based on the sequencing of their distinct hypervariable regions [24]. Furthermore, a metagenomics method to microbiome sequencing has been established to confirm both the phylogenetic activity and the functional repertoire of the gut microbiota gene [25]. However, one of its disadvantages of metagenomic techniques is that microbiome data do not provide insight on whether or not genes are activated at any particular moment. Other optical techniques are developed to solve these constraints, such as sequencing the whole microbial RNA pool of a particular sample, i.e., metatranscriptomic, or investigation of the total protein composition or proteome, i.e., metaprotein. The effectiveness of the latter two technologies is restricted, specifically in the context of the metagenomic method, so many genes or their homologues (and hence their products) are not functionally defined. Finally, metabolomics will develop an overall signature of microbial activity by evaluating the metabolites generated (microbially).

The gold standard methodology for determining microbiota

Most investigations on the intestinal microbiota rely on microbial profile analysis based on the 16S rRNA gene. This gene is made up of nine separate variable regions, namely V1 to V9, each accompanied by evolutionarily conserved DNA sequences which are ideal for binding PCR primers [26]. However, there is no standard method for choosing the best PCR primer combination for amplifying a section of the 16S rRNA for all taxonomy and phylogotypes present in the element [27-30]. Furthermore, none of the currently available DNA sequencing methods enable comprehensive genetic sequencing for many samples that are economically multiplexed at the same time. As previously stated, metagenomic injection sequencing is an ideal alternative for cataloguing GIT using the 16S rRNA gene's microbial profile. This approach ignores the specific gene amplification and possibly broken DNA sequencing. Extracted from the examined environmental sample, containing unclassified microorganisms and viruses. As the microbial profile based on 16S rRNA, the metagenomic shotgun gives a lot more information, including information on the functional features of the microbial population. In this aspect, it is free of the possible bias of the amplification reaction necessary for the gene-based 16S rRNA profile. Shotgun data, in example, may be used to investigate the variety of genes engaged in a broad variety of metabolic activities, like those related to chemical production, such as resource catabolism or short-chain fatty acids, such as carbon sources. Customised databases of functional classification of metagenomic, provide information on a wide range of functional aspects of the gut flora, including conjugated bile salt degradation, prophages, antibiotic resistance and extracellular structures involved in immunomodulation. Furthermore, an assembly-based technique may be utilised to rebuild full or partial genomes of previously uncultivated species, enabling for the investigation of what was previously known as microbes dark matter [31]. To analyse the massive amounts of DNA sequencing data from diverse bacterial communities, like those found in the digestive tract, extensive processing capacity and bioinformatic workflows for sequence, interrogation, and information management are required [18]. It should also be mentioned that underpopulated reference databases and inadequate functional characterization of many genes limit the use of metagenomic techniques used to research gut microbiota.

Novel NGS-based approaches to obtain a high-definition image of The composition of gut microbiota

Microbial profile analyses predicated on the 16S rRNA gene offer information on the diversity of the human microbiome at a taxonomic level far higher than that of species [32]. To overcome this constraint and acquire a more thorough picture of the makeup of the intestinal microbiota, i.e., at the species or even subspecies level, a molecular marker that is substantially more variable at the interspecific level than 16S rRNA must be targeted. The internal transcribed spacer (ITS) sequence, which is located within the rRNA between the 23S and 16S rRNA genes, is a suitable as a genetic marker. An ITS-based procedure known as bifidobacterial profiling analysis was used to acquire a thorough view of bifidobacterial populations. In this regard, it has been demonstrated that the bifidobacterial ITS profile technique detects bifidobacteria strains from the baby microbiota that would have been acquired through vertical transmission (from the respective mother) [33-35].

Decoding the whole genetic profile of each component strain is required for complete microbiome genome analysis. Due to the difficulties of intestinal microbiota, which might consist of hundreds of operational taxonomic units (OTUs), achieving this goal is incredibly challenging. Furthermore, the failure of in vitro testing to recreate the fundamental requirements of ecological niches makes it very hard for most species of the intestinal microbiota to flourish. Single-cell genomics can contribute to the genetic characterisation of the microbiome. Physical separation of microbial cells is followed by chromosomal DNA extraction and amplification of each cell's genetic content [36]. Single-cell genome sequences, in example, may be acquired directly from raw samples, resulting in reference genome sequences for culture-resistant [37, 38] intestinal bacteria or representing uncommon members of the community [39]. However, currently existing unicellular procedures are not especially successful, and the quality of the data acquired, as well as the possibility for contamination, may skew the output results when compared to those produced using normal genomic methods. Unicellular genomics is predicted to address major gaps in existing knowledge of the subject and structure of the human gut microbiome, especially if the technology is improved. However, despite the promising developments in microfluidic technologies for unicellular microbial analysis, it remains very difficult to implement this approach.

A novel strategy of inferring the gut microbiota composition at the strain level without isolation and bacterial strain culture includes reconstructing an individual microbiota member's genome sequence using metagenomic shotgun data [40]. This NGS technique gives not only taxonomic information on lineage identity, but also extremely relevant information on the genetic makeup of the organism, including metabolic and evolutionary information [40]. MetaPhlAn is an intriguing technique for determining the high-resolution (up to the strain level) makeup of the intestinal flora [41].

This programme is based on reading mapped to a pre-computed library of strain-specific marker genes derived from a comparative investigation of all publicly accessible bacterial genome sequences. The fundamental critique of this technique is that it can only profile already sequenced species, neglecting the presence of as-yet-unknown/uncultured individuals in the population.

Approaches of culturalomics

Over the last decade, most of the above-mentioned culture-independent methodologies have been employed to analyse the makeup of human gut microbiota, whereas microbial cultivation techniques have been disregarded to some extent [42]. This has resulted in a significant knowledge gap between bacterial species found in the human gut but not yet isolated and cultured [42]. Approximately 56% of gut bacteria discovered by NGS methods have cultivated representatives [43, 44]. With the introduction of so-called cultural methods, this chasm is closing. Culturomics investigates the microbiota of the human stomach using high-throughput growing settings. Recently, various cultural studies of human stool samples have involved the formulation of complex growth media, which has allowed a considerable number of novel gut microorganisms to be isolated and cultivated [19, 45, 46].

