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# Prenatal and early childhood development of gut microbiota

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**Abstract. – OBJECTIVE: Gut microbiota provide a diverse "organ" or biocenosis responsible for protection against pathogens and the development of both intestinal and immune systems. Microbiota are also responsible for the synthesis of vitamins and short-chain fatty acids, which in turn affect the host's metabolism. It was hypothesized that gut microbiota are influenced by fetal life followed by intensive development throughout the first years of life.**

**MATERIALS AND METHODS: We analyzed the available literature (PubMed, Embase, Google Scholar) on prenatal and early childhood development of gut microbiota.**

**RESULTS: A body of evidence suggests in utero colonization. The main factors determining gut microbiota include the type of delivery and post-natal feeding method. The composition of the intestinal flora is also influenced by fetal age at birth, antibiotic therapy, pre- and probiotic supplementation, and other environmental factors. The multifaceted nature of this process guarantees the uniqueness of its composition for each human being.**

**CONCLUSIONS: Although the composition of intestinal microbiota is subject to continuous and dynamic changes, it seems that the perinatal period is critical for the emergence of its proper pattern, which may guarantee health or otherwise illness in adult life.**

*Key Words:*
Gut microbiota, Bacterial colonization, Infant, Intestinal barrier, Prenatal, Postnatal, Perinatal.

## Introduction

The human body provides a habitat for multiple microorganisms, collectively known as the microbiome or microbiota (with plural reference to many microorganisms). Bacteria play a pivotal role within this ecological community, and begin to colonize ecological niches already during fetal life[1]. Microbial cells have been found in digestive, respiratory, and urinary tracts, as well as in many organs, including skin, hair, ear canals, and mammary glands. However, microbiota of the greatest richness and diversity are localized within the digestive tract. There are as many as 100 trillion microbiotic cells within the alimentary tract, although microbial density strongly depends on the particular location within the gastrointestinal tract[2]. The types and number of bacteria vary according to secretory activity, oxygen availability, and gastrointestinal motility, with the highest concentration found in the colon with $10^{14}$ cells/per g of feces[3].

Of particular interest is that the gut microbiome of every host differs, and is subject to continuous and dynamic changes throughout life. In contrast, however, microbiotic function appears in some respects to be similar among different people and habitats[4]. The complex of bacteria could be described as a very active "organ" or biocenosis which provides metabolic, trophic, and immunological benefits. Microbiota ensure protection against pathogens, regulate the development of the intestine, synthesize vitamins and short-chain fatty acids (SCFAs) and train the immune system through the stimulation of innate and adaptive (humoral and cellular) responses. Consequently, the diversity of the gut microbiome could have a large impact on human bodily functions[5]. An alteration in microbiota composition and therefore function has been recently acknowledged as a strong risk factor for the development of various diseases.

For many years there has been a conviction that the microbiome provides an "organ" function without an "organ" structure. Successive colonization of the intestine by different genera and species of bacteria is the basic process that shapes

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this "organ". Reports[6,7] suggest that the time required to form a fully mature microbiome is about 3 years from the time of birth. This organ however is not stable regarding its structure. Drugs, dietary patterns, exercise, and illnesses serve as environmental factors affecting the composition of this microbial organ. Although the composition of the intestinal microbiome is subject to continuous and dynamic changes, it seems that the perinatal period is critical to the emergence of its future pattern, which may guarantee health or illness in adult life (Figure 1).

## Fetal Microbiota

For more than a century, the fetus was thought to be sterile and colonization with microorganisms was regarded as taking place only during labor and after delivery. However, recent reports have suggested that already in fetal life the human organism may be exposed to both pathogenic and commensal microorganisms. Microorganisms in umbilical cord blood, amniotic fluid, placenta, and fetal membrane were detected following the development of molecular techniques (polymerase chain reaction, PCR; fluorescent *in-situ* hybridization; human intestinal tract chip, HITChip analyses) and obstetric gynecology aimed at establishing sterility status during pregnancy. According to the literature, even under the physiological conditions of pregnancy with no positive infection indices, microorganisms living in the above-mentioned structures may easily be detected[1].

In germ-free mouse models it has been demonstrated that the presence of bacteria in the fetal period is necessary for the proper development of the gastrointestinal tract. Gnotobiotic (sterile or with reduced microbiota) pigs gave birth to animals in which decreased intestinal epithelial cell renewal and reduced mucus production were observed[8,9]. Along with these alterations, abnormalities in gut-associated lymphoid tissue (GALT) and reduced production of antibodies were reported[10]. Concerning additions to microbiota, Gomez de Agüero et al[11] were able to demonstrate that gestational colonization with *E.coli* HA107 resulted in enhanced antibacterial peptide production and elevated metabolism of bacterial molecules. Most importantly, when labelled *E. faecium* – a genus typical for a healthy woman's breast milk - were ingested by pregnant mice, these microbes were then found in amniotic fluid[12].

