Review

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Gut microbiota of full-term and preterm newborns: role in the development of infectious diseases and possibilities for correction (literature review)

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ABSTRACT. ***Introduction.*** The study of the gut microbiota in newborns is an important area of modern medicine, as its development affects immunity, metabolism, and overall child health. This issue is particularly relevant for preterm infants, whose microbiota maturation is delayed, increasing the risk of infections and chronic diseases. The composition and functional state of the microbiota are significantly influenced by the mode of delivery, antibiotic therapy, and type of feeding, although these factors require further investigation. ***Aim*** – to systematize current data on the factors affecting the intestinal microbiota of full-term and premature newborns, and methods of its correction. ***Materials and methods.*** This article presents a review of scientific literature, including clinical studies and systematic reviews. Data were analyzed regarding methods of microbiota assessment, and the influence of delivery mode, antibiotic therapy, breastfeeding, formula feeding, and treatment with prebiotics and probiotics on gut microbiota composition. ***Results.*** A healthy gut microbiota in newborns plays a crucial role in preventing infectious diseases. Disturbances of the intestinal microbiota caused by cesarean section, antibiotic therapy, and formula feeding are associated with reduced colonization resistance and the proliferation of opportunistic bacteria (*Klebsiella* spp., *Enterococcus* spp., *Pseudomonas* spp.), increasing the risk of sepsis and necrotizing enterocolitis. The use of probiotics and prebiotics helps reduce infectious morbidity by restoring microbial balance and strengthening the intestinal barrier, making microbiota correction an important direction in the prevention and treatment of neonatal infections. ***Conclusion.*** The formation of a healthy gut microbiota in newborns is critical for their long-term health. Current evidence highlights the importance of vaginal delivery, breastfeeding, and rational antibiotic use in the care of preterm infants. Probiotics represent a promising approach for correcting microbiota disturbances in newborns; however, further studies are needed to optimize their use.

KEYWORDS: *intestinal microbiota, newborns, preterm infants, infectious diseases, probiotics*

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Микробиота кишечника доношенных и недоношенных новорожденных: роль в развитии инфекционных заболеваний и возможности коррекции (обзор литературы)

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РЕЗЮМЕ. ***Введение***. Изучение микробиоты кишечника новорожденных является важным направлением современной медицины, так как ее становление влияет на иммунитет, метаболизм и здоровье детей. Особенно актуальна эта проблема для недоношенных детей, у которых замедлено развитие микробиоты, что повышает риск инфекций и хронических заболеваний. На состав и функциональное состояние микробиоты существенно влияют способ родоразрешения, антибиотикотерапия и тип вскармливания, хотя это и требует дальнейшего изучения. ***Цель*** — систематизировать современные данные о факторах, влияющих на микробиоту кишечника доношенных и недоношенных новорожденных, и методах ее коррекции. ***Материалы и методы.*** В статье проведен обзор научной литературы, включая клинические исследования и систематические обзоры. Использованы данные о методах изучения микробиоты, влиянии на микробиоту способа родоразрешения, антибиотикотерапии, грудного вскармливания и искусственных питательных смесей, лечения пребиотиками и пробиотиками. ***Результаты.*** Здоровая кишечная микробиота новорожденных играет значительную роль в профилактике инфекционных заболеваний. Нарушение микробиоты кишечника, вызванное кесаревым сечением, антибиотикотерапией и искусственным вскармливанием, сопровождается снижением колонизационной резистентности и ростом условно-патогенных бактерий (*Klebsiella* spp., *Enterococcus* spp., *Pseudomonas* spp.), что повышает риск развития сепсиса и некротизирующего энтероколита. Применение пробиотиков и пребиотиков способствует снижению инфекционной заболеваемости за счет восстановления микробного баланса, укрепления барьерной функции кишечника, что делает коррекцию микробиоты важным направлением профилактики и терапии инфекций у новорожденных. ***Заключение***. Формирование здоровой микробиоты у новорожденных критически важно для их долгосрочного здоровья. Современные данные подтверждают значимость естественных родов, грудного вскармливания и рационального использования антибиотиков при выхаживании недоношенных детей. Пробиотики представляют перспективное направление для коррекции нарушений микробиоты новорожденных, однако необходимы дополнительные исследования для оптимизации их применения.

КЛЮЧЕВЫЕ СЛОВА: *микробиота кишечника, новорожденные, недоношенные дети, инфекционные заболевания, пробиотики*

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INTRODUCTION

The development of gut microbiota in newborns is one of the most promising yet under-researched areas of modern medicine. Although recent studies by scientists such as Y. Chen et al. (2021) have significantly advanced our understanding of the microbiota's impact on human health, many aspects of its impact on newborns remain unclear [1, 2].

