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MICROBIOLOGICAL CHARACTERISTICS OF COLON CAVITY IN BREASTFED INFANTS (ONE TO SIX MONTHS) WITH ACUTE COLIENTERITIS

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Despite the undeniable progress made in recent decades in the study of etiology and pathogenesis of acute intestinal infections in children on microbiological, immunological level that allow to synthesize and successfully use a wide range of remedies and precautions, preventing the spread of the acute intestinal infections and their successful treatment, unfortunately, these achievements should be recognized as relative, because they do not guarantee the determination of a clear etiology and mechanisms of the disease, which makes impossible to conduct a successful therapy and prevention.

To determine the etiology of colienteritis in infants (1-6 months old), the taxonomic composition and population level of pathogenic, conditionally pathogenic microorganisms and representatives of major, additional and accidental microbiota of colon in breastfed infants suffering from colienteritis.

Content of colon cavity of 48 children (one to six months old) sick with colienteritis underwent bacterial and mycological examination.

Etiological structure was determined in 28 (58,33 %) cases. Among them, 14 (29,17 %) were caused by enteropathogenic serotypes O55, O125, O128av, O151, O158 of *E. coli* (EPEC), in other 13 (27,08 %) cases – by enterotoxigenic *Escherichia* and in one child the disease caused by *Citrobacter*. In other cases (41,67 %) etiology could not be defined. In the intestinal microbiocenosis of colon of infants (one to six months old) that were on breastfeeding (breast milk is not infected with pathogenic and conditioned pathogenic microorganisms). Gram-positive asporogenous saccharolytic obligate anaerobic bacteria, mainly bacteria of genera *Bifidobacterium*, *Lactobacillus* and facultative anaerobic bacteria (*Enterococcus*) were dominated as well as gram-negative obligate anaerobic asporogenous bacteria of genus *Bacteroides* and facultative anaerobic and aerobic bacteria of genus *Escherichia*. According to the studies, *Bifidobacteria* are the most important by representation in the content of colon microbiota of breastfed infants and maintain microecological homeostasis. Their population level in the colon cavity reaches $10,87 \pm 0,79$ lg CFU/g. The population level of *Lactobacilli* is lower by 12,29 %, *Bacteroides* – 51,39 %, *Escherichia* - by 36,39 %. *Bifidobacteria* are basic according to its quantitative dominance and role in microbiocenosis. Due to these indicators they differ by 12,29 % and 13,33 % from *Lactobacilli* and from *Bacteroides* their role is higher by 18,48 % and 47,83 %; from *Escherichia* - by 36,38 % and 36,0 % respectively. On the background of the decreased population level of *Bifidobacteria* and *Lactobacilli*, the coefficient of quantitative dominance goes down by 66,27 % and 60,38 % respectively, and coefficient of importance – by 78,95 % and 66,67 %. This helps the contamination of colon with pathogenic *E. coli* Hly+ in 27,08 % patients with acute colienteritis, population level of which reaches $7,79 \pm 0,27$ lg CFU/g. Smaller ($6,50 \pm 0,22$ lg CFU/g) population level is found in enteropathogenic *Escherichia* that contaminate biotope in 29,17 % of patients. The decrease of mucosal colonization resistance on account of the reduction of population level of *Bifidobacteria* and *Lactobacilli* leads to the development of *Staphylococcal* intestinal infections, and as an argument, the increase of population level of *Staphylococci* by 2,10 times. As a result there is an increase in their role in microbiocenosis of colon by 2.07 times. Depression of mucosal colonization resistance promotes colonization of colon by pathogenic *Escherichia* (*E. coli* Hly+, EPEC) and conditionally pathogenic *Enterobacteria* (*Proteus*, *C. diversus*), *Peptococci* that reach a high population levels in biotope. Taxonomic composition of the main microbiota is not changed, but the population level of indigenous obligate anaerobic *Bifidobacteria* and *Lactobacilli* is sharply reduced, that leads to contamination of colon with pathogenic and conditionally pathogenic *Enterobacteria*, *Peptococci* and other bacteria that together contribute to an infectious process and form dysbacteriosis of various stages. Obtained results of studies of taxonomic and population changes of the colon microbiota of one to six months old breastfed children with acute colienteritis certify that in majority of affected children the dysbacteriosis of I stage is formed, while in other (35.41 %) - II-III stages.