The time needed for colonization of the fetus has not been accurately estimated, but data indicate a role in the swallowing of amniotic fluid by the fetus. The composition of amniotic fluid changes during pregnancy and includes fetal ex-

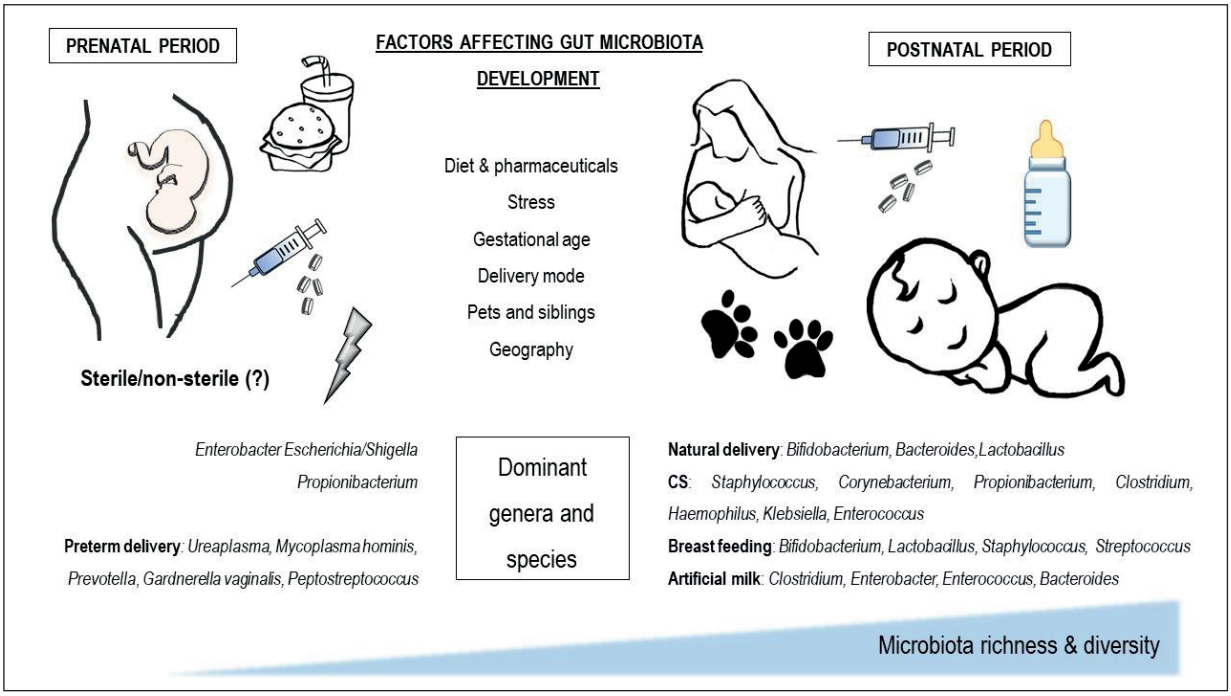


**Figure 1.** Factors influencing gut microbiota development in the prenatal and postnatal period. CS = cesarean section.

creta and secretions, hormones, growth factors, but also bacterial cells and the products of their disintegration[13,14]. The skill of swallowing develops around 10 weeks post fertilization. Theoretically, bacteria could be translocated to the body of the fetus and fetal fluids *via* the bloodstream and/or by ascending from the genital canal. The exact mechanism of putative transmission is not well established, but it has been speculated that immune cells may be involved. Possibly, dendritic cells within the gut barrier may play an important role here, presenting bacteria and their molecular products to the lymphoid cells and then translocating them through the mucosa-associated lymphoid tissue (MALT) system to other organs[15].