The book by V.P. Novikova et al., "The Gut Microbiota as a Regulator of Human Organs and Systems" (2025), demonstrates that gut microbial communities participate in shaping the immune response, regulating inflammatory processes, metabolism, and neurohumoral interactions. Disruptions in their composition are associated with infectious, inflammatory, and metabolic diseases. During the neonatal period, particularly in preterm infants, microbial immaturity and its high sensitivity to external factors determine an increased risk of infectious complications. Therefore, the establishment and regulation of a healthy gut microbiota represent a priority in modern medicine [3].

There are a number of gaps in our understanding of mechanisms influencing delivery methods, dietary characteristics, and the effects of antibiotic therapy on microbiota development during the neonatal period. Furthermore, long-term consequences of impaired intestinal microbiota development, including autoimmune and metabolic diseases, remain poorly understood [4].

Current approaches to restoring and maintaining a healthy microbiota in preterm infants involve the use of probiotics and prebiotics. However, methods for their administration are not yet fully developed, although the results of studies examining their effect on the gut microbiota in infants are encouraging [5–7].

Thus, this topic is important due to its role in preserving children's health, as well as the need to develop effective approaches in order to prevent and treat diseases caused by an imbalance in the microbiota of newborns, which should help reduce morbidity and improve the quality of life for infants.

METHODS FOR ANALYZING THE GUT MICROBIOTA ARE RESEARCH TECHNIQUES, STANDARDIZATION OF SAMPLE COLLECTION AND STORAGE CONDITIONS

Gut microbiota profiling provides an assessment of the qualitative and quantitative composition of the microbial community, as well as its functional capabilities [8]. Traditionally, intestinal microbiota is studied by isolating and culturing bacteria. However, there are difficulties in culturing anaerobic microorganisms, which significantly reduce reliability of the results. Metagenomic sequencing analyzes the DNA of both cultivable and uncultivable microorganisms based on 16S rRNA gene sequencing. The application of next-generation sequencing (NGS) technologies for microbiome research is particularly interesting. The real-time polymerase chain reaction (PCR) allows for determining representation of bacteria of a single species or closely related species using taxon-specific primers. DNA fingerprinting provides a semi-quantitative assessment of the overall diversity of the bacterial community of gut microorganisms. Functional metagenomic profiling provides quantitative information on the relative abundance of individual genes, gene clusters, and metabolic pathways. Microbiome profiling results are used to assess the pathogenic burden of dysbiosis, predict its consequences, and develop targeted measures to correct microbiome imbalances.

Fecal samples are most commonly used in studies of the gut microbiota composition. However, it is important to note that the microbial community in the mucosa of different sections of the colon and in feces differs [9].

Since fresh fecal samples are not always analyzed immediately, there is a need to store them. Freezing at –80 °C without preservatives preserves the microbial composition and is considered the "gold standard" for microbiome studies [10]. Large-scale population studies typically employ optimal sample collection methods; however, maintaining a temperature of –80 °C is not always feasible. This necessitates the search for alternative methods that minimize analytical errors. Swallowed capsules for sample collection are considered one of the most promising options, as they ensure high accuracy and sensitivity of the research data [11].

In addition to freezing, there are other sample storage methods (with or without preservatives) that allow the microbiota to be preserved in a state close to fresh samples. These are fecal occult blood test cards, FTA (Fast Technology for Analysis of Nucleic Acids) cards, Whatman® FTA® cards), and the Genotek DNA kit, which enables the development of microbiome-based clinical

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applications using OMNIgene™ GUT Dx—the first and only FDA-approved (Food and Drug Administration, U.S. Department of Health and Human Services) device for self-collection of stool samples. These methods have now proven effective for storing samples for several days at room temperature [12]. For stable storage of stool samples, 95% ethanol or RNAlater sample preservation solution is recommended as a preservative [13].

G.M. Williams et al. (2019) recommend to collect the first samples within the first 24 hours of life (the first stool, meconium), and then to repeat the collection on days 1, 3, 7, 14, and 30. Collecting meconium allows for assessing initial sterility or early intestinal colonization. However, the bacterial load in meconium is very low, and it is not always possible to obtain a sufficient amount of DNA for sequencing. On the 1st–3rd day of life, after contact with a mother and environment, active intestinal colonization begins. Microbiome studies on days 7, 14, and 30 allow for tracking the dynamics of microbiome formation and the influence of external factors (mode of delivery, feeding methods, etc.) [14].