Main drivers of the infant intestine microbial colonization

Way of delivery

In full-term infants, the manner of delivery is known as a key determinant of early intestinal microbiota [47]. Vaginal delivery delivered neonates come into touch with the mother vaginal and faecal microbiota, leading in the colonization of the gut with vagina-associated bacteria like Lactobacillus and Prevotella [48, 49]. Caesarean section (C-section)-delivered infants, on the other hand, are not directly exposed to maternal microbes and are therefore more likely to be colonized by environmental microorganisms from maternal skin, hospital staff or hospital environment [49-53]. Several studies using different culture-and molecular-based methodologies have described a deviating gut microbiota in these infants, including the recently used high-performance sequencing technologies and metagenomics approaches [49, 50, 53]. The predominant phyla represented during the early days of life were observed to be Proteobacteria and Firmicutes, with *Actinobacteria* showing in the faeces of C-section delivered kids on days 7 to 15 following delivery [54]. Caesarean newborns also have a less complex gut microbiota and are less commonly colonised by microbes such as *Bifidobacterium* and *Bacteroides*, whereas *Clostridium sensustricto* and *Clostridium difficile* [48, 49, 54-59] are more frequently colonised.

These differences gradually decrease between vaginal and cesarean deliveries, but cesarean deliveries remain more heterogeneous than vaginal deliveries for up to 12 months [53, 60]. Notably, permanent variations in gut flora have been found in children as young as 7 years old [57, 58, 61] between C-section and vaginal delivery. In contrast, following the early neonatal period [62, 63], a recent article indicated that C-section had no discernable influence on the early microbiome. The found microbiota differences between vaginally delivered and C-section delivered neonates were connected with the protective benefit of natural delivery, especially because C-section has been indicated to have long-term health repercussions. In fact, levels of several cytokines have been demonstrated to be considerably lower in C-section-born children [56, 64], as well as related with an increased risk of immunological illnesses such as asthma [65], allergies [66], type 1 diabetes (T1D) [67], and obesity [68]. The study suggests that the manner of delivery influences adult health while the impacts on the composition of the intestinal flora decrease during the first years of life, emphasising the relevance of early gut microbiota in the maturation and development of the host's immune system.

Age of gestation at birth

Gestational age is another crucial element in forming the newborn gut microbiota. Preterm neonates are those that are born before 37 weeks of gestation [69]. Depending on the degree of prematureness, preterm newborns may have major health issues at first. They frequently have undeveloped intestines as well as immunological, respiratory, and neurological abnormalities when subjected to lengthy antibiotic and other medication treatments or when neonates are frequently hospitalised for extended periods of time, breathe artificially, feed artificially, or parenterally. All of these events are likely to disrupt the typical pattern of microbiota acquisition and growth, resulting in abnormal establishment or aberration of the gut microbiota. Several studies have found variations in the faecal microbiota between preterm and full-term newborns. Preterm neonates have delayed intestinal colonization with commensal anaerobic microbes like Bifidobacterium or Bacteroides, and their faeces include much more Enterobacteriaceae, Enterococci, and other harmful (opportunistic) germs than mature neonates [59, 70-74]. Gram-positive bacteria, such as *Staphylococcus*, *Enterococcus* and *Clostridia*, dominate the intestinal microbiota of preterm during the first month of life, whereas Gram-negative microorganisms such as *Enterobacteriaceae* and *Veillonella* can vary [75].

In a very low-birth-weight (VLBW) preterm gut ecosystem, a trend of colonization and progression of bacterial taxa ranging from Bacilli through Gammaproteobacteria towards Clostridia was discovered [76]. The microbiota seemed to evolve with phases of dramatic population changes and a common endpoint in the latter study, whereas the preterm gut was discovered to be colonised by anaerobes, mainly clostridia [76].

Though gestation has been postulated as the most critical determinant for the creation of the gut microbiota, there is a significant inter individual variability, which is most likely due to the co-occurrence of the aforementioned variables. It is critical that the observed aberrations produce a preterm baby microbiome rather than the equivalent term, and a preterm baby microbiota is considered to be connected with a delay in the creation of an adult microbiota signature [77]. These modifications can have a significant impact on one's health. Indeed, the combination of the preterm baby's altered microbiota with its immature immune system can promote inflammation and enhance infectious illnesses [78, 79]. Indeed, preterm intestinal microbiota composition has been linked to a higher risk of necrotizing enterocolitis (NEC) or sepsis [80-82]. Furthermore, the preterm newborn bowel microbiota is differentiated not only by its composition, but also by its characteristics. The contents of the major short-chain fatty acids (GCC) generated by the gut microbiota were shown to be lower in stool samples of preterm infants and NBMV than in stool samples of term newborns [72, 83]. Functional inference analyses have also identified pathways potentially affected by prematurity, with a higher frequency of genes related to xenobiotic biodegradation and metabolism and lipogenesis and a slower rate of genes associated with energy metabolism and cofactor and vitamin biosynthesis current in stool samples of premature babies compared to forward counterparts [83]. It has been discovered that preterm babies have an increase in bile acid derivatives, indicating abnormal lipid metabolism. Furthermore, preterm newborns' metabolites urine samples included higher levels of vitamins D and E [59].

Children's feeding mode

Another key component that influences microbial colonization and, as a consequence, the makeup of the neonatal intestinal flora and gastrointestinal function is the type of flow. The microbial makeup of the intestines of mammography and formula-fed children differs [84, 85], with higher levels of *Bifidobacteria* observed in the first cohort of infants. Breastfeeding delivers a combination of nutrients as well as antibacterial and probiotic substances, which supports the development of a "milk-based microbiota.". Breast milk IgAs antibody promotes a stronger tolerogenic and regulating immune system [86]. Human milk oligosaccharides (HMOs) are also found in mother's milk and can help beneficial microorganisms develop and operate (see below). Breastfed infants' gut flora is less diverse than that of bottled newborns [87]. Transcriptomic analyzes of intestinal epithelial cells have shown that the type of feeding of infants also affects the expression of host genes, with the increase in transcriptional supply of genes that are associated with immune activity and metabolic activity [53, 88]. Formula-fed infants were exposed to a variety of bacteria, carbohydrates, and nutrients (micro), resulting in varying patterns of microbial populations of the intestine. Breastfed babies' stools raise the levels of *Lactobacilli* and *Bifidobacteria* and decreased level of potential pathogens than formula-fed babies' stools [3, 58, 60, 87, 89, 90], with the latter being associated with the latter being linked with a more diversified intestinal microbiota dominated by *Bacteroides, Staphylococci, Clostridia, Enterobacteria, Enterococci*, and the genus *Atopobium*. Because of these differences in microbiota, SCFA levels are also different in the faeces of breast-fed infants, with propionate and butyrate being present at higher levels in the latter group [91]. On the other hand, it appears that babies fed on powdered milk may rapidly deviate from those of an adult composition of the microbiota [53]. The newborn microbiota seems to float throughout the period of exclusive nursing, and the phenomena of bacterial succession continues; nevertheless, after weaning, when changed into a microbiota comparable to that of an adult, the microbiota becomes more stable and complicated [92-94]. The influence of weaning on microbiota development has received far less attention than the early feeding (exclusively milk) period [93, 94].