Bacteria which typically live in the intestinal lumen of an adult have been detected in the placenta (*E. coli*)[16], amniotic fluid[13,14], meconium (*Enterococcus* sp.)[17], and in human milk (*Staphylococci, Streptococci, Lactobacillus, Bifidobacteria*)[18]. Of note, the meconium microbiome was shown to potentially consist of bacteria which can be located on the nipple or in breast milk. As evidenced by Jiménez et al[17] who collected stool from the first two hours of life from children not fed by mother's milk which inhibited the contamination with milk/nipple bacteria, *Enterococcus faecalis, Staphyloccocus epidermis*, and *Escherichia coli* might be present in meconium samples. One study with a sample of 15 mother-newborn pairs[14], in whom elective cesarean sections (CS) were conducted, aimed to look for proof of maternal/fetal microbial transfer. They were able to demonstrate the presence of predominantly Proteobacteria (*Enterobacter* and *Escherichia/Shigella* genera*)* in placenta and amniotic fluid, a pattern found to be present in meconium in conjunction with *Propionibacterium*. Four days after delivery, newborn gut microbiota began to resemble that found in colostrum. More recently, placental-tissue bacteria in a group of more than 1300 pregnant women from a low income rural area in Africa were categorized and it was reported[19] that specific bacteria were associated with chorioamnionitis and poor pregnancy outcomes, including low birth mass. The presence of *Fusobacterium nucleatum*, *Ureaplasma sp.*, and *Gemella asaccharolytica* was found to be associated with a shorter pregnancy duration and *Sneathia sanguinegens*, *Prevotella copri*, *Lachnospiraceae* sp., and *Phascolarctobacterium succinatutens* with giving birth to a smaller newborn[19]. Interestingly, in the placenta and in amniotic fluid, bacteria found to be involved in periodontal disease (*Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum ssp., Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, Treponema denticola*) were also detected. It has also been shown that the presence of these may precede premature labor or even preeclampsia[20,21].

The duration of pregnancy may also shape the intestinal microbiota of a child. The gut of a premature baby is colonized by *Clostridium* sp., *Escherichia coli, Enterococcus, Streptococcus, Klebsiella*, and *Staphylococcus* whereas a term-delivered infant's microbiota consists of *Bifidobacterium* and *Lactobacillus* genera[22]. In premature infants, the most commonly reported microorganisms which also reside in amniotic fluid were *Ureaplasma*[23] and anaerobic bacteria which are also associated with bacterial maternal vaginosis, such as *Mycoplasma hominis, Prevotella* spp., *Gardnerella vaginalis, Peptostreptococcus* spp.[24].

Moreover, there is a body of evidence that supports the presence of bacteria in fetal membranes, placental parenchyma, placental basal plate, cervical fluid, and cord blood[16,19,25-30] in cases of spontaneous preterm deliveries. Leon et al[31], who analysed a total of 400 placental specimens collected from non-spontaneous and spontaneous preterm newborns found enrichment of the latter samples with some microbes, especially *Mycoplasma* spp. and *Ureaplasma* spp., independent of the mode of delivery. Of note, *Capnocytophaga,* a genus typically found in the oral cavity and linked to intrauterine infection[32,33], was detected in a proportion of samples. However, as they emphasized, bacterial colonization *via* sample collection may have been responsible for at least some of these genera[31]. Indeed, there is evidence showing the possibility of reducing contamination of the specimens using different extraction procedures[34].

Possibly the mother's state of health, her eating habits, and other environmental factors are crucial for colonization of the fetus. In a study conducted in monkeys, the effect of stress experienced in pregnancy on intestinal microflora in the early infancy period of their offspring was evaluated[35]. The stress factor was acoustic stimulus, which was positively associated with elevation of serum cortisol in pregnant animals from study group compared to controls. During the first six months of life, the overall generic structure of gut microbiota in simian offspring was similar regardless of the stress intervention in their mothers. However, in control group a statistically significant increase in the number of *Lactobacil-*

*lus* genera was observed. In addition, a study on late-prenatal-stress stimuli (between 15 and 21 weeks of gestation) reduced significantly *Bifidobacteria* count at 24 weeks of age in test animals. Stress *via* hormonal changes may therefore affect the intestinal microbiota. Other studies have shed light on the relationship between gut microbiota in early childhood and prenatal antibiotic therapy. One study found that the diversity of offspring gut bacteria may be decreased following treatment of mothers with antibiotics[36].

On the other hand, some observational studies have provided some doubts as to whether the fetal environment actually harbors any bacteria. Kim et al[37] found that microbes were significantly more frequently observed in chorioamniotic membranes with positive amniotic fluid cultures. These specimens were free of microbes in the case of women giving at-term birth. Also, studies on the presence of dead microorganisms, with the presence of microbial by-products or cell walls with pathogen-associated molecular patterns may provide reasons for some proven immune-related effects during prenatal life[38]. Recently, Rehbinder et al[39], evaluated microbial counts before delivery and found that bacterial content was comparable to that in negative controls (operating-room environment samples). The bacterial concentrations were observed to be ten times higher only when the sample was collected in the case of fetal-membrane rupture. The most commonly detected bacterial genera were *Bifidobacterium, Olsenella, Prevotella, Aerococcus, Lactobacillus, Shuttleworthia, Sneathia, Ureaplasma, Caulobacteraceae, and Pseudomonas*, the latter two perhaps especially associated with contamination during labor[40]. Overall, as elegantly summarized by Perez-Muñoz et al[41], *in utero* colonization is doubtful, as molecular techniques may be appropriate to detect small amounts of genetic material but not living organisms. Moreover, lack of contamination controls and lack of proof of bacterial viability contribute to uncertainty in the results. The pros and cons for the theory of in utero colonization are shown in Table I, but we will assume in this article that *in utero* colonization does actually occur.