FORMATION OF THE PRIMARY GUT MICROBIOTA IMMEDIATELY AFTER BIRTH AND ITS CHANGES IN NEWBORNS AND INFANTS

The development of the gut microbiota during the neonatal period is characterized by significant changes in taxonomic composition and biodiversity. This process is determined by a complex of factors: the gestational age at birth, mode of delivery, characteristics of the mother's diet and health, type of feeding, use of antibiotics in premature infants, as well as social and environmental conditions (family members, presence of siblings and pets).

The existence of microbial colonization of the human gut before birth remains a controversial topic. For a long time, the scientific community held the view that the amniotic cavity was "sterile." However, recent years have seen the detection of protein markers of microbial colonization in amniotic fluid from women with preterm rupture of membranes using an antibody microarray via enzyme-linked immunosorbent assay (ELISA) and protein and antibody microchip technology, which supports the possibility of fetal intestinal colonization by maternal microorganisms [15].

The results of microbiological studies of the human placenta are contradictory. Data from K.M. Kennedy et al. (2023) and I. Sterpu et al. (2021) did not confirm the presence of placental microbiota. The authors believe that their findings are most likely the result of "contamination of the material under study", when obtaining samples of fetal tissue during clinical procedures or during DNA extraction and sequencing [16, 17]. At the same time, data from A. Stupak et al. (2024), based on studies of placental proteins obtained during childbirth using liquid chromatography coupled with mass spectrometry, indicated the possible presence of a number of proteins specific to bacteriophages and viruses, whose role as early markers of fetal infection has not been definitively established [18].

Research by Q. He (2020) detected the presence of bacteria in the meconium of newborns, indicating that the intestine is not sterile prior to birth. In the first days and months of life, the number and diversity of bacteria in children's feces were low, but shortly after complementary feeding began and against the backdrop of active physical development, a rapid increase in the number of bacteria and an expansion of their diversity were observed [19].

Composition and functions of the mother's gut microbiota directly influence fetal and newborn microbiota development. Experimental studies in germ-free animals and clinical observations in humans have confirmed that the mother's microbial profile during pregnancy determines the development of the fetus's immune system and its resistance to infections after birth. Disruptions in the mother's microbiota are associated with an increased risk of intrauterine inflammatory reactions and infectious complications in newborns. It has been shown that the intestinal microbiota participates in shaping the fetal "gut-brain axis," mediating the maturation of immune and neuroregulatory mechanisms that provide anti-infectious protection in the early postnatal period [20].

Initially, the gut is colonized by microbes acquired from the mother and the environment. Children born naturally are dominated by bacteria from the mother's birth canal, such as *Lactobacillus spp., Prevotella* spp., and *Sneathia* spp., whereas children born via cesarean section are predominantly colonized by "skin" microorganisms, including *Staphylococcus* spp. and *Propionibacterium*

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spp. During the first week of life, bacteria that are adapted to the intestinal environment predominate, including *Bifidobacterium* spp., *Bacteroides* spp., and *Escherichia coli* spp. [21].

A single-study analysis of the gut microbiota in 11 healthy infants in their first year of life, conducted by N.V. Gonchar et al. (2017) using sequencing of amplified *16S* rRNA gene regions, revealed a predominance of Gram-positive anaerobic bacteria (71±23%). The quantitative distribution of phyla decreased in the following order: the phylum *Firmicutes* – 43±15% (represented by *Clostridium* spp., *Blautia* spp., *Lactobacillus* spp., *Enterococcus* spp., and *Veillonella* spp.), the phylum Actinobacteria – 38±10% (more than 90% of OTUs represented by *Bifidobacterium* spp.), and the phylum *Proteobacteria* – 15±8% (represented by the family *Enterobacteriaceae*). Representatives of the phylum Bacteroidetes were identified (7–15%) in three samples only. All samples were characterized by low species diversity; the Shannon index and α-diversity criterion ranged from 1.5 to 4.2 and 3 to 20, respectively. Breastfed infants had a higher abundance of *Proteobacteria* compared to formula-fed infants. The influence of adverse factors during the mothers' pregnancies (antibiotic use, respiratory infection) on infants' gut microbiota composition was noted [22].

According to data from A.V. Kaplina et al. (2023), intestinal dysbiosis may serve as a predisposing factor for necrotizing enterocolitis. Antibiotic therapy leads to a predominance of facultative anaerobes, such as *Enterococcus* spp. and *Enterobacteriaceae*, as well as a decrease in *Bifidobacteriaceae* in the gut microbiome of preterm newborns. Antimicrobial therapy disrupts the physiological process of colonization, and the formation of normal microbiota (its "maturation") may be delayed. However, they note that dysbiosis is not the primary cause of necrotizing enterocolitis, and the mother's health status, as well as the characteristics of fetal development and the early postnatal period, play a significant role [23].