Due to the concurrent introduction of a range of alpha bacteria during weaning, Proteobacteria and Actinobacteria are replaced as prominent members of the baby microbiota by Firmicutes and the phylum Bacteroidetes [92, 93]. A research of the development of the gut microbiota between 9 and 18 months after birth found a rise in the relative abundance of certain of the main bacteria families, such as Lachnospiraceae, Ruminococcaceae, Eubacteriaceae Rikenellaceae, or Sutterellaceae [95]. Bifidobacteriaceae, Actinomycetaceae, Veillonellaceae, Enterobacteriaceae, Lactobacillaceae, Enterococcaceae, Clostridiales incertae sedis XI, Carnobacteriaceae, and Fusobacteriaceae, on the other hand, decreased in relative abundance during infant passage at the infant stage [95], which is consistent with previous reports [53]. Protein consumption was linked to an increase in Lachnospiraceae and a decrease in saccharolytic bacteria, such as members of the Bifidobacteriaceae family, which are normally associated in breast milk and baby feeding, while fibre intake was linked to higher levels of Prevotellaceae [95].

Faecalibacterium prausnitzii and *Akkermansia muciniphila*, which are missing or present at extremely low levels at the start of infancy, respectively, grow for adult levels at 12 months and 24 months. This rise in the latter example might be due to a progressive increase in mucin production, the major carbohydrate fermented by *A. muciniphila* and which is present at extremely low levels throughout early development.

Stopping nursing and moving to more diverse and robust diets is thought to boost the gut microbiota's alpha diversity [53, 95-98]. Furthermore, the change from breast milk toward formula milk (i.e. bovine) has a massive effect on the intestinal microbiota's growth. There was a rise in distribution and abundance of the taxa *Bacteroides, Blautia,* and *Ruminococcus*, and also a drop in distribution and abundance of *Bifidobacterium*, *Lactobacillus*, and *Enterobacteria*, within 5 days following breast milk [99]. There was also an elevation in alpha diversity and faecal pH.. Furthermore, the observed increase in bacterial diversity adds to functional alterations. There has been a documented rise in overall SCFA concentrations, particularly butyrate [60, 93]. The establishment of a mature microbiota comprising genes responsible for the digestion of complex carbohydrates, starch, and xenobiotics, as well as vitamin synthesis, is induced by switching completely from milk-based diets to solid foods [93]. To digest plant-derived polysaccharides from adult meals, the adult microbiota is more functionally sophisticated and organised, giving mutual advantages to the host and the microbe [96].

Mother diet

There is rising interest in studying the impact of maternal mortality and BMI on the baby's gut microbiome [52]. Recently, it has been discovered that the baby's faecal microbial composition is regulated by the mother's BMI and weight growth during pregnancy [100]. In the first 6 months of age, faecal bacteria and *Staphylococcus* concentrations were significantly higher in babies of overweight mothers; however, *Bifidobacteria* counts were higher in babies of non-obese mothers. Other authors [101] have not confirmed these observations. Furthermore, there is currently no information on the impact of breastfeeding and its relationship to weight and the number of mothers. These variables can be perplexing, indicating the need for further investigation.

Environment (life style and location of the family)

Family members and near relatives (siblings) have also been recognised as an important environmental element that may impact the colonization pattern of the baby's gut microbiota [51], although the effects of family size, structure, and birth order have yet to be demonstrated [52]. Newborns aged one month who were enrolled from the KOALA Birth Cohort Study in the Netherlands and had older siblings had a larger amount of bifidobacteria in their gut microbiome than infants without siblings [58]. It was also found, in this instance as apart of the allergy flora research, that children who did not have older siblings had an increased proportion of non-*Escherichia coli*, *Clostridia* such as *Enterobacteria* in the gut, as well as an altered facultative anaerobic-anaerobic ratio [55]. A recent Danish cohort research concluded that the prevalence of family members were related with greater gut diversity and microbial richness throughout childhood, but the involvement of domestic animals had less determined on the gut microbiome [102]. The "brother effect" is a notion that can help with creation. The theory is still debatable, and further research is needed in this field.

Geographic location can also impact the microbiome, such as microbiota differences seem to be related to dietary habits and lifestyle in a specific area [51]. In addition, different ethno geographic populations have distinct regions. cultural practices [103]. Infants residing in rural hamlet in Africa, for example, vary from infants residing in an urban location in Italy [104], and numerous other studies have examined the spatial influence of ethnicity and/or nutrition on microbiological composition and diversity [84, 92, 105-107]. Infants living on the street in Bangladesh have an intestinal flora that differs dramatically from infants with the same age group in upper middle class suburbs in the U.S. In instance, the faecal bacterial community and composition of infants from these two different regions differed, with Bangladeshi children's microbiota being abundant in *Prevotella* and reduced in *Bacteroides* when compared to American children [106]. Other research children from South-East and Northern Europe observed discrepancies in composition of the group of bacteria, with the species *Bifidobacterium* and the group *Bacteroides-Prevotella* being present in greater abundance in African children [105]. In general, it appears that the construction of the house and familiar settings (rural vs. urban) influence colonization of the gut microbiota after birth, however further research is needed.

Genetics host

More and more scientific data suggests that the host's genetics impact the improvement and maintenance of the infant's gut microbiota [108-110]. In this regard, the significance of the host genotype in modelling the composition and organisation of the microbiome was investigated in human and familial twins. In this regard, a study of children under the age of ten found that genetically similar twins had greater rates of similarity than identical and fraternal and independent controls [111]. However, subsequent analyzes by other authors have failed to identify significant differences in bacterial diversity between monozygotic and dizygotic twins [112, 113]. A recent research of a large cohort (1,539 persons aged 18 to 84 years) discovered a connection between host genotype and the prevalence of different bacterial taxonomies in adulthood. The authors [114] revealed that genetic polymorphisms (SNPs) at the LCT locus (involved in the production of human lactase) are linked to the frequency of *Bifidobacterium* and identified a link among host genetics and dairy product intake. This emphasises the need for more study into the interaction of human genetics, nutrition, and microbiome growth.

