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Study of Microbiocenosis of Intestine in Children of Different Age Category

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Abstract
In order to study the microbiocenosis in children of different age categories a total of 1891 clinical samples have been investigated. It was determined that in all groups the dysbiotic disorders were evident. In 84% of children the dysbiosis were characterized with the presence of conditionally pathogenic bacteria. Mono- and mixed infections were observed in 76,5% and 23,5% of cases respectively. In the age category I and II monoinfections were 15% less than in category III and IV, while frequency of isolation *Klebsiella spp.* and *Proteus spp.* was higher in children under 1 year. Leading position in all groups was held by hemolytic *E.coli*.
Mixed infections were mentioned predominantly in children of category I and II and characterized by various microbial associations. A comparison of different age categories of children found out that major prevalence was for children I and II category, including those under one year – with mixed infections.

Keywords: monoinfection, mixed infection, dysbiosis

Introduction

The human gastrointestinal microbiota (microflora) consists in a group of microorganisms that live in the digestive tract. They comprise a metabolically active and complex ecosystem, consisting of hundreds of thousands of microorganisms (bacteria, viruses, and some eukaryotes) that colonize the digestive tract soon after birth. [1]

The newborn intestine is an aerobic environment where only facultative anaerobes, such as members of the *Enterobacteriaceae* family can grow. In a matter of days, however, the intestinal lumen turns anaerobic, allowing for strict anaerobes, such as *Bifidobacterium*, *Clostridium*, and *Bacteroides* to colonize. During the first few weeks, the microbiota of the newborn gut resembles the maternal skin and vaginal microbiome, with *Enterococcaceae*, *Streptococcaceae*, *Lactobacillaceae*, *Clostridiaceae*, and *Bifidobacteriaceae* being predominant bacterial taxa. In the ensuing 12–30 months, the infant gut microbiota progresses into an adult-like gut microbiota and plays many critical roles in the body. The microbiota in children under 3 years of age fluctuates substantially and is more impressionable to environmental factors than the adult microbiota. Normal intestine flora may exist only in a normal physical condition of an organism establishing a dynamic association of mutual benefits (symbiosis) with the human organism, involved in stimulation of the immune system, synthesis of vitamins, enhancement of GIT motility and function, digestion and nutrient absorption, inhibition of pathogens, production of short-chain fatty acids (SCFAs) and polyamines. [2, 3, 4]

There are many areas of host health that can be compromised when the microflora is drastically altered and results in the suppressing of normal immunological, metabolic, and motor functions, as well as correct nutrient digestion and absorption. The dynamic changes are markedly different among different individuals, but the common effect is a macrobalance. [5, 6]

As soon as pathological changes occur in the organism, the structure and properties of intestinal microflora are changed and its function is disordered which leads to the

dysbacteriosis. Enteral dysbacteriosis is a disease determined by qualitative and quantitative disorders in normal microbiota. In such situation *Klebsiella, Proteus,* etc. attain pathogenic properties, which induced inflammatory and destructive alterations of enteral mucosa, which, in its turn, results in deteriorated enzymatic and motor activity in gastric-intestinal tract.

Dysbacteriosis as a disease most frequently develops in early babyhood. These children may be considered a risk-group in regard of enteral dysbacteriosis. In elder age groups high probability of enteral dysbacteriosis is predicted in children with frequent acute respiratory infections, allergic reactions, long-lasting treatment with antibiotics. Thus, knowledge of the detrimental changes to the microflora is becoming increasingly important. [7, 8, 9]

Objective of the present investigation is the study of microbicenosis in children of different age categories.

Material and method

While studying enteral microflora, the sowing incidence of separate microorganism species and their quantitative indices per 1 g of feces were determined. As a health standard the following indices were accepted: *Bifidobacteria* – 10^9-10^{10} microbial cell per 1g, *Lactobacteria* – 10^6-10^8, *Enterococcus* – 10^5-10^6, number of typical *Escherichia* – 10^7-10^8, *Staphylococcus spp.,* conditionally-pathogenic enterobacteria $\leq 10^4$, fungi of *Candida* genus $\leq 10^4$. Bacteriological examination of 1891 clinical material (feces) isolated from children was made according to methodological recommendations. Morphological and biochemical properties of strains were studied by standard methods using different selected media (1,5% nutrient agar, nutrient broth, Endo medium, 5% Blood agar, Kligler agar, Manitol-salt agar and etc.) and Api-test systems.[10, 11]

Result and Discussion

Total of 1891 clinical material (feces) isolated from children were examined during 2014-2015. Children were divided in different age categories: I - under 1 years (855); II – from 1 up to 6 years (663); III – from 6 to 10 years (240) and IV – from 10 to 16 years (133).

Category I: Dysbiotic disturbance of intestinal microbiocenosis in children were observed in 72% cases. 32% of dysbiosis were caused by significant decrease of *Lactobacillus;* 16% by *Bifidobacterium* and 20% of important reducing were mentioned at the same time with both *Lactobacillus + Bifidobacterium* microorganisms. In 40% *E.coli* with reduced fermentative properties has been detected. Approximately in 89% dysbiotical disorders of intestinal microflora were characterized by the presence of conditionally-pathogenic bacteria of genus *Escherichia, Klebsiella, Proteus, Staphylococcus* and other. Monoinfections were observed in 68% cases. Leading position were held by hemolytic *E.coli* - 70%. At a lesser degree were isolated hemolytic *Enterococcus spp*. – 9,4%, *Proteus spp.* – 8,3%, *Klebsiella spp.* – 7,7%. Unitary cases of isolation were found for *P.aeruginosa, Staphylococcus spp.* Mixed infections were mentioned in 32% of clinical material. Out of microbial associations were dominated the same combinations: hemolytic *E.coli +S.aureus* - 29%; hemolytic *E.coli +Proteus spp*. - 20%; hemolytic *E.coli +Klebsiella spp*. - 17%; hemolytic *E.coli* +hemolytic *Enterococcus spp* - 13,3%.