S. Graspeuntner et al. (2024) investigated the gut microbiota in infants under 90 days of age with late-onset sepsis using 16S rRNA sequencing of stool samples. They found a decrease in the number of *B. longum* and an increase in the number of *Bacteroidia* spp. in patients with lactic acidosis upon hospital admission. Authors suggested that previously healthy infants developing sepsis exhibited gut microbiota dysbiosis combined with undefined specific immunological patterns. The study demonstrated that the healthy gut microbiota of infants is characterized by the absence of overgrowth of opportunistic microorganisms (OM). Furthermore, it plays a crucial role in the development and stable functioning of the immune and digestive systems [24].

A dissertation study by G.N. Chistyakov (2024) identified informative immunological markers such as absolute white blood cell count, relative counts of CD14+HLA-DR+ and CD14+CD11b+ monocytes in umbilical cord blood. These markers make it possible to predict the risk of intestinal colonization by *K. pneumoniae*, an opportunistic pathogen from ESKAPE group in premature infants during the neonatal period [25].

A systematic literature review by C. Alcazar et al. (2022) attempted to confirm the association between the gut microbiota in infants aged 0 to 12 months, assessed using genomic sequencing, and respiratory diseases of infectious and allergic nature in children under 18 years of age. The authors suggest that low α-diversity of the microbiota and the relative abundance of certain genera of commensal gut bacteria (*Bifidobacterium, Faecalibacterium, Ruminococcus*, and *Roseburia*) are associated with respiratory diseases in children. However, the study results were contradictory, as there were significant limitations, including insufficient characterization of bacterial taxa at the species level, inconsistent interpretation of the data, residual confounding factors, and a small sample [26].

A study by Y. Gao et al. (2021) provides evidence that the maternal gut microbiome influences the risk of allergic diseases and asthma in children. Similarly, a study by X. Yuan et al. (2022) demonstrates a link between gut microbiota dysbiosis in early childhood and an increased risk of developing metabolic diseases, such as obesity and type 1 diabetes [27, 28]. These findings have served as the basis for developing therapeutic strategies aimed at early correction of gut dysbiosis using probiotics and prebiotics [29].

As shown in the study by Q. Jia et al. (2022), preterm newborns exhibit slower gut microbiota establishment compared to term infants, accompanied by higher species diversity of the gut microbiota. These findings provide new insights

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into the need for individualized interventions to modulate the microbiota composition regarding varying gestational ages at different periods after birth [30].

Y. Chen et al. (2023) analyzed changes in microbial composition in fecal samples from 92 preterm infants treated in the intensive care unit, starting at birth and continuing at 1, 3, 7, 14, 21, 28, and 60 days of life using 16S rRNA sequencing. The results showed that the main types of gut bacteria in preterm infants were *Proteobacteria, Firmicutes, Actinobacteria, Tenericutes*, and *Bacteroidetes*, collectively accounting for 99.7% of the microbial community. *Pseudomonas* spp., *Staphylococcus* spp., and *Klebsiella* spp. predominated at the genus level, along with unclassified members of the *Enterobacteriaceae* family and the *Clostridium-sensu-stricto-1* group, which together accounted for 90.9% of the total microbiota. The abundance of *Proteobacteria*, including the genus *Pseudomonas*, initially decreased, reaching a minimum on the 14th day of life, after which it increased again. Concurrently, there was a decrease in the number of *Firmicutes*. At the same time, in formula-fed infants, the proportion of *Proteobacteria* (at the phylum level) and *Streptococcus* spp. (at the genus level) was significantly higher compared to breastfed infants [31].

The study by C. Chi et al. (2019) aimed to compare gut microbiome composition in infants with very low birth weight (VLBW) and normal birth weight (NBW) during the first three months of life using 16S rRNA gene sequencing. Fecal samples were collected at 0 and 3 days, 2 and 6 weeks, and 3 months of age (defined as microbiota development stages 1–5). The authors found that α-diversity decreased over time in both groups. The Shannon index revealed significant differences between the groups at stages 1–3. The structure of the gut microbiota in LBW infants differed significantly from that in NBW infants throughout the entire observation period. In children with LBW, an increase in Firmicutes and a decrease in *Proteobacteria* were observed; at birth, *Enterococcus* spp., *Klebsiella* spp., and *Streptococcus* spp. predominated, whereas *Haemophilus* spp. were underrepresented [32].

E.W. Blakstad et al. (2019) investigated the effect of feeding practices on the development of healthy gut microbiota in infants with very low birth weight (VLBW) and extremely low birth weight (ELBW). Fifty preterm newborns were divided into an experimental group receiving enhanced nutrition and a control group. Fecal samples from 45 infants were analyzed using 16S rRNA sequencing. The group receiving enhanced nutrition showed a more significant increase in gut microbiota diversity and a decrease of staphylococci, while no increase in the number of pathogenic microorganisms was observed. The relative abundance of bifidobacteria increased more in the control group, where better weight gain was also observed. In preterm newborns, the establishment of the *Bifidobacterium* spp. population was characterized by delayed colonization and low abundance, requiring more time to achieve an optimal composition [33].