Some studies have been conducted to evaluate the impact of prenatally administered probiotics (ingested by pregnant mothers) on intestinal microbiota of their newborns. Rautava et al[46] found that *Lactobacillus* spp. were present in the placenta and in the stool of full-term newborns whose mothers had taken probiotics during pregnancy. *Lactobacilli* are commensal bacteria that build a proper microbiotic environment in the vagina. Their protective role in premature newborns has been reported; in infants colonized with the *Lactobacilli*, even in the presence of pathogenic microbial cells, lower concentrations of biochemical markers of inflammation were found[24]. In a randomized clinical study conducted by Lahtinen et al[51], with a group of 122 mothers who were supplemented with *Lactobacillus rhamnosuss* GG (LGG) from the 36$^{th}$ week of pregnancy, the microbiomes of infants evaluated at 90 days post delivery were enriched in *Bifidobacterium longum*. Gueimonde et al[52] confirmed that *Bifidobacterium* counts at 5 days of age were increased if mothers received LGG probiotic administration during pregnancy. The stability of microbiota in stools collected from infants in the first month of life[53] has been shown to be independent of the type of delivery and nutrition. *Proteobacteria* and *Firmicutes* were the main bacteria present at every stage in the early period of life. It seems that these types of bacteria might dominate the fetal microbiome and may provide its "core" model.

## Newborn Microbiota

A variety of factors affect the colonization of the gastrointestinal tract of a newborn and contribute to differences in the composition of intestinal microbiota. One of the most important factors is the time of delivery. Microbiome of premature newborns may contain pathogenic microorganisms, be less diverse, and depleted in SCFAs[54,55]. A clinical trial in which microbiota structure during the first 30 days of life was determined in 14 preterm babies[56], utilized two methods of stool evaluation: a culture-dependent technique and HITchip analysis. Just after birth, the major inhabitants of the intestine were *Proteobacteria* and *Firmicutes*, which dominated the gut during the first week of life. Later, a greater diversity of bacteria was observed. The authors emphasized the relationship between the occurrence of *Serratia* (*Proteobacteria* type) in newborns treated with antibiotics and subjected to mechanical ventilation. Madan et al[57] found a lower diversity of bacteria in meconiums collected from premature newborns that had developed sepsis compared to healthy age-matched controls. The latter had more diverse microbiota enriched in *Clostridium, Klebsiella, and Veillonella*, whereas *Staphylo-*

**Table I.** Pros and cons for the theory of *in utero* colonization.

| PROS/method of identification | Reference | CONS/method of identification | Reference |
|---|---|---|---|
| Bacteria detected in 70% of placenta samples/FISH | 42 | Bacterial species enumeration uncertainty: in one single meconium sample number of species was within a range of 1-5/Bacterial culture, 16S rDNA sequencing | 17 |
| Bacteria detected in 94% of placenta samples/culturing and PCR of 16S rRNA | 43 | No bacteria in CS labors and bacterial DNA present in half of vagina deliveries/PCR of 16S rRNA | 44 |
| Bacteria detected in 91% of studied meconium samples regardless of the type of the delivery/454 pyrosequencing | 45 | Two-fold risk increase for intracellular bacteria in very preterm birth/histology and gram staining | 29 |
| Placenta Lactobacillus DNA present in 100% and Bifidobacterium DNA in 41% of placentas; selected bacterial DNA in 43% of amniotic fluids/qPCR | 46 | Bacteria in placenta present due to history of antenatal infection/16S rRNA sequencing and WGS metagenomics | 16 |
| Bacteria present in 100% of meconium samples/16S rRNA sequencing | 47 | Only 12.7% of placenta from patients with preeclampsia were positive for bacterial DNA/ Standard PCR, 16S rRNA sequencing | 48 |
| Bacteria pesent in 67.3% of meconium samples/16S rRNA sequencing | 49 | | |
| More than 40 bacterial phylotypes are common within meconium, amniotic fluid, and placenta/Cultures, 16S rRNA pyro-sequencing, qPCR, DGGE | 14 | | |
| Bacteria detected in 95% of meconium samples/RT-qPCR | 50 | | |

DGGE, denaturing gradient gel electrophoresis; FISH, fluorescent *in situ* hybridization; qPCR, quantitative Polymerase chain reaction; WGS, whole genome sequencing; CS, cesarian section.