Acute colienteritis in one to six months old children, which are on natural feeding, develops on the background of prevailing colon dysbiosis of I – III stages is formed by reducing she number of *Bifidobacteria* and *Lactobacilli* and the contamination of colon with *E. coli* Hly +, enteropathogenic *Escherichia* and *C. diversus*, *Proteus*, *Peptococi*, *Staphylococci* and *Peptostreptococci*, on the background of growth of the population level of *Bacteroides* and *Escherichia*.

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DEPENDENCE OF STRUCTURE-AL-ANTIMICROBIAL ACTIVITY OF A NUMBER OF NEW 2,4-DISUBSTITUTIVE 1-ARYL-IMIDAZOLE-5-METHYLCARBONYLS AND 5-CARBALDEHYDES

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Imidazole derivatives are one of the classes of medical preparations possessing a wide spectrum of their use in medicine and are extremely advaced for the search of new effective antimicrobial means. In this respect many countries

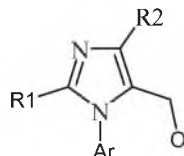


of the world synthesize new imidazole derivatives and their biological properties are examined including those antimicrobial [Zirngibl L., 1998; Hagiwara K. et al., 2000; Assmann L. et al., 2000; Basappa M.P. 2003; Sadashiva M.P. et al., 2005]. The search of new medical preparations including antifungal and antibacterial means in the group of carbon-functionalized imidazole derivatives in particular has been proved to be rather prospective [Chornous V. et al., 2014; Chornous V. et al., 2015].

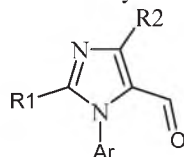
Considering the fact that a great number of functionalized imidazole derivatives including carbon-functionalized ones possess antibacterial and antifungal action, a directed construction of their new bioactive representatives remains of first-priority.

Was to study the effect of chemical structure of 2,4-disubstitutive 1-aryl-imidazole-5-methylcarbonyls and 2,4-disubstitutive 1-aryl-imidazole-5-carbaldehydes in their antimicrobial activity.

13 new derivatives of 2,4-disubstitutive 1-aryl-imidazole-5-methylcarbonyls and 10 new derivatives of 2,4-disubstitutive 1-aryl-imidazole-5-carbaldehydes were selected for the study with their general formula:



derivatives of 2,4-disubstitutive 1-aryl imidazole-5-methylcarbonyls



derivatives of 2,4-disubstitutive 1-aryl- imidazole-5-carbaldehydes

The antimicrobial properties of the examined compounds were studied by means of common methods of two-time series dilution in a liquid nutrient medium. Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923), gram-negative bacteria (*Escherichia coli* ATCC 25922) and yeast-like fungi (*Candida albicans* ATCC 885-653) were used as reference-strains. All the experiments were accompanied by appropriate controls: control of the medium and sterility, control of culture growth in the medium without a compound.

Conducted microbiological examinations enabled to detect that synthesized compounds possess a moderate antimicrobial activity. Thus, minimal bacteriostatic concentration of the prevailing majority of the derivatives of 2,4-disubstitutive 1-aryl-imidazole-5-methylcarbonyls is within the limits of 125 – 250 mcg/ml. It should be noted that in the majority of cases the compounds examined possess higher antibacterial activity concerning gram-positive *S. aureus* ATCC 25923 as compared to gram-negative *E. coli* ATCC 25922. Minimal bactericidal concentration of the prevailing majority of the derivatives of 2,4-disubstitutive 1-aryl-imidazole-5-methylcarbonyls was twice as much as minimal bacteriostatic concentration and was within the ranges of 250 – 500 mcg/ml.