DELIVERY METHOD AND ITS IMPACT ON GUT MICROBIOTA IN NEWBORNS

A systematic review by L. Princisval et al. (2021) showed that neonates born vaginally acquire a gut microbiota in the first weeks of life that is similar in composition to their mother's. At the same time, newborns delivered via cesarean section have a gut microbiota composition that more closely corresponds to the environment. During the first 90 days of life, infants born via vaginal delivery exhibit increased bacterial diversity in the *Actinobacteria* and *Bacteroidetes phyla*, including *Bifidobacterium* spp. and *Bacteroides*, compared to those born via cesarean section [34].

According to a study by Y. Chen et al. (2021), infants born via cesarean section exhibited a decrease in α-diversity of the microbiota. A particularly notable decrease was observed in the abundance of *Bifidobacterium* spp. during the first three months of life, whereas representatives of the genera *Enterococcus* spp., *Klebsiella* spp., *Streptococcus* spp., *Haemophilus* spp., and *Veillonella* spp. demonstrated active colonization from the first week of life up to one month. Among bacterial families, *Veillonellaceae* and *Enterobacteriaceae* were dominant [35].

The lack of contact with the maternal vaginal microbiota in children born via cesarean section is considered one of the reasons for the increased prevalence of bacteria from the *Firmicutes* phylum and reduced colonization by mem-

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bers of *Bifidobacterium* spp. and *Bacteroidetes*. All of these are crucial for carbohydrate digestion and the development of the immune system. This change is associated with a reduced ability of the microbiota to metabolize carbohydrates, including oligosaccharides found in breast milk [36].

The work of L. Li et al. (2021) shows that elective cesarean section is considered the most important factor that can delay the onset of breastfeeding and shorten its duration [37]. Research by L.M. Mallick et al. (2024) also shows that women who underwent both elective and emergency cesarean sections are generally more likely to discontinue breastfeeding than women who gave birth vaginally [38]. This negatively affects the diversity and structure of infant gut microbiota, reducing the colonization of *Lactobacilli* spp. and *Bifidobacterium* spp., and increases the risk of developing autoimmune diseases (inflammatory bowel disease, rheumatoid arthritis, celiac disease) and metabolic disorders due to a feedback mechanism between systemic inflammation and dysregulation of the gut microbiota [36].

FEEDING OF NEWBORNS IN RELATION TO THE INTESTINAL MICROBIOTA

Active research continues, focusing on how microbial populations develop in the intestines of infants depending on their age and feeding method. N. Chin et al. (2021) found that adding formula to breast milk affects the gut microbiota and the immune system of infants. The study included 24 infants who, by one month of age, were either exclusively breastfed or formula-fed. The analysis involved 16S rRNA gene sequencing of stool samples and flow cytometry of blood samples from birth to six months of age, as well as classification of *Bifidobacterium* spp. strains. The results showed that the addition of formula caused temporary changes in microbial composition, which disappeared by six months of age. At the same time, *B. longum*, a species associated with breastfeeding, decreased significantly upon formula introduction during the first month of life. No significant immunological differences, including the development of T- and B-cell subpopulations or monocytes, were identified [39].

L. Beller et al. (2021) conducted a study of changes in microbiota composition during the first year of life in 8 infants, analyzing 303 samples. In order to assess the transition from infant to adult microbiota, the authors compared the obtained data with samples from participants in the "Flemish Gut Microbiota" project (1,106 samples). Formula feeding reduced the likelihood of *Bifidobacterium* spp. presence compared to breastfeeding. In some infants, *Bifidobacterium* spp. appeared only after the introduction of solid foods, whereas in their absence, *Bacteroides, Clostridia*, and *E. coli* typically dominated [40].

A study by I. Cuxart et al. (2022) provides additional data on the enzymatic processing of oligosaccharides by the gut microbiota in the first months of life. Breast milk oligosaccharides (BMOs), such as galacto-oligosaccharides, play a leading role in microbiota development; these are partially digested in the small intestine and fermented in the large intestine, promoting the growth of *Bifidobacterium* spp. It has been established that lacto-N-biosidase (LnbB), produced by *B. bifidum*, plays a crucial role in the breakdown of human milk oligosaccharides in the intestines of breastfed infants [41]. This process leads to the formation of short-chain fatty acids; the latter inhibit opportunistic pathogens, including members of the *Clostridiaceae, Enterobacteriaceae*, and *Staphylococcaceae* families. Breastfed infants show an increase in *Bifidobacterium* spp., a decrease in BMO in stool, and elevated levels of acetic and lactic acids. These findings confirm the prebiotic effect of breast milk, which promotes the selective enrichment of the microbiota with *Bifidobacterium* spp. [42].