Category II: 70% dysbiotic disorderes of intestinal microflora were observed in children of these group. 50% of dysbiosis were caused by significant decrease of *Lactobacillus;* 21,4% by *Bifidobacterium* and 29% at the same time with both *Lactobacillus + Bifidobacterium*. In 35% *E.coli* with reduced fermentative properties has been detected. Approximately in 83% dysbiotical disorders of intestinal microflora were characterized by the presence of conditionally-pathogenic bacteria. Monoinfections were observed in 70% cases. Among them 77% of infections were caused by *E.coli*, for 12,3% cases were responsible hemolytic *Enterococcus spp.* and 4% were share for *Proteus spp.* and *Klebsiella spp.* Mixed infections were mentioned in 30% of clinical material. Out of microbial associations were dominated the combinations of: hemolytic *E.coli* +hemolytic *Enterococcus spp* - 38%; hemolytic *E.coli +Proteus spp*. - 20%; hemolytic *E.coli +S.aureus* – 18,6%; hemolytic *E.coli +Klebsiella spp*. – 11,6%.

Category III: Dysbiotic disorders of intestinal microbiocenosis were observed in 78% cases. 55% of were caused by considerable decrease of *Lactobacillus,* 17% - by *Bifidobacterium* and 28% at the same time with both *Lactobacillus + Bifidobacterium.* In 32% *E.coli* with reduced fermentative properties were observed. 83% dysbiotical disorders of intestinal microflora were caused by conditionally-pathogenic bacteria of genus *Escherichia, Klebsiella, Proteus, Staphylococcus* and other. The leading position among 82% of observed monoinfections were held by hemolytic *E.coli* - 83%. Relatively small amounts were fixed for hemolytic *Enterococcus spp*. – 21% and *Klebsiella spp.* – 4%. Mixed infections were mentioned in 18% of clinical material. Among of microbial associations were dominated the same combinations: hemolytic *E.coli* +S.aureus - 29%; hemolytic *E.coli +Proteus spp*. - 20%; hemolytic *E.coli +Klebsiella spp*. - 17%; hemolytic *E.coli* +hemolytic *Enterococcus spp* - 13,3%.

Category IV: 74% dysbiotic disorderes of these group in 47% were caused by important reduction of *Lactobacillus,* 11,2% by *Bifidobacterium* and 42% with both *Lactobacillus + Bifidobacterium*. In 27% *E.coli* with reduced fermentative properties has been detected. 81% dysbiotical disorders of intestinal microflora were found out by the presence of conditionally-pathogenic bacteria. Monoinfections were mentioned in 86% cases. 82% of these infections were caused by *E.coli*, for 16% cases were responsible hemolytic *Enterococcus spp.* Mixed infections were fixed in 14% of clinical material and major microbial association was: hemolytic *E.coli* +hemolytic *Enterococcus spp.* - 53%. In children of these age category mixed infections were not characterized with microbial diversity.

The results of the experiment carried out determined that in all groups of children the dysbiotic disorders of enteral microbiocenosis were evident. 46% of dysbiosis were caused by significant decrease of *Lactobacillus,* 16,4% by *Bifidobacterium* and 30% of important reducing were mentioned at the same time with both *Lactobacillus + Bifidobacterium* microorganisms. Approximately in 84% dysbiotical disorders of intestinal microflora were characterized by the presence of conditionally-pathogenic bacteria of genus *Escherichia, Klebsiella, Proteus, Staphylococcus* and other.

Monoinfections were observed in 76,5% cases. Leading position were held by hemolytic *E.coli* - 78% and at a

lesser degree were isolated hemolytic *Enterococcus spp*. – 15%, *Proteus spp.* – 3%, *Klebsiella spp.* – 4%. In the age category I and II cases of monoinfections were 15% less than in category III and IV, while frequency of isolation of *Klebsiella spp.* and *Proteus spp.* was higher, particularly in children under 1 year.

Mixed infections were mentioned in 23,5% of clinical material. Out of microbial associations were prevailed the same combinations: hemolytic *E.coli* +hemolytic *Enterococcus spp.* - 37%; hemolytic *E.coli +S.aureus* - 16%; hemolytic *E.coli +Proteus spp*. - 13%; hemolytic *E.coli +Klebsiella spp*. - 12%. Mixed infections were mentioned predominantly in children of category I and II and characterized by various microbial associations.

A comparison of different age categories of children with mono and mixed infections, found out that major prevalence for children I and II category, including those under one year – with mixed infections.

Conclusions

Our investigation has revealed alterations of enteral microbiocenosis in children of different age groups. A connection was noted between the presence in faces of associations of conditionally pathogenic microorganisms and the deficiency or complete absence of bifido- and lactobacilli. Along with decreased bifido- and lacto-flora, representatives of *Enterobacteriaceae* family dominated in intestinal microbiocenosis. Especially low resistance to conditionally pathogenic flora in the newborn children was manifested in significant dysbiotic disorders in normal intestinal microflora. Mixed infections were observed in twice time more cases in children under 6 year and characterized by diversity of microbial species.

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