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Intestinal Microbiota in Premature Children — the Modern State of the Problem (Literature Analysis)

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The problem of impact of intestinal microflora (microbiota) on health of infants has gained particular relevance in the past few years. On the one hand, it is related to significant human environmental deterioration, on the other hand, to high incidence of digestive disorders in children, especially in the premature ones. Introduction of modern highly informative molecular-genetic examination methods (PCR amplification with gene sequencing) allowed to reveal the initial stage of microbial colonization of a human – antenatal ontogenesis – and decipher the microbiotic structure in neonates and under-1 infants in detail. It has been established that quantitative evidence and qualitative composition of intestinal microbiota is influenced by the mother's microbiotic composition, which depends on the presence/absence of both inflammatory and metabolic diseases (obesity). A reliable dependence of a neonate's microbiotic composition on their mother's mode of delivery (a more favorable composition is observed after normal delivery) has been observed; it ought to be noted that the mentioned differences persist for a few months after birth. One of the main factors impacting microbiota from the first days of life is nutrition; most studies convincingly confirm the role of breastfeeding in the development of optimal microbiocenosis in infants. Antibacterial therapy undergone by the mother and/or the child negatively affects microbial colonization of the intestines with symbionts. Negative external factors impacting microbiota are especially significant in premature infants, primarily in infants with very low or extremely low body weight. Ontogenesis of these infants is particularly complicated with negative factors (infections and need in massive antibacterial therapy, hypoxia, operative delivery and forced formula feeding) secondary to general prematurity, including immature nocifensors. Targeted correction of microbiota in premature infants is an important condition of prevention and treatment of such severe diseases as sepsis and necrotizing enterocolitis. That is why use of probiotic drugs is seen as one of the promising spheres of practical neonatology. The article presents an example of a probiotic therapy efficacy study on premature infants with a multisystem perinatal pathology.
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Relevance

Ontogenetic process in humans are defined by interaction of the macroorganism's genetic code and internal and environmental factors. A set of such factors includes interactions of humans and microbes. Interactions of such physiological processes have not been sufficiently studied yet; a

considerable variety of microbiotic microbes developed in the course of a long-term evolution of the balance of the host's immune system and microbial growth [1].
Human microflora (microbiota) is a set of bacteria colonizing surfaces and open cavities of the body – skin, airways, genitourinary system and gastrointestinal tract. Intestinal microbiota is the largest human microbial community: intestinal microbiota contains 10 times more cells and 150 times more genes than the human body itself. Many microbiologists consider the combination of all microbes colonizing a human body a "superorganism" [2].
It has been established that intestinal microbiota plays one of the key roles in maintaining a human's health by affecting metabolic and immunological processes. Intestinal microbiota composition is unique: scientists distinguish between 3 enterotypes depending on the prevalence of specific species of microbes [3]; such a prevalence leads to an individual immunological tolerance to specific bacteria.
It has been established in recent years that intestinal microbiota disorders lead not only to pathologies of the gastrointestinal tract, but are also indirectly connected with diseases of the cardiovascular and endocrine systems (obesity, pancreatic diabetes), atopic pathology and even with oncologic, mental and autoimmune disorders [4-8].
Impaired microbiotic formation in infants is of particular significance since various influences in critical ontogenetic periods create a background for a delayed pathology connected primarily with dysmaturity of the intestinal immune system [9, 10]. It has been established that low microbiotic diversity in infancy increases the risk of atopic disease [11] and promotes faulty metabolic programming by means of failed regulation of epitheliocyte growth [12].
Until recently there has been almost no information on the gastrointestinal tract's (GIT) microflora in children with very low body weight, as traditional cultural methods allow to identify only 20% of the colonizing microbes [13]. Achievements in the sphere of molecular technologies helped to considerably increase knowledge of human microflora, as well as of microflora of premature neonates.
Gastrointestinal microflora has the key role in maintaining integrity of the intestinal barrier in premature infants, as damage thereof may cause sepsis, necrotic enterocolitis (NEC) and systemic inflammatory response syndrome [14]. Long-term consequences of organ damage at systemic inflammatory response syndrome are connected with severe diseases in premature infants, including brain lesion (periventricular leukomalacia) and pulmonary injury (chronic pulmonary diseases) [14]. Composition of GIT microflora associated with optimal health has not been established; however, the general conclusion of trials of infants with very low body weight (VLBW) is decrease in the GIT microbiotic diversity and increase in the pathogenic load [13, 15-18].