*coccus* was more abundant in the low diversity microbiota of infants with sepsis[57]. Antibiotic therapy in these cases aggravated the abnormal microbiotic patterns. The author emphasized that the meconium from infants was not sterile and was colonized with *Lactobacillus*, *Staphylococcus (Firmicutes*), and the *Enterobacteriacae (Proteobacteria*) family. They suggested the existence of a "healthy microbiome" in premature newborns that protects them from infection. The study was however conducted with a small group, and such research should be continued.

In a study by Arboleya et al[54] colonization of the intestine in the first three months of life was assessed in 21 premature infants (30-35 weeks of gestational age) and 20 full-term infants. In all samples the gut had already begun to be colonized with relatively aerobic bacteria. This was most likely due to increased access to oxygen in the intestinal lumen after delivery (the fetal intestinal environment has low oxygen concentrations). Relatively aerobic bacteria consuming oxygen created conditions for further colonization by anaerobic microorganisms. When the microbiota compositions of both groups of newborns were compared there were significantly more *Enterobacteriaceae* and *Enterococcaceae*, with a limited number of obligate anaerobic *Bifidobacterium* and *Bacteroides* in premature babies. An elevated count of primary facultative anaerobic colonizers that are potential pathogens may explain the increased risk for developing necrotizing enterocolitis (NEC) and other intestinal problems in preterm infants. Claud et al[58], while looking for an association between microbiota and the development of NEC, examined ten premature newborn stools and demonstrated that in patients with NEC *Proteobacteria* and *Firmicutes* may enrich the gut 3 weeks before the disease manifests itself. This corresponds with results from meta-analyses on the efficacy of probiotic therapy in the prevention of NEC in premature infants[59-61]. Researchers have also shown that children exposed to prolonged hospitalization (e.g., due to prematurity) have an increased amount of *Clostridium difficile*, which is present in the hospital environment in spore form[62].

Despite various studies, it has still not been possible to create a model for the "healthy intestinal microbiome" of a newborn. Even among healthy newborns born naturally and exclusively breast-fed there is significant variation in the structure of gut microbiomes. In a study aimed at looking for a proper composition of a gut microbiome, the colonization of healthy newborns after natural delivery was assessed[63]. Newborns were divided into those with a mass suitable for gestational age (AGA) and newborns with large mass relative to gestational age (LGA). It was noticed that in AGA children, gut Gram positive *Firmicutes: Lactobacillus, Staphylococcus*, and *Clostridium (Proteobacteria*) dominated and provided a greater variety of colonizing bacteria. Gram negative *Proteobacteria*, mainly *Escherichia coli* dominated in LGA group. Therefore, even among children from groups with potentially "healthy microbiota" there are significant differences in composition, confirming that colonization of the intestine depends on many factors.

## Intestinal Microbiota of Infants

The age at which a fully mature intestinal microbiome is formed has not been undeniably established. It seems that this takes place during the preschool period at around the age of 2.5 to 3 years[6,7]. In one study, samples of stools collected from pregnant women and their children up to the age of 4 years were analyzed[64]. The authors demonstrated that 4-year-olds had a microbiotic pattern similar to maternal composition that is present during the first trimester of pregnancy. Along with the expansion of a child's nutritional repertoire, a stable intestinal microbiota consisting of *Bacteroidetes* and *Firmicutes* phyla typical for mature human microbiota could be developed. Bergström et al[65] found that stabilization of intestinal microfloral structure takes place between 18 and 36 months of age[65]. Its further development depended on the previous type of labor[66], the feeding method: artificial *vs.* natural[67], as well as the infant's diet[68], use of antibiotics[69], and other environmental factors[70].

## Type of Delivery

For many years, the route of birth has been considered as one of the most important factors shaping microbiota, not only of the gut but also of other sites colonized with microorganisms, namely skin, nose/throat, and ear. During natural birth, a newborn is exposed to a greater number and diversity of bacteria compared to newborns born *via* cesarean section. Naturally-born newborns have been found to be colonized by bacteria living in the mother's vagina and anus. Following the guidelines of the Polish Neonatal Society direct contact between newborns and their mothers (skin to skin) for at least 2 h after delivery should be arranged. It is also a good time to start breastfeeding[71].