Overall, the almost unlimited combinations of these well-known genetic and environmental elements are responsible for the sole bacterial community protected by each individual's gut.

From infancy to adulthood

It is a subject of great discussion in the scientific community to learn how to differentiate the bacterial vaccine in different microbiological ecosystems in several habitats associated with different ecologies. The first step to understand this aspect is to know what are the "founder" reference types of the nascent microbiota, then the next operational taxonomic units are obtained. This evolution can be interpreted using the tools of ecological sequence theory developed by plant ecologists. Little is known about the developmental mechanisms of the human microbiological ecosystem during the early stages of life in founding communities [93, 115-117]. As mentioned earlier, neonates born after natural delivery have a gut microbiota that mirrors the mother's vaginal microbiota, as evidenced by meconium swabs [48, 118, 119]. Accordingly, the first acquisition of bacteria is apparently driven by vertical transmission from mother to child; only then does it develop into differential CDs associated with multiple anatomical regions

[120], although the ultimate effect of the host genome on the microbiome remains unclear. Indeed, in a follow-up study in 2009, 31 pairs of monozygotic twins and 23 pairs of monozygotic twins, Turnbau et al. reports that microbiota monozygotic twins are not significantly more similar to these twins. Thus, to date, no study has proven the inheritance of the human microbiome in the digestive tract. One possible reason for this discrepancy in results is that twin studies have compared common communities, perhaps underestimating the dynamics of known subsets in the community.

While the pioneers of the team from zero moment pollution (delivery) from the cutaneous, vaginal or intestinal ecosystems of the mother, is of uncertain origin [121] Voltage diversity is increasing rapidly during the first years of life, but with a considerable degree of instability [93, 116]. However, the reason for the growth of this diversity is not known. New bacteria can enter a microbiota ecosystem at a constant rate, depending on how they have lived in the region's ecosystem or whether they need to grow in such a complex system. different niches, or their origin is larger than the individual niches [122]. Studies have shown that large islands are home to many species, that increasing functional complexity itself brings taxonomic complexity to balance, characterized by the "adult" microbiotype (mainly Firmicutes and Bacteroidetes), which consists of a number of characteristics: about a year and a half.

Although studies of individual children have shown significant diversity in colonizing eggs, consortia of bacterial taxa are unpleasant to a child, indicating that microorganisms depend on each other to form a consortium [116]. This microbiological flexibility may be an adaptive diet that is selectively beneficial for a wide variety of variations in the physiological development of children. It is not known how lifestyle, geographic location circumstances, puberty, disease, and other variables affect the stability of the microbiota throughout an individual's life [104, 123]. The process of monitoring bacteria in early childhood, which varies from person to person [124], as already mentioned, can be a model for understanding the process of recolonization in adults after antibiotic therapy.

Conclusion

With the development of metagenomics, scientists can now define the function and structure of the growing microbiome community. Although cultivation-based approaches were formerly the gold standard for assessing the microbiota, they have substantial drawbacks, such as the inability to recognize fastidious and low-abundant species. Metagenomics can discover organisms that are not cultivated by culture-based methods, as well as accurately analyse and determine amounts of these species that were previously under-evaluated. Due to the finding of bacteria in previously sterile tissues, dogmas such as the "sterile womb" notion are being challenged by metagenomics. However, it's unknown if these bacteria invade the foetus or are only there for the purpose of priming the foetal immune system.

Over the course of the first few years of life, the neonatal microbiome develops, with an increasing diversity of bacterial flora that grow into specialised body niches. Interestingly, whereas the manner of delivery was previously thought to have an important impact in the establishment of the newborn microbiome, this is no longer the case. Further research has revealed that this may not be the most important element determining the offspring microbiome's formation and development. Instead, maternal nutrition, breastfeeding vs formula feeding, and gestational age have the greatest impact on the newborn microbiota.

Changes in the newborn microbiota have the potential to cause serious health problems and illness. Despite the fact that metagenomics has allowed for the identification of healthy and dysbiotic microbiomes, many uncertainties remain. Hypothesis-driven research is critical to further probe disease pathophysiology as a result of metagenomics findings; this will have a substantial influence on illness prevention and therapy through modification of the human microbiome.

References

1. Lind AL, Pollard KS. Accurate and sensitive detection of microbial eukaryotes from whole metagenome shotgun sequencing. *Microbiome*. 2021;9(1):58. https://doi.org/10.1186/s40168-021-01015-y
2. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci*. 2012;13(10):701-712. https://doi.org/10.1038/nrn3346
3. Gritz EC, Bhandari V. The human neonatal gut microbiome: a brief review. *Front Pediatr*. 2015;3:17. https://doi.org/10.3389/fped.2015.00017
4. Berrington JE, Stewart CJ, Cummings SP, Embleton ND. The neonatal bowel microbiome in health and infection. *Curr Opin Infect Dis*. 2014;27(3):236-243. https://doi.org/10.1097/QCO.0000000000000061
5. Putignani L, Del Chierico F, Petrucca A, Vernocchi P, Dallapiccola B. The human gut microbiota: a dynamic interplay with the host from birth to senescence settled during childhood. *Pediatr Res*. 2014;76(1):2-10. https://doi.org/10.1038/pr.2014.49
6. Benno Y, Sawada K, Mitsuoka T. The intestinal microflora of infants: composition of fecal flora in breast-fed and bottle-fed infants. *Microbiol Immunol*. 1984;28(9):975-986. https://doi.org/10.1111/j.1348-0421.1984.tb00754.x
7. Mata LJ, Urrutia JJ. Intestinal colonization of breast-fed children in a rural area of low socioeconomic level*. *Ann the N Y Acad Sci*. 1971;176(1):93-109. https://doi.org/10.1111/j.1749-6632.1971.tb34996.x
8. Rotimi VO, Duerden BI. The development of the bacterial flora in normal neonates. *J Med Microbiol*. 1981;14(1):51-62. https://doi.org/10.1099/00222615-14-1-51
9. Stark PL, Lee A. The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. *J Med Microbiol*. 1982;15(2):189-203. https://doi.org/10.1099/00222615-15-2-189
10. Yoshioka H, Iseki K, Fujita K. Development and differences of intestinal flora in the neonatal period in breast-fed and bottle-fed infants. *Pediatrics*. 1983;72(3):317-321. https://doi.org/10.1542/peds.72.3.317
11. Finegold SM, Sutter VL, Mathisen GE. Normal Indigenous Intestinal Flora. In: Human Intestinal Microflora in Health and Disease. *Elsevier*. 1983:3-31. https://doi.org/10.1016/B978-0-12-341280-5.50007-0
12. Adlerberth I, Carlsson B, Man PD, et al. Intestinal colonization with enterobacteriaceae in Pakistani and Swedish hospital-delivered infants. *Acta Paediatr*. 1991;80(6-7):602-610. https://doi.org/10.1111/j.1651-2227.1991.tb11917.x
13. Adlerberth I, Wold AE. Establishment of the gut microbiota in Western infants. *Acta Paediatr*. 2009;98(2):229-238. https://doi.org/10.1111/j.1651-2227.2008.01060.x
14. Persson KMS, Bjerre B, Elfström L, Polberger S, Forsgren A. Faecal carriage of group B streptococci. *Eur J Clin Microbiol*. 1986;5(2):156-159. https://doi.org/10.1007/BF02013972
15. Duranti S, Longhi G, Ventura M, van Sinderen D, Turroni F. Exploring the ecology of bifidobacteria and their genetic adaptation to the mammalian gut. *Microorganisms*. 2020;9(1):8. https://doi.org/10.3390/microorganisms9010008
16. Mevissen-Verhage EA, Marcelis JH, de Vos MN, Harmsen-van Amerongen WC, Verhoef J. Bifidobacterium, Bacteroides, and Clostridium spp. in fecal samples from breast-fed and bottle-fed infants with and without iron supplement. *J Clin Microbiol*. 1987;25(2):285-289. https://doi.org/10.1128/jcm.25.2.285-289.1987
17. Hall MA, Cole CB, Smith SL, Fuller R, Rolles CJ. Factors influencing the presence of faecal lactobacilli in early infancy. *Arch Dis Child*. 1990;65(2):185-188.