Contemporary methodological approaches to studying intestinal microbiome

In the previous century, the studies of GIT microflora were based on cultural methodology defined by determination of the genus, species and even the strain by means of a range of morphological and biochemical tests. However, up to 80% of intestinal microbes remained extremely difficult for identification by means of cultural methods [13]. Molecular genetic technologies developed in the last ten years have become a revolutionary step in microbiome studies.
High-performance method of sequencing ribosomal RNA (rRNA) gene *16S* is the most common approach used to characterize a microbial community. The segment containing rRNA gene *16S* is a small part of RNA present in all organisms and contains regions of highly conservative sequences in comparison with hypervariable regions. Selective amplification of these hypervariable regions with subsequent sequencing is an effective contemporary method of characterizing microbial communities [19].
In order to obtain information on the amount and functional activity of microbial communities metagenome, metatranscriptome, metaproteome and metabolome approaches are becoming

widely used along with *16S* rRNA-sequencing [20]. Metagenome consists of the totality of genomes of the whole community, including bacteria, fungi, viruses and protozoa. In order to analyze a metagenome, DNA is extracted for obtaining genome sequences and further determination of the functional and biochemical potential of the microbial community. DNA of two strains of any given bacterial species may differ by as much as 25%; this determines their effect on the macroorganism – harmless, commensal or pathogenic.
The next step consists in determining not only the genes present in the microbiome, but also of gene (metatranscriptomics) or protein expression (metaproteomics) in mRNA [20]. Currently, metatranscriptome analysis of the GIT microbiome is restricted due to inherent RNA instability and complexity of extraction from the biological environment. In this case, metaproteomics is an attractive alternative, although it is not devoid of restrictions, either; these restrictions are caused by analytical problems due to insufficient volume of the reference database. Metabolome analysis is conducted by means of mass spectrometry or nuclear magnetic resonance-based spectrometry for monitoring the whole set of small molecules produced by microbes or host cells in the samples [20].
More than 1 000 bacterial species were identified in the GIT of adult humans by means of contemporary methods; they are distinguished into three main genera: *Bacteroides*, *Actinobacteria* and *Firmicutes*.

Intestinal microbiota in neonates

The main body of knowledge about GIT microbiota formation in neonates was obtained by studying term infants: only few studies involved premature and low-weight neonates.
It has been considered until recently that GIT of a healthy term neonate is sterile; however, new evidence of intrauterine bacterial translocation [21] and presence of microbial ribosomal RNA (rRNA) in neonatal meconium confirm that intestinal system of a neonate is colonized before birth [22, 23]. Detection of bacterial liposaccharide in umbilical blood of premature neonates indicates the presence of an intrauterine translocation process [24]. Oral introduction of genetically marked *Enterococcus faecium* to pregnant mice led to detection thereof in meconium of the infant mice delivered by means of operative delivery; this demonstrates the possibility of prenatal microbial transfer [21].
Active bacterial colonization of a neonate's GIT starts immediately after birth. In healthy term neonates, indigenous bacteria colonize mucosal surfaces and the intestinal system in a typical succession, which consists of four phases [25, 26].
Phase I is the initial stage of colonization and lasts 2 weeks from birth. In this phase, streptococci and intestinal bacteria, i.e. aerotolerant flora, are prevalent in the intestinal system. Gram-positive non-spore-forming anaerobes appear later and include mainly bifidus or lactic bacteria (depending on the type of feeding – breastfeeding and artificial feeding, respectively). *Clostridium* and *Bacteroides* are also found, although in smaller amounts than at later stages of postnatal development.
Phase II consists of the breastfeeding period and lasts from the end of phase I to the beginning of introduction of solid food to the diet. The amount of *Bacteroides* gradually increases during phase II.
Phase III is the period from the beginning of supplemental feeding not covered by previous phases to complete weaning. This stage ends when a child's flora is completely developed (**phase IV**) [27].
Infantile microbiota is more variable in terms of composition and less stable in the course of time in comparison with an adult's microbiocenosis [13, 27-29]. Relative stability of adult microbiota was called into question in a recent study, which confirmed that a diet change lasting for one week is sufficient to affect composition of intestinal microbiota [30].

Microbial colonization in infants may be significantly affected by exogenous factors, including mode of delivery, type of feeding, use of antibiotics and peculiarities of supplemental feeding introduction.