Breast milk is referred to as a complex food matrix with numerous components that can potentially influence the composition of an infant's microbiota, promoting the growth of indigenous bacteria and limiting the growth of opportunistic pathogens. It has also been shown that *Bifidobacterium* spp., particularly *B. infantis*, correlate with increased levels of secretory immunoglobulin A (sIgA), ensuring adequate local immunity in the gut. The synergistic effects of BMO and *Bifidobacterium* spp. on infants promote the diversity and maintenance of gut microbiota balance, as well as the strengthening of the immune system [43].

Research by G. Boudry et al. (2021) indicates that breastfeeding in early life opens a "window

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of opportunity" for health development through microbial modulation. The authors described the composition of breast milk and established the role of its components in shaping the gut microbiota. Among the most frequently mentioned genera, *Staphylococcus* spp. and *Streptococcus* spp. were identified as universally predominant in breast milk. Other bacterial genera are also frequently mentioned, including *Corynebacterium* spp., *Bifidobacterium* spp., *Propionibacterium* spp., *Bacteroides* spp., *Enterococcus* spp., *Faecalibacterium* spp., *Lactobacillus* spp., *Veillonella* spp., *Serratia* spp., *Ralstonia* spp., *Acinetobacter* spp., *Rothia* spp., and several representatives of the families *Lachnospiraceae* and *Ruminococcaceae* [44]. Studies by J.M. Vélez-Ixta et al. (2024) show that breast milk-mediated colonization is a specialized pathway through which bacteria selectively migrate into the newborn's gut, playing a crucial role in its development, as breast milk contains a wide range of microorganisms, including "skin" and "non-skin" Gram-positive bacteria [45].

Studies by V. Notarbartolo et al. (2022) have established that breast milk contains not only bacterial but also viral, fungal, and archaea components. The composition of breast milk can be influenced by various factors, such as the mother's body mass index and diet, antibiotic use, the timing and method of delivery, as well as the method of breastfeeding. At the same time, indigenous microorganisms such as streptococci (*S. mitis* and *S. salivarius*) and coagulase-negative staphylococci, which predominate in breast milk, compete for space and resources with opportunistic microorganisms, such as *S. aureus* [46].

K. Ingram et al. (2024) analyzed 46 breast milk samples from 15 women, collected 1, 4, 7, and 10 months postpartum. The authors used a metagenomic approach to assess the bacterial microbiota and found that microbiota composition in breast milk changes at different stages of lactation. They believe that data on breast milk bacteria can be used to predict and reduce health risks for infants [47]. The results of a study by Y. Komatsu et al. (2022) established that oligosaccharides disappear from infants' feces at the beginning of the fourth month of breastfeeding, when a shift in the species of Bifidobacterium occurs (from *B. breve* to *B. longum* subsp. *infantis*). This process is accompanied by fluctuations in the levels of several metabolites in infant feces, including acetate and butyrate. Consequently, breast milk components modulate gut microbiota composition and metabolite production (e.g., short-chain fatty acids) and may indirectly influence the immune and metabolic responses of infants [48].

Although breastfeeding is the gold standard for infant nutrition, there is a growing need to devote more effort to the production of infant formula, which must closely resemble the composition of breast milk and act similarly. One way to achieve these goals is to add various bioactive ingredients to formula, including probiotics, prebiotics, vitamins, and trace elements. Scientific data on beneficial effects of infant formula indicate its overall positive impact on the gut microbiota and its metabolic activity [49].

ANTIBIOTIC THERAPY AND GUT MICROBIOTA OF NEWBORNS

Antibiotics remain a key method for treating infectious diseases. However, irrational use of such drugs, particularly during pregnancy and in the first months of a child's life, can lead to adverse long-term consequences. These include developing resistance to antibiotics, as well as persistent disruptions in the structure and activity of gut microbiome, which may negatively affect health in future [50].

T.H. Dierikx et al. (2020) found that antibiotic therapy in mothers before or during childbirth negatively affects intestinal microbiota establishment in newborns, reducing microbial diversity, lowering the proportion of *Actinobacteria* and *Bacteroidetes*, and increasing *Proteobacteria*. It has been shown that these changes in the gut microbiota are distinctly pronounced in children born vaginally and may persist for up to 12 months of life [1].

