ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

# Role of Colonization Resistance of Gastric and Intestinal Mucosa in the Development of Gastrointestinal Bacterial Infections

**Chicherin IYu[1]*, Pogorelsky IP[2], Kolodkin AM[1], Lundovskikh IA[2], Shabalina MR[2] and Kolevatykh EP[3]**

[1]*Scientific society «Microbiota», Sergiev Posad, Moscow Region, Russian Federation*

[2]*Department of Microbiology, Institute of Biology and Biotechnology, Vyatka State University, Kirov, Russian Federation*

[3]*Department of Microbiology and Virology, Kirov State Medical University, Kirov, Russian Federation*

***Corresponding Author:** Chicherin IYu, Scientific society «Microbiota», Sergiev Posad, Moscow Region, Russian Federation.

**Received:** July 17, 2021 **Published:** August 25, 2022

## Abstract

The aim of the article is to analyze the role of colonization resistance of the gastric and intestinal mucosa in the development of bacterial gastrointestinal infections in conventional white mice. We created the experimental models of intestinal yersiniosis, pseudotuberculosis, salmonellosis and escherichiosis in conventional white mice by oral administration of bacterial suspensions containing pathogenic strains of *Yersinia enterocolitica* KM1407, *Yersinia pseudotuberculosis* KM07, *Salmonella enteritidis* KM08, *Escherichia coli* EG, isolated from patients with manifesting infectious diseases. Bacteriological methods were used to confirm bacterial engraftment and generalization of the infectious process. Pathological changes in the stomach and small intestine of dead animals were evaluated using histological sections of the stomach and small intestine. We measured the concentration of bacteria in the stomach and intestine after their single administration at a dose, required to cause symptomatic disease. Oral administration of the metaprebiotic Stimbifid plus (both preventive and therapeutic) to infected animals completely stopped the infectious process. Our findings indicate high therapeutic potential of metaprebiotic Stimbifid plus and its ability to preserve commensal microbiota and colonization resistance that play an essential role in the prevention of intestinal infections and ensure their termination in the early stage.

***Keywords:*** *Intestinal Yersiniosis; Pseudotuberculosis; Salmonellosis; Escherichiosis; Microbiota; Colonization Resistance; Stimbifid Plus; White Mice*

## Abbreviations

CFU: Colony Forming Units

## Introduction

Currently, the fight against infectious diseases, despite the significant achievements of health care, does not lose its relevance. This fully applies to acute intestinal infections, which, according to clinicians, ranked second among the causes of death in children under 5 years of age [1]. The unfavorable course of acute intestinal infections in most cases is associated with untimely or inadequate treatment. It is important to note that in recent years in most countries of the world in the etiological structure of acute intestinal infections, viral diarrhea predominates, and among them (mainly in children under 5 years of age) - rotavirus infections [2,3].

At the same time, etiological agents of other acute intestinal infections are not disregarded, because almost all of them proceed with symptoms of acute intoxication, with a significant change in the composition of the normal microbiota of the stomach and intestines, the

development of dysbiosis of varying severity, translocation of various types of intestinal microbiota into other biotopes of the organism and their excessive growth [4,5]. To this it should be added that very often emerging and aggravating metabolic disorders are associated with microecological disorders in the consortium of normal microbiota of the digestive tract, which provoke the progression of the pathological condition with a rapid lethal outcome [6].

The stomach is the second barrier after the oral cavity on the path of pathogenic microorganisms, the mucous membrane of which is sensitive to their entry and rapid reproduction to critical populations. Depending on which mucosa of which organ of the digestive tract is inflamed, all intestinal infections can proceed in the following forms: gastritis, enteritis, colitis, gastroduodenitis, gastroenterocolitis.

Pseudotuberculosis, intestinal yersiniosis, salmonellosis and escherichiosis are among the infectious diseases in which infectious disease doctors, epidemiologists and microbiologists face a serious problem when making a diagnosis at the initial stage of the development of the infectious process. Pseudotuberculosis is an acute zoonotic bacterial infection caused by the bacteria *Yersinia pseudotuberculosis*, with a septic form of which the mortality rate reaches 25 - 50% [7,8]. Intestinal yersiniosis is also an acute zoonotic bacterial infection caused by microorganisms *Yersinia enterocolitica* [8,9]: mortality in the septic form of the disease reaches 30 - 60% [10,11]. Salmonellosis is a zoonotic-anthroponous bacterial infectious disease with a fecal-oral mechanism of transmission of the pathogen, affecting the organs of the digestive system, and in a chronic form causing arthritis and pneumonia. The disease is caused by facultative anaerobic bacteria *Salmonella enteritidis*, *Salmonella infantis*, *Salmonella papuanta*, *Salmonella gallinarum*, belonging to the *Enterobacteriacea* family [12,13]. Escherichiosis is a group of infectious diseases caused by bacteria of the genus *Escherichia*. In more than 90% of cases, *Escherichia coli* is the causative agent of escherichiosis, otherwise the infections of this group are caused by the bacteria *Escherichia ferqusonii*, *Escherichia hermanii*, *Escherichia vulneris*. Diseases occur in the form of infections of the gastrointestinal tract, urinary tract, bacteremia and meningitis of newborns and are associated with four groups of pathogens - enterotoxigenic, enteroinvasive (enteroadhesive), enteropathogenic and enterohemorrhagic *E. coli* [13, 14].