Effect of the mode of delivery on the composition of intestinal microbiota in neonates

Composition of GIT microbiota in the infants delivered by means of cesarean section and the children delivered naturally is different; some of these difference persist throughout infancy [15, 28, 29, 31-33]. After vaginal delivery, neonates fairly quickly develop the initial intestinal microbiome, which consists mainly of maternal vaginal and intestinal microbes. On the contrary, the infants delivered by means of cesarean section are subjected to initial contamination with environmental bacteria (flora of medical personnel and mother's skin) [34].

After vaginal delivery, term infants feature bacterial diversity (number of different species) of the composition of GIT microflora and homogenous distribution of microbial species [31, 32]; they have more *Bacteroides* and *Bifidobacteria* microbes than the infants delivered by means of operative delivery. A range of data indicates that the children delivered by means of operative delivery preserve fewer and less diverse intestinal microbes by the age of 4 months than the infants delivered naturally [32].

According to numerous specialists, the composition of intestinal microbiota in the children delivered by means of cesarean section (high specific weight of anaerobes, particularly, of *Clostridium difficile*) is reliably associated with high risk of development of allergic diseases (bronchial asthma, atopic dermatitis) and metabolic disorders in children [11, 35].

At the same time, Azad et al. [32] demonstrated that *Escherichia* and *Shigella* are few, whereas Bacteroides are not detected at the age of 4 months in the neonates delivered on term by means of cesarean section (in comparison with the children delivered by means of vaginal delivery). In contradiction to the aforementioned authors, the authors did not observe any differences in the distribution of *C. difficile*, as well as in the proportion of *Bifidobacterium* or *Clostridium* depending on the mode of delivery.

Effect of antibacterial therapy on the composition of intestinal microbiota in neonates

Most children with VLBW receive broad-spectrum antibiotics in the early neonatal period; this leads to inadequate intestinal colonization in phase I [36] with observable reverse correlation of the antibacterial therapy course duration in the 1st postnatal moth and microbial diversity, as well as the total count of intestinal bacteria [37]. Studies of the initial intestinal microbiota demonstrated that colonization by beneficial bacteria, such as *Lactobacillus*, is significantly impaired in the event of antibacterial therapy [38, 39] and that antibiotic use promotes growth of *Staphylococcus*.

Very recently F. Fouhy et al. [40] have demonstrated that term neonates receiving ampicillin and gentamycin parenterally within the first 48 postnatal hours had a significant decrease in the number of *Actinobacteria* (including *Bifidobacterium*) and *Firmicutes* (including *Lactobacillus*) bacteria, as well as the growth of *Proteobacteria* (including *Enterobacteriaceae*). Domination of *Proteobacteria* and reduced microbial diversity persisted for at least 8 weeks after antibiotic use.

7-day-long clindamycin treatment in the framework of a study of adult patients led to reduced bacterial diversity, which persisted for 2 years [41]. Given instability of a neonate's GIT microflora, we may expect the antibiotic use to profoundly affect the microbial community and have long-term consequences [41-43].

French neonatologists used two protocols of treating children with suspicion of intrauterine infection [44]. Effect of antibiotics on fecal flora was evaluated in 3 groups of neonates: 10 children with suspicion of intrauterine infection were receiving amoxicillin and netilmicin, 10 – amoxicillin, cefotaxime and netilmicin; 10 children composing the control group were not

receiving antibiotics. Amoxicillin-tolerant *Klebsiella oxytoca* and *Escherichia coli*, *E. faecium* and coagulase-negative staphylococci were observed in group 1. Homogenous intestinal flora with rapid colonization with staphylococci and *Candida* fungi was observed in group 2. These facts indicate the need in individualizing antibacterial therapy.

Effect of the type of feeding on the composition of intestinal microbiota in neonates

Healthy term neonates are initially colonized by a large number of *Enterobacter* and *Streptococcus* regardless of the type of feeding [27]. It is assumed that these bacteria are responsible for preparing the GIT environment appropriate for colonization by anaerobes Bacteroides, Bifidobacterium and Clostridium in the period from postnatal day 4 to postnatal day 7. It has been proven that beneficial bifidus and lactic bacteria are prevalent in the composition of microbiota by postnatal day 7 in breastfed healthy term neonates [45].