Examination of anti-candidiasis activity of the synthesized derivatives of 2,4-disubstitutive 1-aryl-imidazole-5-methylcarbonyls found their higher activity as compared to antibacterial one – minimal fungistatic concentration of the indicated compounds was within the ranges from 62,5 to 125 mcg/ml, and minimal fungicidal concentration - from 125 to 250 mcg/ml.

The examination of antibacterial and antifungal activity of the derivatives of 2,4-disubstitutive 1-aryl-imidazole-5-carbaldehydes found their lower antimicrobial action as compared to the derivatives of 2,4-disubstitutive 1-aryl-imidazole-5-methylcarbonyls. Thus, minimal bacteriostatic concentration of the prevailing majority of 2,4-disubstitutive 1-aryl-imidazole-5-carbaldehydes derivatives concerning both gram-positive *S. aureus* ATCC 25923, and gram-negative *E. coli* ATCC 25922 was on the level of 250 mcg/ml, and their minimal bactericidal concentration – 500 mcg/ml.

The investigation of the effect of the chemical structure of 2,4-disubstitutive 1-aryl-imidazole-5-methylcarbonyls and 2,4-disubstitutive 1-aryl-imidazole-5-carbaldehydes derivatives on their antimicrobial activity detected that the value of biological activity is effected by the type of a substitute in the position of 5 imidazole cycle and substitutes in the positions 1, 2 and 4.

The compounds of a similar structure, in particular, having alcohol radical in the position 5 demonstrated twice as much activity as compared to the compounds with aldehyde group. Introduction of an aryl substitute of lipophilic fluorine atom into the aromatic cycle reduces bactericidal action, while introduction of a metal group intensifies it. The change of hydrogen atom into chlorine atom in the position 2 does not practically effect on the volume of antimicrobial action of the compounds.

The derivatives of 2,4-disubstitutive 1-aryl-imidazole-5-methylcarbonyls and 2,4-disubstitutive 1-aryl-imidazole-5-carbaldehydes reveal a moderate antimicrobial action enabling to recommend further search of effective antimicrobial means among this group of chemical compounds including purposeful synthesis of new compounds with predicted antimicrobial properties.

The study of the effect of the chemical structure of 2,4-disubstitutive 1-aryl-imidazole-5-methylcarbonyls and 2,4-disubstitutive 1-aryl-imidazole-5-carbaldehydes derivatives on their antimicrobial activity detected that the most optimal parameters of the molecule ensuring antibacterial and antifungal action is availability of an aryl substitute in its



content in the position 1 without lipophilic groups, absence of chlorine in the position 2 in case of methylcarbonyl group available in the position 5.

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STATE OF CAVITARY MICROBIOTA OF COLON IN ONE TO SIX MONTHS OLD CHILDREN WITH ACUTE COLIENTERITIS BREASTFED WITH INFECTION OF BREAST MILK

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Despite the significant progress made in the field of acute colienteritis in children, many questions of etiology, pathogenesis, microecological relations, formation of intestinal dysbacteriosis/dysbiosis and taxonomic composition and population-level of major, additional and accidental microbiota of intestinal microbiocenosis and other issues of colienteritis require study, taking into account bacteriological and immunological researches of today. This is especially related to children one to six months of age (that are breastfed), sick with colienteritis. Normally, breast milk is sterile, but in inflammation process in breast, it can be infected, so the disease poses a threat to a child that uses this milk, and there is a threat to the life of the mother. The first reaction to such feeding will be from a side of cavitary microbiota of a colon that characterizes the relevance of study of the colon microbiota in infants suffering from colienteritis.

Contents of colon cavity of 53 children one to six months of age with colienteritis (control - 35 children) who were breastfed and, in fact, breast milk, which breastfed children, underwent bacteriological and mycological examination.