https://doi.org/10.1136/adc.65.2.185
18. Biteen JS, Blainey PC, Cardon ZG, et al. Tools for the microbiome: nano and beyond. *ACS Nano*. 2016;10(1):6-37. https://doi.org/10.1021/acsnano.5b07826
19. 19. Browne HP, Forster SC, Anonye BO, et al. Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. *Nature*. 2016;533(7604):543-546. https://doi.org/10.1038/nature17645
20. Rajilić-Stojanović M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiol Rev*. 2014;38(5):996-1047. https://doi.org/10.1111/1574-6976.12075
21. Langille MGI, Zaneveld J, Caporaso JG, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol*. 2013;31(9):814-821. https://doi.org/10.1038/nbt.2676
22. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010;7(5):335-336. https://doi.org/10.1038/nmeth.f.303
23. Schloss PD, Westcott SL, Ryabin T, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol*. 2009;75(23):7537-7541. https://doi.org/10.1128/AEM.01541-09
24. Clarridge JE 3rd. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin Microbiol Rev*. 2004;17(4):840-862. https://doi.org/10.1128/CMR.17.4.840-862.2004
25. Gilbert JA, Dupont CL. Microbial metagenomics: beyond the genome. *Annu Rev Mar Sci*. 2011;3(1):347-371. https://doi.org/10.1146/annurev-marine-120709-142811
26. Neefs JM, Van de Peer Y, De Rijk P, Chapelle S, De Wachter R. Compilation of small ribosomal subunit RNA structures. *Nucl Acids Res*. 1993;21(13):3025-3049. https://doi.org/10.1093/nar/21.13.3025
27. Sundquist A, Bigdeli S, Jalili R, et al. Bacterial flora-typing with targeted, chip-based Pyrosequencing. *BMC Microbiol*. 2007;7:108. https://doi.org/10.1186/1471-2180-7-108
28. Claesson MJ, Wang Q, O'Sullivan O, et al. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic Acids Res*. 2010;38(22):e200. https://doi.org/10.1093/nar/gkq873
29. Turroni F, Peano C, Pass DA, et al. Diversity of bifidobacteria within the infant gut microbiota. *PLoS One*. 2012;7(5):e36957. https://doi.org/10.1371/journal.pone.0036957
30. Milani C, Hevia A, Foroni E, et al. Assessing the fecal microbiota: an optimized ion torrent 16S rRNA gene-based analysis protocol. *PLoS One*. 2013;8(7):e68739. https://doi.org/10.1371/journal.pone.0068739
31. Rinke C, Schwientek P, Sczyrba A, et al. Insights into the phylogeny and coding potential of microbial dark matter. *Nature*. 2013;499(7459):431-437. https://doi.org/10.1038/nature12352
32. Chakravorty S, Helb D, Burday M, Connell N, Alland D. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *J Microbiol Methods*. 2007;69(2):330-339. https://doi.org/10.1016/j.mimet.2007.02.005
33. Milani C, Lugli GA, Turroni F, et al. Evaluation of bifidobacterial community composition in the human gut by means of a targeted amplicon sequencing (ITS) protocol. *FEMS Microbiol Ecol*. 2014;90(2):493-503. https://doi.org/10.1111/1574-6941.12410
34. Milani C, Mancabelli L, Lugli GA, et al. Exploring vertical transmission of bifidobacteria from mother to child. *Appl Environ Microbiol*. 2015;81(20):7078-7087. https://doi.org/10.1128/AEM.02037-15
35. Milani C, Turroni F, Duranti S, et al. Genomics of the genus bifidobacterium reveals species-specific adaptation to the glycan-rich gut environment. *Appl Environ Microbiol*. 2016;82(4):980-991. https://doi.org/10.1128/AEM.03500-15
36. Dean FB, Nelson JR, Giesler TL, Lasken RS. Rapid amplification of plasmid and phage DNA using Phi29 DNA polymerase and multiply-primed rolling circle amplification. *Genome Res*. 2001;11(6):1095-1099. https://doi.org/10.1101/gr.180501
37. McLean JS, Lombardo MJ, Badger JH, et al. Candidate phylum TM6 genome recovered from a hospital sink biofilm provides genomic insights into this uncultivated phylum. *Proc Natl Acad Sci USA*. 2013;110(26):e2390-e2399. https://doi.org/10.1073/pnas.1219809110
38. Fodor AA, DeSantis TZ, Wylie KM, et al. The "most wanted" taxa from the human microbiome for whole genome sequencing. *PLoS One*. 2012;7(7):e41294. https://doi.org/10.1371/journal.pone.0041294
39. Marcy Y, Ouverney C, Bik EM, et al. Dissecting biological "dark matter" with single-cell genetic analysis of rare and uncultivated TM7 microbes from the human mouth. *Proc Natl Acad Sci USA*. 2007;104(29):11889-11894. https://doi.org/10.1073/pnas.0704662104
40. Lugli GA, Milani C, Mancabelli L, et al. Ancient bacteria of the Ötzi's microbiome: a genomic tale from the Copper Age. *Microbiome*. 2017;5(1):5. https://doi.org/10.1186/s40168-016-0221-y
41. Segata N, Waldron L, Ballarini A, Narasimhan V, Jousson O, Huttenhower C. Metagenomic microbial community profiling using unique clade-specific marker genes. *Nat Methods*. 2012;9(8):811-814. https://doi.org/10.1038/nmeth.2066
42. Hugon P, Dufour JC, Colson P, Fournier PE, Sallah K, Raoult D. A comprehensive repertoire of prokaryotic species identified in human beings. *Lancet Infect Dis*. 2015;15(10):1211-1219. https://doi.org/10.1016/S1473-3099(15)00293-5
43. Goodman AL, Kallstrom G, Faith JJ, et al. Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proc Natl Acad Sci USA*. 2011;108(15):6252-6257. https://doi.org/10.1073/pnas.1102938108
44. Walker AW, Duncan SH, Louis P, Flint HJ. Phylogeny, culturing, and metagenomics of the human gut microbiota. *Trends Microbiol*. 2014;22(5):267-274. https://doi.org/10.1016/j.tim.2014.03.001
45. Lagier JC, Khelaifia S, Alou MT, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. *Nat Microbiol*. 2016;1:16203. https://doi.org/10.1038/nmicrobiol.2016.203
46. Lagier JC, Armougom F, Million M, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect*. 2012;18(12):1185-1193. https://doi.org/10.1111/1469-0691.12023
47. Munyaka PM, Khafipour E, Ghia JE. External influence of early childhood establishment of gut microbiota and subsequent health implications. *Front Pediatr*. 2014;2:109. https://doi.org/10.3389/fped.2014.00109
48. Biasucci G, Rubini M, Riboni S, Morelli L, Bessi E, Retetangos C. Mode of delivery affects the bacterial community in the newborn gut. *Early Hum Dev*. 2010;86(1):13-15. https://doi.org/10.1016/j.earlhumdev.2010.01.004
49. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci USA*. 2010;107(26):11971-11975. https://doi.org/10.1073/pnas.1002601107
50. Bokulich NA, Chung J, Battaglia T, et al. Antibiotics, birth mode,