Children actively adapt to enteral feeding in the first hours and days of postnatal life, which is why the type of feeding at that period is the main factor of development of peculiarities of the intestinal microbiota. The bifidus bacteria count in breastfed children is twice as large as in the artificially fed infants; within several weeks bifidus flora becomes dominant. Flora diversity is also observed: enterococci, enteric bacteria and veillonella are observed along with *Bifidobacterium breve*, *Bifidobacterium bifidum* and *Bifidobacterium longum*. Bacteroides, clostridia, enteric bacteria and streptococci are observed along with bifidus bacteria in the artificially fed children [46].

A more mature type of microflora [33, 36, 47] along with the abundance of potentially pathogenic bacteria (*C. difficile* and colibacilli) [17, 45] is common in case of artificial feeding. The latest studies by Azad et al. [32] demonstrate reduction in microbiotic bacterial diversity in 4-month-old children receiving milk formulae in comparison with breastfed children. Later, the proportion of *C. difficile* increased in the artificially fed children, but not in breastfed children.

Similar data were obtained by other authors [48] who noticed that breastfed children have twice as much bacterial cells in the composition of their microbiota than their peers receiving milk formulae who have a higher count of *Atopobium* in the setting of lower count of *Bifidobacterium* and higher proportion of *Bacteroides*.

It is assumed that the differences in intestinal colonization in breastfed children and the infants receiving milk formulae may be caused by the presence of the specific rich microbiome and oligosaccharides in breastmilk; they selectively stimulate growth and/or activity of *Bifidobacterium* and *Lactobacillus* [49]. It has been shown that in the event of breastfeeding the child's mouth cavity bacteria enter breast milk ducts and contribute to the breastmilk microbiota [50]. Extraction of rRNA gene *16S* from breastmilk even after disinfection of the breast with iodine solution confirms the presence of microbial flora in milk [49, 51].

Another mechanism of breastmilk colonization and differences in the composition of microbiota of the children undergoing different types of feeding is the ability of bacteria or their components to actively migrate from the mother's intestinal system as macrophages or dendritic cells to mammary glands and breastmilk [52].

Contemporary studies indicate that peculiarities of the mother's phenotype and mode of delivery may affect composition and diversity of microbiota in breastmilk.

Thus, R. Cabrera-Rubio et al. [49] studied breastmilk microbiota in three benchmarks of the lactation period in the mothers with different body mass index (BMI), weight gain during pregnancy and mode of delivery.

They established correlation of a mother's BMI and microbiotic composition of her breastmilk. High BMI in mothers was associated with high amount of *Lactobacillus* in colostrum. In a similar way, increase in the amount of Staphylococcus and lower amount of Bifidobacterium in breastmilk 6 months after birth were associated with higher mother's BMI.

Excessive weigh gain during pregnancy was accompanied by increase in the amount of Staphylococcus (including staphylococcus aureus) bacteria in the breastmilk of women within the first month of lactation, as well as by higher count of *Lactobacillus* and lower count of *Bifidobacterium* in breastmilk after 6 months of lactation.

It has been determined that mother's endocrine disorders – body mass index before pregnancy and inadequate weight gain during pregnancy – significantly affect intestinal microflora of neonates and infants under 6 months of age. It has been established that a woman's body mass index, weight gain during pregnancy and number of bacteroides, clostridia and staphylococci in intestinal microbiota of a neonate are in significant direct correlational relationship; at the same time, these mother's peculiarities are in reverse correlation with the child's bifidus bacteria level.

The mothers who have delivered children vaginally have a different taxonomic composition of breastmilk bacteria from the one in the women who have delivered their children by means of cesarean section. The mothers who have undergone operative delivery featured a significant change of breastmilk microflora: lower count of *Leuconostocaceae* and higher count of *Carnobacteriaceae* in comparison with the women who have delivered children naturally. This difference is observable as early as in colostrum and remains in breastmilk after 1 and 6 months of lactation.

The bacterial count in the colostrum is the same after both term and urgent operative delivery. Composition of transitional and mature breastmilk of the mothers who have delivered children by means of urgent cesarean section was similar to the composition of milk of the mothers who have delivered children naturally.

Thus, results of a study conducted by R. Cabrera-Rubio et al. demonstrate that a woman's anthropometric data (BMI) before and during pregnancy are associated with taxonomic composition and diversity of breastmilk microbiota. Colostrum and breastmilk of obese women in the first month of lactation have lower bacterial diversity than in mothers with normal weight, even though this difference disappears by the time the child reaches 6 months of age.