In CS-born newborns, the sterile operating-room environment, delayed contact with the mother, and delayed initiation of breastfeeding create favorable conditions for hospital flora to colonize the newborn's various ecological niches. In addition, these newborns were found to be at higher risk for developing respiratory disorders (e.g., transient respiratory disorders) which require increased supervision in Intensive Care Units (ICUs), also reducing the promotion of a physiological pattern of colonization[72,73]. It has been shown that the intestinal microbiota of newborns born naturally is similar to maternal intestinal microbiota. In the case of newborns born *via* CS, this relationship was not found[74]. In parallel, it has been suggested that external perinatal factors, such as conditions in the operating room and/or the health of persons looking after the infant may be more significant for the colonization process as compared to CS itself[75,76].

Interestingly, it was reported to be very important whether the CS was performed electively or preceded by the physiological beginning of labor. The latter has been found to be associated with more gut microbiota similarities when compared to infants born naturally. In contrast, in infants born *via* elective CS, the intestine microbiota pattern more resembled the bacterial flora of the mother's skin[77]. These differences could be traced in *Lactobacillus*, *Clostridium*, and *Bacteroides* genera. In the intestinal microbiome of newborns born naturally *Lactobacillus* bacteria were shown to be more abundant (37% of bacterial content in meconium of children born naturally *vs.* 6% in infants born surgically) and this difference was demonstrated to persist up to 3 years of age[50]. In another study[62], natural labor increased the counts of *Bacteroides fragilis* and diminished the abundance of *Clostridium difficile*. These results come from a study conducted in the Netherlands, where one rule is that natural birth should take place at home. However, the difference in the number of *Bacteroides* was also confirmed in Swedish children, in whom a similar composition

pattern was shown to last until the 2nd year of life. Importantly, *Bacteroides* sp. are typical residents of mothers' guts. The above-mentioned findings were confirmed in a meta-analysis consisting of seven studies addressing this issue. While pooling the results, authors demonstrated that CS-born children have significantly more *Firmicutes* (*Clostridium*) bacteria in the first three months of life and in contrast less *Actinobacteria* and *Bacteroidetes*. In children after physiological delivery, more *Actinobacteria* (*Bifidobacterium*) and *Bacteroidetes* bacteria were enumerated. In addition, the diversity of *Actinobacteria, Proteobacteria, Bacteroidetes,* and *Firmicutes* at the age of 6 months was no longer related to the type of labor. At this age, infants from both groups were found to be colonized by almost the same bacteria, although there were quantitative differences[78]. Recently, Akagawa et al[79], following previous reports, demonstrated that CS resulted in less diverse gut microbiota, which started to resemble that of vaginally-born infants by 1 month of age. There are also reports[54] in which no relationship was found between the mode of delivery and the intestinal microbiota of the newborn, but these were carried out mainly among preterm-born infants.

In conclusion, it should be stated that CS delivery disturbs the colonization of the newborn's intestine, results in a smaller number of anaerobic bacteria (e.g., *Bacteroidetes*) and a smaller diversity of microbiota, which can result in a significantly more frequent occurrence of atopic diseases[80] and metabolic disorders[81]. In an era of increasing numbers of CSs with no significant medical indication, the importance of perinatal microbiota of children is emphasized. Constant alertness of medical staff and parents is extremely important for shaping the intestinal flora and thus the health of society.

## Nourishment

The composition of milk, as the first food that children receive at birth, is critical to the functioning and colonization of the digestive tract. Food supplies not only nutrition, but also immunomodulatory substances and microorganisms capable of colonizing the intestine of a newborn. The microbiome of breast milk contains a number of species of bacteria typical for a given environment, which are subject to large variation depending on the period of lactation. Milk microbiota diversity increases in the first 6 months after giving birth[82].

The microbiota typical for colostrum has been found in the meconium of breastfed newborns[14]. Fragments of bacterial DNA (*Streptococcus thermophilus, Staphylococcus epidermidis, Bifidobacterium longum*) of breastmilk origin have been discovered in stools of breastfed infants[15]. There is a close resemblance between the bacterial flora of infant stools, female breast milk, and the nipple skin of nursing mothers[83], and the intestinal microflora of children fed with mother's milk is enriched in *Bifidobacterium* species[84]. *Bifidobacterium longum* rDNA sequences were also found in the blood of mothers, mother's stools, and milk[83]. Martín et al[84] suggested that mother's milk is the main source of gut *Lactobacilli* in newborns.