and diet shape microbiome maturation during early life. *Sci Transl Med*. 2016;8(343):343ra82. https://doi.org/10.1126/scitranslmed.aad7121
51. Rodríguez JM, Murphy K, Stanton C, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb Ecol Health Dis*. 2015;26(S2):26050. https://www.tandfonline.com/doi/pdf/10.3402/mehd.v26.26050%40zmeh20.2015.26.issue-s2?needAccess=true
52. Fouhy F, Ross RP, Fitzgerald GF, Stanton C, Cotter PD. Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps. *Gut Microbes*. 2012;3(3):203-220. https://doi.org/10.4161/gmic.20169
53. Bäckhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe*. 2015;17(5):690-703. https://doi.org/10.1016/j.chom.2015.04.004
54. Del Chierico F, Vernocchi P, Petrucca A, et al. Phylogenetic and metabolic tracking of gut microbiota during perinatal development. *PLoS One*. 2015;10(9):e0137347. https://doi.org/10.1371/journal.pone.0137347
55. Adlerberth I, Strachan DP, Matricardi PM, et al. Gut microbiota and development of atopic eczema in 3 European birth cohorts. *J Allergy Clin Immunol*. 2007;120(2):343-350. https://doi.org/10.1016/j.jaci.2007.05.018
56. Jakobsson HE, Abrahamsson TR, Jenmalm MC, et al. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by Caesarean section. *Gut*. 2014;63(4):559-566. https://doi.org/10.1136/gutjnl-2012-303249
57. Neu J, Rushing J. Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. *Clin Perinatol*. 2011;38(2):321-331. https://doi.org/10.1016/j.clp.2011.03.008
58. Ratsika A, Codagnone MC, O'Mahony S, Stanton C, Cryan JF. Priming for life: early life nutrition and the Microbiota-Gut-Brain Axis. *Nutrients*. 2021;13(2):423. https://doi.org/10.3390/nu13020423
59. Hill CJ, Lynch DB, Murphy K, et al. Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome*. 2017;5(1):4. https://doi.org/10.1186/s40168-016-0213-y
60. Martin R, Makino H, Cetinyurek Yavuz A, et al. Early-life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. *PLoS One*. 2016;11(6):e0158498. https://doi.org/10.1371/journal.pone.0158498
61. Salminen S, Gibson GR, McCartney AL, Isolauri E. Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut*. 2004;53(9):1388-1389. https://doi.org/10.1136/gut.2004.041640
62. Falony G, Joossens M, Vieira-Silva S, et al. Population-level analysis of gut microbiome variation. *Science*. 2016;352(6285):560-564. https://doi.org/10.1126/science.aad3503
63. Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nat Med*. 2017;23(3):314-326. https://doi.org/10.1038/nm.4272
64. Almanzar G, Schönlaub J, Hammerer-Lercher A, Koppelstaetter C, Bernhard D, Prelog M. Influence of the delivery modus on subpopulations and replication of lymphocytes in mothers and newborns. *Early Hum Dev*. 2015;91(12):663-670. https://doi.org/10.1016/j.earlhumdev.2015.09.010
65. Darabi B, Rahmati S, HafeziAhmadi MR, Badfar G, Azami M. The association between caesarean section and childhood asthma: an updated systematic review and meta-analysis. *Allergy Asthma Clin Immunol*. 2019;15:62. https://doi.org/10.1186/s13223-019-0367-9
66. Pistiner M, Gold DR, Abdulkerim H, Hoffman E, Celedón JC. Birth by cesarean section, allergic rhinitis, and allergic sensitization among children with a parental history of atopy. *J Allergy Clin Immunol*. 2008;122(2):274-279. https://doi.org/10.1016/j.jaci.2008.05.007
67. Cardwell CR, Stene LC, Joner G, et al. Caesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies. *Diabetologia*. 2008;51(5):726-735. https://doi.org/10.1007/s00125-008-0941-z
68. Flexeder C, Thiering E, Kratzsch J, et al. Is a child's growth pattern early in life related to serum adipokines at the age of 10 years? *Eur J Clin Nutr*. 2014;68(1):25-31. https://doi.org/10.1038/ejcn.2013.213
69. Ruiz L, Moles L, Gueimonde M, Rodriguez JM. Perinatal microbiomes' influence on preterm birth and preterms' health: influencing factors and modulation strategies. *J Pediatr Gastroenterol Nutr*. 2016;63(6):e193-e203. https://doi.org/10.1097/MPG.0000000000001196
70. Rougé C, Goldenberg O, Ferraris L, et al. Investigation of the intestinal microbiota in preterm infants using different methods. *Anaerobe*. 2010;16(4):362-370. https://doi.org/10.1016/j.anaerobe.2010.06.002
71. Jacquot A, Neveu D, Aujoulat F, et al. Dynamics and clinical evolution of bacterial gut microflora in extremely premature patients. *J Pediatr*. 2011;158(3):390-396. https://doi.org/10.1016/j.jpeds.2010.09.007
72. Arboleya S, Binetti A, Salazar N, et al. Establishment and development of intestinal microbiota in preterm neonates. *FEMS Microbiol Ecol*. 2012;79(3):763-772. https://doi.org/10.1111/j.1574-6941.2011.01261.x
73. Arboleya S, Sánchez B, Milani C, et al. Intestinal Microbiota Development in Preterm Neonates and Effect of Perinatal Antibiotics. *J Pediatr*. 2015;166(3):538-544. https://doi.org/10.1016/j.jpeds.2014.09.041
74. Cong X, Xu W, Janton S, et al. Gut microbiome developmental patterns in early life of preterm infants: impacts of feeding and gender. *PLoS One*. 2016;11(4):e0152751. https://doi.org/10.1371/journal.pone.0152751
75. Aujoulat F, Roudière L, Picaud JC, et al. Temporal dynamics of the very premature infant gut dominant microbiota. *BMC Microbiol*. 2014;14:325. https://doi.org/10.1186/s12866-014-0325-0
76. La Rosa PS, Warner BB, Zhou Y, et al. Patterned progression of bacterial populations in the premature infant gut. *Proc Natl Acad Sci USA*. 2014;111(34):12522-12527. https://doi.org/10.1073/pnas.1409497111
77. Groer MW, Gregory KE, Louis-Jacques A, Thibeau S, Walker WA. The very low birth weight infant microbiome and childhood health: VLBW Infant Microbiome and Childhood Health. *Birth Defects Res C Embryo Today*. 2015;105(4):252-264. https://doi.org/10.1002/bdrc.21115
78. Collado MC, Cernada M, Neu J, Pérez-Martínez G, Gormaz M, Vento M. Factors influencing gastrointestinal tract and microbiota immune interaction in preterm infants. *Pediatr Res*. 2015;77(6):726-731. https://doi.org/10.1038/pr.2015.54
79. Watkins C, Stanton C, Ryan CA, Ross RP. Microbial therapeutics designed for infant health. *Front Nutr*. 2017;4:48. https://doi.org/10.3389/fnut.2017.00048
80. Madan JC, Salari RC, Saxena D, et al. Gut microbial colonisation in premature neonates predicts neonatal sepsis. *Arch Dis Child Fetal Neonatal Ed*. 2012;97(6):F456-F462. https://doi.org/10.1136/fetalneonatal-2011-301373
81. Mai V, Torrazza RM, Ukhanova M, et al. Distortions in development of intestinal microbiota associated with late onset sepsis in preterm infants. *PLoS One*. 2013;8(1):e52876. https://doi.org/10.1371/journal.pone.0052876