Taking into consideration the fact that breastmilk bacteria are virtually the first bacterial cells entering an infant's gastrointestinal tract, change of the milk's bacterial composition may be a factor promoting transfer of bacterial flora shifts from mothers to infants. Thus, the obtained results may indicate an additional mechanism, which explains high risk of obesity in the children of women suffering from obesity or excess weight. Moreover, results of a study conducted by R. Cabrera-Rubio et al. demonstrated that breastmilk of the mothers who have delivered children by means of regular cesarean section has considerable compositional differences from breastmilk of the women who have delivered children by means of an urgent operative delivery or *per vias naturales*. These differences are observable as early as in colostrum and remain in breastmilk after 1 and 6 months of lactation; this shows that bacterial composition shifts have a long-term effect. It is assumed that physiological (e.g., hormonal) changes taking place in a mother's body during labor may affect microbiotic composition. Intestinal microbial community in the infants receiving mixed feeding are similar to the microbiotic composition of the children receiving artificial feeding (when they start receiving supplemental feeding) [53].

Results of studies confirm that breastfeeding affects formation and development of intestinal microflora in infants, while breastmilk is one of the most important factors in the postnatal period as it modulates metabolic and immunological programming and impacts the child's health in the long term. The latest studies resulted in new data on microbiota formation in breastmilk, where the bacterial count is 10^3-10^4 CFU/ml [54].

Breastmilk of the women who have delivered children by means of premature delivery has a specific composition characterized by a higher level of oligosaccharides, which may affect the microbial growth [55].

During the first year of life, the intestinal system of term neonates becomes colonized with microbes, the number whereof reaches ca. 10^4 CFU/ml of intestinal contents. Most anaerobes are located in the anorexia-exposed distal segment of the small intestine and in the large intestine, where only anaerobes and facultative bacteria are capable of surviving. However, some

anaerobes and aerobes may also colonize upper segments of the gastrointestinal tract. Microbiome of a term infant develops throughout the first 3 years of life until it forms the adult composition [15, 46].

Intestinal colonization in premature VLBW infants and connection thereof with infectious-inflammatory pathologies

Colonization of gastrointestinal tract in VLBW children depends on a range of factors: mode of delivery, antibiotic use by a mother or her child, latency period duration, parenteral feeding, enteral feeding introduction delay, food transit stretching, gestational age, birth weight, duration of stay at a resuscitation and intensive care unit, contamination with hospital pathogenic flora in the absence of skin-to-skin contact with the mother and the mother's breastmilk microbiome [13, 15-18, 31, 36, 52].

Molecular studies of VLBW neonates, including the ones with NEC, helped to reveal significant differences in the composition of intestinal microbiota of premature and term infants: low bacterial diversity, increase in the amount of pathogenic flora potentially associated with the development of NEC, high eukaryotic and viral diversity [15, 18, 42].

Peculiarities of intestinal microbiota in premature infants are defined by numerous factors: the factor of immaturity – lower gestational age by birth characterized by increased permeability of the intestinal barrier, which results in high risk of bacterial translocation outside of the intestinal system – is of high importance (along with the aforementioned, i.e. mode of delivery, nature of the mother's pathology, type of feeding and delivered therapy) [56]. Premature infants, especially with very low and extremely low body weight, are characterized by immaturity of both local and general immune system; this results in high risk of sepsis and NEC development.

As has been mentioned previously, modern methods of molecular microbiology (PCR-amplification) helped to reveal intrauterine bacterial colonization of amniotic fluid (if fetal membranes remain whole) and fetal meconium; initially, premature infants are characterized by lower microbial diversity, lower volume of bifidus and lactic flora, as well as prevalence of staphylococci and enteric bacteria. Direct correlation of the initial microbial colonization of the intestinal system and gestational age has been established [13].

According to the contemporary understanding, the microbial community (microbiota) are located on the intestinal mucosa in the form of bacterial biofilm, which is balanced in terms of species composition and constantly changes (some species are replaced by the other). In their turn, microecological disturbance due to immature immune mechanisms negatively affects the latter: as the macroorganism loses the ability to identify microbes as friends or foes, microflora is replaced with mixed polymicrobial biofilms of opportunistic microflora – polyresistant strains of staphylococcus, Enterobacter, klebsiella, Escherichia, pseudomonas, Acinetobacter etc. [57]. These causative agents are etiologically significant for the development of infectious-inflammatory diseases and are usually considered hospital-acquired flora (healthcare-associated infections) in premature infants hospitalized to resuscitation intensive care units and the 2nd stage inpatient hospitals.