Intestinal bacteria utilize fiber as a source of energy for their own development. In the fermentation process, microbiota by-products are released close to the surface of the intestinal barrier which ensures proper development of the host. These metabolites include SCFA and vitamin B12, vitamin K and folic acid[67]. The main SCFA in breast-fed children is ethanoic acid, found to act as a stimulant for the growth of *Bifidobacterium* and *Lactobacillus* genera. A body of research[62] suggests that infants fed with mother's milk and infants born vaginally start to be colonized on the 6th day of life mainly by *Bifidobacterium*. In infants born prematurely, even with a relatively early supply of mother's milk, *Bifidobacterium* bacteria start to colonize the intestine after the 3rd week of life[85]. In a study by Lee et al[86], five basic colonizing bacteria were identified in four-week-old Korean newborns. These were *Streptococcus salivarius, Streptococcus lactarius, Streptococcus pseudopneumoniae, Bifidobacterium longum,* and *Lactobacillus gasseri*. Their presence was, however, independent of the type of feeding. They were therefore considered to be the indigenous commensal intestinal microbiota for infants of this particular descent. The feeding method influenced the counts of individual species. In breastfed infants *Streptococcus pseudopneumoniae, Bifidobacterium longum,* and *Lactobacillus gasseri* were found to be more abundant, while in infants fed with artificial milk *Streptococcus salivarius* and *Streptococcus lactarius* dominated.

The intestinal microbiota of children fed with artificial milk is much more diverse. It contains *Escherichia coli, Clostridium difficile, Bacteroides fragilis,* and *Lactobacillus*[62]. In infants fed by mixed methods (mother's and artificial milk) there

was a greater number of *Bifidobacterium* bacteria found in comparison to infants fed with artificial milk only[36]. Importantly, the composition of intestinal microbiota in infants fed only once a day artificially in the first week of life was similar to the gut microbiome structure found in infants fed exclusively artificially[87]. On the other hand, the intestinal microbiota of artificially-fed children was more stable and contained anaerobic bacteria.

In breastfed infants, aerobic bacteria predominate, and the microbiota in the first year of an infant's life is highly variable[86]. The introduction of solid foods into an infant's diet results in a gradual blurring of previous differences between microbiota composition (depending on method of feeding: natural *vs.* artificial) and start to resemble a pattern typical for adults[88]. During the period of weaning, there is a significant decrease in *Bifidobacterium* and *Bacteroides* and overgrowth of *Clostridium group* XIVa. The variation in the bacterial community is less spectacular in infants fed with artificial milk, but in the early period of weaning the amount of *Bifidobacterium* was also reported to decrease. The largest change in microbiota in these infants is attributable to the weaning period and the introduction of solid food. These events resulted in the elevation of *Escherichia coli* counts. These data were confirmed by Fallani et al[89] who examined stools of 531 children at 6 weeks of age and 4 weeks after the introduction of solid diet. The authors were able to demonstrate a tendency for a reduction in the abundance of *Bifidobacterium* and an increase in the population of *Clostridium coccoides* and *Clostridium leptum* after adult-type foods were administered. As evidenced by Pannaraj et al[82], after an infant is predominantly breastfed for a month, its gut may receive more than 25% of bacteria from breast milk and approximately 10% from areolar skin in a dose-dependent manner, even after expanding the nutritional repertoire with solid foods.

Following the development of nutrigenomics, many studies have evaluated the efficacy of enriching artificial milk with prebiotics, as substances that stimulate the development of normal intestinal flora. The main role is played by oligosaccharides, which, when supplemented in artificial milk, differ from the oligosaccharides of human milk. Intestinal bacteria use undigested colon prebiotic oligosaccharides to produce SCFAs that stimulate bowel movements. Expert opinion (ESPGHAN) suggests that adequate supplementation of short-chain galactooligosaccharides (GOS) and long-chain fructo-oligosaccharides (FOS) increases the amount of *Bifidobacterium* in the infant gut. Additionally, at least a few researches were able to show that artificial mixtures supplemented with oligosaccharides have the potential to modulate an infant's gut microbiota, in particular to increase the amount of *Bifidobacterium* in the first months of life[90].

Intestinal microflora is also affected by probiotics. In a randomized double-blind study in which 1310 premature newborns received *Bifidobacterium breve* BBG-001 or a placebo, a probiotic strain was found to be present in the gut of 85% of infants from the intervention group and 37% from placebo group. However, there were no differences in the diversity and richness of intestinal microbiota between studied groups[91].

## Antibiotic Therapy

The fetus is exposed to antibiotics either because of therapy of a pregnant mother, or because infants born *via* CS have perinatal prophylaxis against infection. Therefore, with an increasing percentage of surgical deliveries this has resulted in an increasing number of newborns treated with antibiotics. In a recent study pre- and postnatal antibiotic therapy decreased the *Bifidobacterium* abundance and gut microbiota of these ICU infants, namely *Klebsiella, Escherichia*/*Shigella*, and *Enterococcus*[92]. Surprisingly, the gut microbiota of infants treated with aminoglycoside and vancomycin was only affected temporarily and quantitatively, without influencing gut richness[93].