82. Cernada M, Bäuerl C, Serna E, Collado MC, Martínez GP, Vento M. Sepsis in preterm infants causes alterations in mucosal gene expression and microbiota profiles compared to non-septic twins. *Sci Rep*. 2016;6:25497. https://doi.org/10.1038/srep25497
83. Arboleya S, Sánchez B, Solís G, et al. Impact of Prematurity and perinatal antibiotics on the developing intestinal microbiota: a functional inference study. *Int J Mol Sci*. 2016;17(5):649. https://doi.org/10.3390/ijms17050649
84. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature*. 2012;486(7402):222-227. https://doi.org/10.1038/nature11053
85. O'Sullivan A, Farver M, Smilowitz JT. Article commentary: The influence of early infant-feeding practices on the intestinal microbiome and body composition in infants. *Nutr Metab Insights*. 2015;8(Suppl 1):1-9. https://doi.org/10.4137/NMI.S29530
86. Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. *Nature*. 2012;489(7415):231-241. https://doi.org/10.1038/nature11551
87. Bezirtzoglou E, Tsiotsias A, Welling GW. Microbiota profile in feces of breast-and formula-fed newborns by using fluorescence in situ hybridization (FISH). *Anaerobe*. 2011;17(6):478-482. https://doi.org/10.1016/j.anaerobe.2011.03.009
88. Moore RE, Townsend SD. Temporal development of the infant gut microbiome. *Open Biol*. 2019;9(9):190128. https://doi.org/10.1098/rsob.190128
89. Harmsen HJM, Wildeboer–Veloo ACM, Raangs GC, et al. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr*. 2000;30(1):61-67. https://doi.org/10.1097/00005176-200001000-00019
90. Guaraldi F, Salvatori G. Effect of breast and formula feeding on gut microbiota shaping in newborns. *Front Cell Infect Microbiol*. 2012;2:94. https://doi.org/10.3389/fcimb.2012.00094
91. Le Huërou-Luron I, Blat S, Boudry G. Breast-v. formula-feeding: impacts on the digestive tract and immediate and long-term health effects. *Nutr Res Rev*. 2010;23(1):23-36. https://doi.org/10.1017/S0954422410000065
92. Fallani M, Amarri S, Uusijarvi A, et al. Determinants of the human infant intestinal microbiota after the introduction of first complementary foods in infant samples from five European centres. *Microbiology*. 2011;157(5):1385-1392. https://doi.org/10.1099/mic.0.042143-0
93. Koenig JE, Spor A, Scalfone N, et al. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci USA*. 2011;108(Suppl1):4578-4585. https://doi.org/10.1073/pnas.1000081107
94. Vallès Y, Artacho A, Pascual-García A, et al. Microbial succession in the gut: directional trends of taxonomic and functional change in a birth cohort of Spanish infants. *PLoS Genet*. 2014;10(6):e1004406. https://doi.org/10.1371/journal.pgen.1004406
95. Laursen MF, Andersen LBB, Michaelsen KF, et al. Infant gut microbiota development is driven by transition to family foods independent of maternal obesity. *mSphere*. 2016;1(1):e00069-15. https://doi.org/10.1128/mSphere.00069-15
96. Bergström A, Skov TH, Bahl MI, et al. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. *Appl Environ Microbiol*. 2014;80(9):2889-2900. https://doi.org/10.1128/AEM.00342-14
97. Nuriel-Ohayon M, Neuman H, Koren O. Microbial changes during pregnancy, birth, and infancy. *Front Microbiol*. 2016;7:1031. https://doi.org/10.3389/fmicb.2016.01031
98. Levin AM, Sitarik AR, Havstad SL, et al. Joint effects of pregnancy, sociocultural, and environmental factors on early life gut microbiome structure and diversity. *Sci Rep*. 2016;6:31775. https://doi.org/10.1038/srep31775
99. Davis MY, Zhang H, Brannan LE, Carman RJ, Boone JH. Rapid change of fecal microbiome and disappearance of Clostridium difficile in a colonized infant after transition from breast milk to cow milk. *Microbiome*. 2016;4(1):53. https://doi.org/10.1186/s40168-016-0198-6
100. Collado MC, Isolauri E, Laitinen K, Salminen S. Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: a prospective follow-up study initiated in early pregnancy. *Am J Clin Nutr*. 2010;92(5):1023-1030. https://doi.org/10.3945/ajcn.2010.29877
101. Chu DM, Antony KM, Ma J, et al. The early infant gut microbiome varies in association with a maternal high-fat diet. *Genome Med*. 2016;8(1):77. https://doi.org/10.1186/s13073-016-0330-z
102. Laursen MF, Zachariassen G, Bahl MI, et al. Having older siblings is associated with gut microbiota development during early childhood. *BMC Microbiol*. 2015;15:154. https://doi.org/10.1186/s12866-015-0477-6
103. Arrieta MC, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The intestinal microbiome in early life: health and disease. *Front Immunol*. 2014;5:427. https://doi.org/10.3389/fimmu.2014.00427
104. De Filippo C, Cavalieri D, Di Paola M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA*. 2010;107(33):14691-14696. https://doi.org/10.1073/pnas.1005963107
105. Grześkowiak Ł, Collado MC, Mangani C, et al. Distinct gut microbiota in southeastern African and northern European infants. *J Pediatr Gastroenterol Nutr*. 2012;54(6):812-816. https://doi.org/10.1097/MPG.0b013e318249039c
106. Lin A, Bik EM, Costello EK, et al. Distinct distal gut microbiome diversity and composition in healthy children from Bangladesh and the United States. *PLoS One*. 2013;8(1):e53838. https://doi.org/10.1371/journal.pone.0053838
107. Stearns JC, Zulyniak MA, de Souza RJ, et al. Ethnic and diet-related differences in the healthy infant microbiome. *Genome Med*. 2017;9(1):32. https://doi.org/10.1186/s13073-017-0421-5
108. 108. Benson AK, Kelly SA, Legge R, et al. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc Natl Acad Sci USA*. 2010;107(44):18933-18938. https://doi.org/10.1073/pnas.1007028107
109. Leamy LJ, Kelly SA, Nietfeldt J, et al. Host genetics and diet, but not immunoglobulin A expression, converge to shape compositional features of the gut microbiome in an advanced intercross population of mice. *Genome Biol*. 2014;15(12):552. https://doi.org/10.1186/s13059-014-0552-6
110. Turpin W, Espin-Garcia O, Xu W, et al. Association of host genome with intestinal microbial composition in a large healthy cohort. *Nat Genet*. 2016;48(11):1413-1417. https://doi.org/10.1038/ng.3693
111. Stewart JA, Chadwick VS, Murray A. Investigations into the influence of host genetics on the predominant eubacteria in the faecal microflora of children. *J Med Microbiol*. 2005;54(12):1239-1242. https://doi.org/10.1099/jmm.0.46189-0
112. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature*. 2009;457(7228):480-484.