Unlike term infants, VLBW premature infants develop a very versatile, "thinned" microbiome. This scarce microflora has a very low content of anaerobic bacteria and is characterized by prevalence of such microbes as coagulase-negative Staphylococcus, Enterococcus, Enterobacteriaceae and yeast fungi [58].

Thus, VLBW premature infants demonstrate delayed development of microflora (unlike term infants). Colonization differences and changes in the beginning of life may promote gastrointestinal diseases and immune balance shifts, which lead to atopic disease and development of nervous system disorders in children and adults [59].

A recent longitudinal study of two VLBW premature infants revealed that intestinal microbiome forms under the influence of the antibiotic-resistant microbes colonizing an infant's body in an intensive care unit [15]. Antibiotic resistance genes may be transferred between microbes,

including pathogenic microbes. Another longitudinal study of a VLBW child delivered by means of cesarean section demonstrated a shift towards strict anaerobes (they exceeded the proportion of one of the most common microbes – *E. coli*) in 9 stool samples taken during the 3rd postnatal week [60].

One of the latest studies described a microbial sequence in 58 VLBW neonates grouped by gestational age: < 26 weeks, 26-28 weeks, > 28 weeks [61]. 922 fecal samples were prospectively analyzed as infants grew and developed. The obtained results confirmed a preprogrammed and nonrandom succession of the microflora development process – from *Bacillus* to *Gammaproteobacteria* and later to Clostridia with dominant anaerobic colonization by week 33-36 of post-conceptual age.

Scientists observe frequent and sharp unpredictable changes in the microbiotic composition dynamics due to external factors, such as breastfeeding, use of antibacterial drugs and mode of delivery. These studies are especially significant for analyzing further development of children: e.g., they will help to determine how peculiarities of microbiome formation are connected with early development of pediatric diseases.

Information on the production of certain signaling molecules affecting brain synaptogenesis by normal representatives of intestinal microbiota are of high interest. A recent study demonstrated that intestinal microbiome may regulate the development of cephalic neural networks [62]. This regulation has time restrictions with a critical window in the early postnatal period, wherein intestinal microflora may modulate synaptogenesis by means by changing expression of the genes the products whereof affect neurotransmitter modulation in the nervous system. Microbial colonization process modulates the signal mechanisms affecting the neural networks responsible for control of response to stress signals.

Microflora considerably affects brain functions and vice versa. The brain may change microbiotic composition by modulating intestinal secretion, permeability and motility, removal of excessive bacteria from the intestinal lumen and prevention of bacterial growth [63]. Signaling molecules are expressed into the intestinal lumen by the proper mucous plane cells controlled by the central nervous system and may change intestinal motility, secretion and permeability, thus changing conditions of bacterial existence in the gastrointestinal environment.

Thus, the issue of condition of intestinal microbiota and correction of its disorders becomes especially relevant in neonates, as their bodies are constantly dynamically changing and characterized by the highest possible plasticity (the so called critical window) [9]. The methods of affecting microflora used in this period may have not only short-term, but also long-term effects, reduce risk of metabolic disorders in the future and even improve the cognitive development level.

Use of probiotics in neonates, including premature infants

Strains of lactobacillus are thermophile streptococcus developed by I.I. Mechnikov, on the basis whereof he created a lactate product that he proposed to use for therapeutic purposes, should be considered the first probiotics in history. We may conditionally divide modern probiotics into the following groups: primarily containing lactic bacteria, bifidus bacteria, other lactic acid bacteria (streptococci, enterococci) and non-lactic acid microbes.

The most important quality of probiotic bacteria is securement of colonization resistance of the intestinal system due to antagonism (competitive adhesion) with opportunistic and pathogenic microbes and participation in the local and systemic immune protection. Immunological mechanisms of probiotic action include activation of the macrophage antigen presentation function, increase in the production of secretory immunoglobulin A and change of cytokine profiles, which induces tolerance to food allergens. Moreover, probiotics create an adverse environment for adhesion of pathogenic microbes (change local pH), produce bacteriocins that suppress the growth thereof, stimulate production of epithelial mucus and inactivate pathogenic microbial toxins; this improves the barrier function of the intestinal system [64].