According to data from C. Argentini et al. (2022), as well as C. Ozkul et al. (2020), antibiotic therapy in newborns, particularly the combination of amoxicillin and clavulanic acid, significantly disrupts gut microbiota, reducing its species diversity and triggering metabolic disorders and dyspeptic symptoms [51, 52]. At the same time, since resistance to amoxicillin among *Bifidobacterium* spp. is rarely observed (and this is considered a species-independent trait), its use reduces the potential influence of bifidobacteria [53].

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Intrapartum antibiotic therapy has become a common practice in obstetrics and is used in 40% of assisted deliveries. A systematic review by P. Zimmermann and N. Curtis (2020) demonstrated how ampicillin, prescribed to pregnant women with a positive GBS status (*group B Streptococcus*), negatively affects the gut microbiota of infants. The authors identified six studies involving 272 infants. A total of 502 stool samples were analyzed from children under 3 months of age. Infants exposed to intrapartum antibiotic therapy for group B streptococcal infection had fewer bacteria in their gut microbiota, fewer actinobacteria, particularly bifidobacteria, and more proteobacteria compared to infants who did not receive intrapartum antibiotic treatment [54]

Understanding the impact of widely used antibiotic therapy on the gut microbiota and butyrate-producing microorganisms in infants can aid in making therapeutic decisions in neonatal intensive care units. According to data from J. Qiu et al. (2025), antibiotic therapy in newborns negatively affects the initial development of the gut microbiota: it leads to a decrease in diversity, a reduction in the relative abundance of butyrate-producing bacteria (especially *Clostridiaceae*), and an increase in *Enterococcidae* [55].

According to R. Ventin-Holmberg et al. (2022), even a single course of antibiotics during the period of gut microbiota formation in infants caused a shift in the fungal composition of the gut. The shift was characterized by an increased relative abundance of *Candida* as well as diversity and abundance of fungal species, which may be one of undesirable long-term health effects of antibiotics [56].

EFFECTS OF PROBIOTICS IN NEWBORNS, INCLUDING PRETERM NEONATES

There is ongoing discussion regarding differences in gut microbiota between breastfed and formula-fed infants. It is hypothesized that these differences partly explain why breastfeeding is associated with a reduced risk of numerous infectious and non-infectious diseases in early life. Recent studies by T.K. Das et al. (2022) and A. Blanchetière et al. (2023) focus on the effects of probiotic microorganisms, including members of the genera *Lactobacillus* spp., *Bifidobacterium* spp., and *Saccharomyces* spp. *Lactobacillus* spp. is the most studied and widely used bacteria in neonatology. The beneficial effects of *Lactobacillus* are associated with their ability to enhance colonization resistance, inhibit the growth of opportunistic bacteria by producing organic acids and bacteriocins, and modulate the innate and adaptive immune response. Thus, it reduces the risk of infectious diseases and necrotizing enterocolitis in preterm newborns. At the same time, the literature describes potential negative effects, which are most often associated with transfer of genetic determinants of antimicrobial resistance from *Lactobacillus* to Gram-negative microbiota. Furthermore, heterogeneity of *Lactobacillus* strains and the lack of a unified classification complicate clinical data interpretation, underscoring the need for rigorous strain selection and an individualized approach to their use in neonatal practice [6, 7, 57].

Studies by E.C. Davis et al. (2022) and Q.S. Damaceno et al. (2023) explore opportunities for using probiotic bacteria isolated from breast milk to develop new infant formulas [58, 59]. Based on the results of these studies, the strains *Lacticaseibacillus rhamnosus* and *Leuconostoc mesenteroides* have been proposed as potential probiotics in a formula mimicking breast milk [59].

Studies by M. Nguyen et al. (2023) provide a strong rationale for using probiotics in neonatal intensive care units. They found that premature infants who received the activated strain *B. infantis* EVC001 at a dose of 8×10^9 CFU per day rapidly developed a high degree of *Bifidobacteriaceae* colonization, the majority of which consisted of *B. infantis*. There was also a decrease in the number of antibiotic resistance genes and a reduction in the number of taxa containing them, such as *Enterobacteriaceae* representatives like *E. coli* and *K. pneumoniae*. Children receiving *B. infantis* EVC001 demonstrated reduced levels of enteritis inflammation markers, including calprotectin, IL-8, IL-17A, and TNFα [60].

Experience of using probiotic Enterococcus strains in long-term nutritional support for preterm infants also appeared to be successful. N.V. Gonchar et al. (2021) confirmed the efficacy and safety of long-term use of the domestic strain *E. faecium* L3 with a titer of at least 10^8 CFU/mL, which was administered orally to infants at a dose of 1 mL twice daily during meals.