https://doi.org/10.1038/nature07540
113. Turnbaugh PJ, Quince C, Faith JJ, et al. Organismal, genetic, and transcriptional variation in the deeply sequenced gut microbiomes of identical twins. *Proc Natl Acad Sci USA*. 2010;107(16):7503-7508. https://doi.org/10.1073/pnas.1002355107
114. Bonder MJ, Kurilshikov A, Tigchelaar EF, et al. The effect of host genetics on the gut microbiome. *Nat Genet*. 2016;48(11):1407-1412. https://doi.org/10.1038/ng.3663
115. Connell JH, Slatyer RO. Mechanisms of succession in natural communities and their role in community stability and organization. *Am Nat*. 1977;111(982):1119-1144. https://doi.org/10.1086/283241
116. Davis EC, Wang M, Donovan SM. The role of early life nutrition in the establishment of gastrointestinal microbial composition and function. *Gut Microbes*. 2017;8(2):143-171. https://doi.org/10.1080/19490976.2016.1278104
117. White RA, Bjørnholt JV, Baird DD, et al. Novel developmental analyses identify longitudinal patterns of early gut microbiota that affect infant growth. *PLoS Comput Biol*. 2013;9(5):e1003042. https://doi.org/10.1371/journal.pcbi.1003042
118. Gosalbes MJ, Llop S, Vallès Y, Moya A, Ballester F, Francino MP. Meconium microbiota types dominated by lactic acid or enteric bacteria are differentially associated with maternal eczema and respiratory problems in infants. *Clin Exp Allergy*. 2013;43(2):198-211. https://doi.org/10.1111/cea.12063
119. Azad MB, Konya T, Maughan H, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ*. 2013;185(5):385-394. https://doi.org/10.1503/cmaj.121189
120. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science*. 2009;326(5960):1694-1697. https://doi.org/10.1126/science.1177486
121. Vaishampayan PA, Kuehl JV, Froula JL, Morgan JL, Ochman H, Francino MP. Comparative metagenomics and population dynamics of the gut microbiota in mother and infant. *Genome Biol Evol*. 2010;2:53-66. https://doi.org/10.1093/gbe/evp057
122. Kirkby MJ, MacArthur RH, Wilson EO. The Theory of Island Biogeography. *Geogr J*. 1968;134(4):592. https://doi.org/10.2307/1796430
123. Bäckhed F. Programming of host metabolism by the gut microbiota. *Ann Nutr Metab*. 2011;58(Suppl 2):44-52. https://doi.org/10.1159/000328042
124. Xu J, Bian G, Zheng M, et al. Fertility factors affect the vaginal microbiome in women of reproductive age. *Am J Reprod Immunol*. 2020;83(4):e13220. https://doi.org/10.1111/aji.13220