For disclosure mechanisms of contamination of colon of children one to six months of age, in patients with acute colienteritis there has been used ecological method that allowed to carry out the specific characteristics of coexistence of taxons of ecosystem "host - microbiota" and identify the direction of disorders of colon microecology on the background of acute colienteritis in children who were breastfed with breast milk infected with bacteria of the genus *Staphylococcus*. The results of bacteriological studies have shown that in 49 (92.45 %) samples *Staphylococcus aureus* (was isolated and identified), and in 4 (7.55 %) women - *Staphylococcus epidermidis*. Population level of the selected strains was 3,0 - 7,0 lg CFU/ml ($M \pm m = 4,37 \pm 0,38$ lg CFU/ml).

Breastfeeding with milk infected by *Staphylococcus* leads to a decrease of population of *Bifidobacterium* by 43.03 % (3 orders), *Lactobacillus* - by 32.24 %, (2 orders). With the decline of population of *Bifidobacteria* and *Lactobacilli* in the intestinal microbiocenosis the colonization resistance of intestinal mucosa becomes disturbed, which promotes colonization of the intestine, especially the colon, with opportunistic *Enterobacteria* - hemolytic *Escherichia* (*E. coli* Hly+) in 16.48 % of children with acute colienteritis, *Proteus* - in 22.53 %, *Citrobacter* - in 3.77 %, *Peptococci* - in 15.09 %, *Peptostreptococci* - in 55.42 % and *Staphylococcus* - in 91.43 % of cases. In addition, in the colon of children with acute colirnteritis breastfed with the infected milk there is an increase of population levels of *Bacteroides* by 32.59 % (2 orders). Furthermore, there is increasing population levels by 61.50 % (3 orders) of *Peptostreptococci* in the colon and contamination with *Peptococci* ($9,13 \pm 0,37$ lg CFU/g) occurs.

Reduction of the concentration of *Bifidobacteria* and *Lactobacilli* also, among other components of ecosystem, leads to an increase of the population level of *E. coli* by 19.07 % (2 orders) in the colon of children with colienteritis. In the experimental group of children *Staphylococci* were isolated from all sick children in concentration $6,16 \pm 0,12$ lg CFU/g, that is 2.92 times higher than in healthy children fed naturally with uninfected milk. Thus, the growth of the population level of *Staphylococci* in the colon of infants suffering from colienteritis may be linked, according to our point of view, on the one hand, with the constant contamination of the gastrointestinal tract due to breastfeeding with milk containing ($4, 37 \pm 0,38$ lg CFU/g) *Staphylococci*, and on the other hand, with changes of colonization resistance of the mucous membrane of the colon as a result of decline of population level of the major part of the large intestine microbiota - bacteria of genera *Bifidobacterium* and *Lactobacillus*.

The obtained data shows that the acute colienteritis in infants who were breastfed with *Staphylococci*-positive milk develops on the background of the prevailing dysbiosis in all children. Most affected children formed the second degree dysbiosis and 2 patients developed children the third degree.

Breastfeeding with milk infected by *Staphylococcus* in children aged from one to six months leads to the formation of *Staphylococcal* dysbacteriosis of the first (up to 43.40 %), second (up to 52.83 %) and third (3.77 % of cases) stages, and on its background colienteritis due to association of pathogenic (*E. coli* Hly+) and opportunistic *Enterobacteriaceae* (*E. coli*, *Proteus*, *Citrobacter*), *Staphylococci* (in population level $6,16 \pm 0,12$ lg CFU/g), *Bacteroides*, *Peptococci*, *Peptostreptococci* and other bacteria develops. Colienteritis in infants breastfed with milk infected by *Staphylococci* (*S. aureus*, *S. epidermidis*) is an infectious processes of mixed etiology, provocative factor of which is the association of opportunistic gram-negative *Enterobacteria*, *Bacteroides* and gram-positive *Staphylococci*, *Peptostreptococci* and *Peptococci*.

Dysbacteriosis formed in infants suffering from acute colienteritis is characterized by a reduction of a part of *Bifidobacteria* and *Lactobacilli* in intestinal microbiota and increasing number and value in microbiocenosis (by a rate of quantitative dominance and significance) of gram-negative opportunistic *Enterobacteriaceae*, *Bacteroides* and gram-positive opportunistic bacteria of the genera *Staphylococcus*, *Peptostreptococcus* and *Peptococcus*.