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Probiotic nutritional therapy for infants with the *E. faecium* L3 strain, as part of a rehabilitation program for preterm newborns whose inpatient care had already concluded, demonstrated the potential to improve the nutritional status and neurocognitive development of preterm infants [61].

According to data from G.C. Deshpande et al. (2011), the greatest efficacy of probiotics is observed in the first week of life, provided the infant is ready for enteral feeding. Early administration of probiotics significantly reduces all-cause mortality and the incidence of necrotizing enterocolitis in preterm infants and contributes to improved food tolerance [62].

Research by N. van Best et al. (2020) showed that probiotics may be most effective in preterm newborns before stable colonization of the gut microbiota is established. Administration of probiotic formulas in the experimental group was associated with a higher relative abundance of *Bifidobacteria* and *Enterobacteria*, combined with a lower abundance of *Escherichia*, *Enterococcus*, and *Klebsiella*. Probiotic *Bifidobacteria* colonization was observed in 50% of infants, although it was often transient. Newborns receiving probiotic formulas showed a significant reduction in the incidence of necrotizing enterocolitis [63].

In a randomized intervention study by J. Samara et al. (2022) examining the effect of a probiotic product containing four strains of *Bifidobacterium* and one strain of *Lacticaseibacillus*. The researchers evaluated its impact on composition and functional trajectory of the gut microbiota in preterm infants who received the product daily starting at the onset of feeding until a gestational age of 36–37 weeks. The results demonstrated an accelerated transition to a mature microbiota similar to term infants. The results of this study indicate that *Bifidobacterium* strains, acting as "ecosystem engineers," accelerate microbiota maturation and exert an immunological effect on extremely preterm infants [64].

Currently, the use of probiotic supplements in preterm infants is considered a promising method for achieving initial "healthy colonization" of the gut during the neonatal period. Clinical data confirm their beneficial long-term effects, including anti-inflammatory effects [65, 66]. A systematic review by V.K. Batta et al. (2023), encompassing 67 randomized controlled trials, and a meta-analysis by H. Wang et al. (2023) of 70 studies showed that the use of probiotics in preterm newborns, especially those containing *B. infantis* strains, significantly reduces the risk of necrotizing enterocolitis and late-onset sepsis, thereby lowering the overall mortality rate in preterm infants [67, 68].

Despite the encouraging results obtained from the use of probiotics in newborns, questions remain unresolved regarding the algorithm for selecting strains, dosages, and regimens for probiotic use.

CONCLUSION

The gut microbiota plays a crucial role in protecting infants from infectious diseases from the very first days of life. The formation of microbial communities begins immediately after birth and depends on the mode of delivery, type of feeding, and use of antibiotics. These factors determine resistance to pathogens and effectiveness of the immune response.

Premature newborns experience a delay in microbiota establishment. Reduced diversity and stability of microbial communities increase the risk of developing severe infections, including sepsis and necrotizing enterocolitis. Intestinal dysbiosis at this age is often accompanied by overgrowth of opportunistic bacteria (*Klebsiella* spp., *Enterococcus* spp., *Pseudomonas* spp.), leading to inflammatory complications and systemic infection.

A key focus of infection prevention in newborns is maintaining a healthy microbiota. The use of probiotics and prebiotics in newborns has proven effective in reducing the incidence of late-onset neonatal sepsis and necrotizing enterocolitis, especially in preterm infants. Probiotic strains (*Bifidobacterium* spp., *Lactobacillus* spp., *E. faecium* L3) help restore normal microbial balance, strengthen the intestinal barrier function, and reduce inflammation.

Nevertheless, questions regarding the selection of optimal probiotic strains, dosages, and duration of use remain subjects for further research.

Thus, studying the development of the gut microbiota in newborns is a pressing issue and serves as the foundation for preventing infectious complications and improving disease outcomes in children at risk of dysbiosis. A deep understanding of microbiota-immune system interactions will enable the development of personalized strategies to prevent and treat infectious diseases in the early neonatal period.

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ADDITIONAL INFORMATION

Author contribution. D.M. Konyukhov — development of the article concept, search and analysis of literary sources, and writing of the article; N.V. Gonchar — development of the article concept, assistance in writing, and editing of the article; K.D. Ermolenko — development of the scientific concept of the article and editing of the text; A.N. Suvorov — participation in research planning, editing of the article, and approval of the final version.

All authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Д.М. Конюхов — разработка концепции статьи, поиск и анализ литературных источников, написание статьи; Н.В. Гончар — разработка концепции статьи, содействие в написании и редактирование текста статьи; К.Д. Ермоленко — разработка научной концепции статьи и редактирование текста; А.Н. Суворов — участие в планировании исследования, редактирование статьи и одобрение финальной версии.

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