Newborns from women in whom vaginal colonization of *Streptococcus agalactiae* is found during pregnancy face antibiotic therapy. This problem affects about 30% of pregnant women. Following global recommendations, this group of women is also subjected to antibiotic prophylaxis before delivery[94]. Consequently, most of these newborns, with no signs of infection, are exposed to antibiotics even before birth. In another group of children, premature newborns, at least 40% have intrauterine infections[95]. In addition, in infants born prematurely, nosocomial infections are much more common, which also results in antibiotic therapy. Based on numerous studies[96-99] it has been shown that the use of antibiotics in the prenatal and neonatal period increases the risk for developing asthma in children, obesity, inflammatory bowel diseases and other inflammatory, and/or allergic diseases. There is a hypothesis that the development of these diseases may result from disturbances in colonization of the gastrointesti-

nal tract in the earliest period of life[69]. Based on two cohort studies conducted in newborns born at term and naturally, a decrease in bacterial diversity and a reduction in *Actinobacteria* and *Bacteroidetes* in children's stools were found when antibiotic perinatal prophylaxis was implemented. In this group of children, a smaller amount of *Bifidobacteriacae* was also observed, and increased *Firmicutes* and *Proteobacteria* counts[94,99,100].

### Geography

The development and diversity of intestinal microbiota seem to be closely related to location. Researchers conducted a multi-center study among 606 infants from five European countries: Sweden, Scotland, Germany, Italy, Spain[89], and assessment of stool microbiota of six-week-old children by means of FISH and flow cytometry techniques was carried out. Feces of children born in northern countries contained significantly higher numbers of bacteria of the *Bifidobacterium* genus. The gut microbiota of children living in southern European countries was more diverse and the *Bacteroidetes* phylum was overrepresented. The study excluded the influence of the type of feeding when evaluating the results by means of PCAs - principal component analyses[89].

In another cohort study, it was noted that regional differences in gut microbiota do occur but depend on age[7]. The study involved 531 persons aged 0 to 70 years. The children qualified for the study were exclusively fed with mothers' milk. The author showed that gut microbiota typical for a US population was less diverse compared to the inhabitants of Venezuela and Malawi, but the differences were significant only in the group of children over 3 years of age and in adults. The authors also emphasized that the composition of intestinal microbiota was strongly associated with genes responsible for the metabolism of cobalamin (vitamin B12) and folic acid.

### Siblings and Pets

Penders et al[62] showed that there are higher *Bifidobacterium* counts in guts of children who have siblings. The authors of one study[100], by means of qPCR, analyzed a cohort of 24 children and reported a relationship between microbiota composition and both siblings and pets. Researchers were able to show that the gut microbiota of children with siblings (13 children) contained fewer *Peptostreptococcus* (phylum *Firmicutes*) bacteria, and the intestinal microflora was less rich. In contrast, gut microbiota of children with a pet (15 children) was found to be of increased richness and contained more *Peptostreptococcus* bacteria and less *Bifidobacterium*. The population of *Peptostreptococcaceae* increased, especially if the pet was a domestic cat.

## Conclusions

The human digestive tract is inhabited by an ecological community unique for each individual. It consists primarily of *bacteria*, but also *fungi*, *arechaea*, *viruses*, and *eukaryota*. This biocenosis is referred to as the microbiome or microbiota. Intestinal microorganisms co-evolved with *Homo sapiens*, suggesting that many physiological processes might be conditioned by their presence. Intestinal microbiota is involved in metabolic, trophic, and immunological functions and, importantly, the products of particular biochemical transformations may serve as substrates for subsequent reactions.

It seems that the intestinal flora is permanently programmed in early childhood. However, there are many environmental factors that can have a significant impact on the variability of intestinal microbiota in adulthood. The intestinal microbiome consists of approximately 1000 species of bacteria and is unique for each of us. The main factors affecting the intestinal ecosystem in adulthood include food and medicines. The "westernization" of life through consumption of processed food, small amounts of vegetables and fruits, and high content of simple sugars, has impoverished intestinal flora[101]. A diet rich in proteins was found to contribute to a reduction in the amount of butyrate-producing bacteria (e.g., *Bifidobacterium)* that inhibit histone deacetylation[102]. The second main factor having a destructive effect on intestinal flora is drugs such as proton-pump inhibitors, H2-blockers, antibiotics, prokinetics, opioids, laxatives, and statins[103]. Although the composition of intestinal microbiota is subject to continuous and dynamic changes throughout life, it seems that the perinatal period is critical to the emergence of its proper pattern, and may be a guarantor of health or illness in adult life. We need to remember, therefore, that the programmed "indigenous" microbiotic pattern in childhood should be nurtured throughout life.

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### Conflict of Interests

The Authors declare that they have no conflict of interests.

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