Most probiotic preparations for infants contain only a few species of the well-studied master seed strains of the following microbes – *Lactobacillus rhamnosus* GG, *Bifidobacterium lactis* Bb-12 and *Streptococcus thermophilus*.

Start of probiotic preparation use in clinical neonatology (the first national probiotic Bifidumbacterin appeared in the 1980s) was synchronous with the change of spectrum of microbial causative agents of infectious-inflammatory diseases in neonates (broader participation of gram-negative flora). In this setting, by the XXI century numerous studies demonstrated clinical effectiveness of probiotics for complex treatment of various pathologies in children – acute and chronic GIT diseases, allergic diseases [64].

A considerable number of recent publications was dedicated to NEC prevention and treatment in premature infants, including children with very low and extremely low body weight. Thus, use of a co-formulated peroral probiotic in VLBW children (randomized controlled study) helped to reduce NEC rate 4 times (in comparison with the control group) and prevent NEC-associated fatal outcomes [65].

The common probiotics used in premature infants contain *Lactobacillus* and *Bifidobacterium*. Cochrane meta-analysis of 20 randomized and non-randomized studies (n > 5,000 children) demonstrated that administration of probiotics to premature infants significantly reduces both total mortality rate [relative risk – 0.65 (0.52-0.81)] and NEC rate [relative risk – 0.43 (0.33-0.56)] [66]. The most significant reductions concern severe NEC rate, enteric feeding intolerance and hospital stay duration.

Other multicenter studies of children demonstrated that a co-formulated probiotic containing *B. bifidum* and *Lactobacillus acidophilus*, helped to reduce the rate of NEC 3.5 times and the lethality thereof 5 times. It has been established that use of probiotics also reduces rates of all infectious-inflammatory diseases, primarily, of sepsis, in small premature infants and contributes to earlier introduction of enteric feeding thereto [67].

In general, publications of most meta-analyses indicate that probiotics significantly reduce the risk of stage II or severer NEC development, advance transfer of a child to completely enteric feeding, promote general decrease in neonatal lethality, primarily of premature infants; however, it has been observed that the risk of late-onset neonatal sepsis development wouldn't decrease. The most significant mechanisms of sanogenetic effectiveness of a drug in premature infants are pathogenic microflora suppression, immune response modulation, intestinal barrier function induction and blood flow improvement. Immunomodulating effect of probiotics is realized by means of contact of their ligands with Toll-like receptors of the macroorganism's cells.

An effectiveness study of a co-formulated probiotic containing *B. lactis* Bb-12 (10^8 CFU) and *S. thermophilus* TH-4 (10^7 CFU) in terms of development of early adaption mechanisms and prevention of functional GIT disorders in premature infants with overlapping perinatal pathologies was conducted at the premature baby unit of the Scientific Center of Children's Health. The open-label longitudinal study involved 25 5-6-days-old children with antibiotic-associated dysbiosis, risk of NEC development and functional digestive disorders born ≤ week 34. Children were blindly randomized to the probiotic group (n = 15) and the control group (n = 10). A complex follow-up clinical-bacteriological study of patients was conducted. According to the results of the study, a satisfactory average daily weight gain was observed in both groups; however, faster recovery of the initial body weight and slightly earlier development of sucking reflex were observed in the group of children receiving a probiotic. By treatment day 7-9, functional digestive disorders in this group of patients were eliminated and coprological analysis data normalized; evaluation of microbial landscape revealed a significant increase in the counts of bifidus and lactic flora and reduction in the concentration of expressed enterococcus [68]. All these facts indicate a sufficient probiotic effectiveness of the combination of these strains represented in Russia by a probiotic complex for neonates and infants Bifiform Baby. The preparation is easy to use at inpatient hospitals for low-weight and premature infants as it reduces labor costs of the medical personnel and is used once a day in the form of suspension. The daily probiotic dose is pre-marked on the measuring pipette.

Thus, safety of the analyzed probiotic strains in neonatology is almost doubtless; other probiotic preparations require further studying.

Review of contemporary studies dedicated to the analysis of development and correction of microbiota in neonates, including premature infants born with VLBW indicates that condition of a neonate's microbiota is one of the most important factors defining his/her health both in neonatality and on subsequent age stages. Further detailed studies of development and age dynamics of microbiota using contemporary molecular-genetic methods will allow developing focused preventive measures against short- and long-term consequences of perinatal pathologies in premature infants.

CONFLICT OF INTEREST

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