The initial stage of development of pseudotuberculosis, intestinal yersiniosis, salmonellosis and escherichiosis largely depends on the realization of the potential of the pathogenicity factors of these pathogens of infectious diseases, which often has a polydeterminant nature. Thus, it was found that plasmid-free strains of *Y. enterocolitica* do not initiate the infectious process and immune restructuring of the sensitive organism during enteral infection. The *inv* mutants that do not synthesize invasin are unable to penetrate into the intestinal epithelial cells at the initial stage of intestinal yersiniosis [15,16].

The data obtained using experimental models of *Yersinia* infection [16,17] turned out to be very informative in terms of microbiological and morphological assessment of the nature of the interaction of *Yersinia* with a sensitive organism [17,18]. With the natural (enteral) route of administration of virulent strains of *Y. enterocolitica* into a sensitive organism, as well as with the enteral administration of bacteria of a closely related pseudotuberculosis microbe (*Y. pseudotuberculosis*) [7,8], the most important stage of the infectious process is the interaction of the pathogen with the intestinal mucosa.

The high colonization resistance of the intestinal mucosa and its resistance to adhesion and colonization by pathogenic and opportunistic microorganisms is an integral part of the normal intestinal defense mechanisms. In the scientific literature, the integrative function of the gastrointestinal tract to protect the body from pathogenic microorganisms and harmful substances is associated with colonization resistance, which is fully associated with such morphofunctional formation as the microbial-tissue complex. This is a complex evolutionary polyfunctional association consisting of the mucosal microbiota colonizing the parietal zone of the gastrointestinal tract mucous microbiota and the underlying tissue structures of the stomach and intestines [20]. The inseparability of the gastrointestinal microbiota and colonization resistance took shape in the appropriate formulation as a set of mechanisms that ensure the ability of the microbiota and macroorganism, cooperatively interacting, to protect the ecosystem of the gastrointestinal tract from the effects of pathogenic microbiota [21-24]. In other words, colonization resistance is a complex of mechanisms that provide protection against pathogen access to the epithelium of the stomach and intestines and their subsequent penetration into the body. The quantitative and qualitative composition of

the microbiota of the gastrointestinal tract, as well as the state of its habitat, is one of the determining conditions for the effectiveness of colonization resistance [25,26].

The creation of immunity to intestinal pathogens can be achieved, as demonstrated by the example of yersiniosis [27,28], by increasing the colonization resistance of the mucosa of the gastrointestinal tract of experimental animals when vaccinated with a recombinant vaccine strain of a pseudotuberculosis microbe that stably inherits the genes that determine F1 biosynthesis protecting white mice and guinea pigs from infection with the causative agents of plague, pseudotuberculosis and intestinal yersiniosis, or use for this purpose immunization with porin, a preparation isolated from bacterial cells of *Y. pseudotuberculosis* [28].

Another method, which implements the approach of targeted action on different links of the infectious process, is the method of efferent therapy, including the use of antiadhesive drugs - means that prevent the attachment of the causative agent to the mucous membranes of the gastrointestinal tract, first tested in experiments with plague. anthrax and salmonellosis [29].

In experimental infectious diseases, a promising method of monotherapy of acute helicobacteriosis with the metaprebiotic Stimbifid plus, tested on animals and human volunteers, has been proposed [30,31]. The positive effect of monotherapy for acute helicobacteriosis is associated with the implementation of two interrelated mechanisms of restriction (i.e. limitation) of *Helicobacter pylori* bacteria, the causative agent of an infectious disease, triggered by the metaprebiotic Stimbifid plus: direct action on the pathogen and indirect action associated with the stimulation of the reproduction of gastric microbiota that produces exometabolites, and restoration of natural colonization resistance. Both mechanisms of action of the metaprebiotic ultimately lead to the eradication of the pathogen. To this should be added the recently obtained data on antimicrobial peptides, which are expressed by the gastric mucosa in patients with helicobacteriosis, regardless of the clinical state of the patients [32].

Obviously, by influencing the pathogen, promoting the revival of the gastric microbiota, restoring and increasing the colonization resistance of the gastric mucosa, stimulating the expression of anti-*Helicobacter* peptides, the task was achieved: complete eradication of the pathogen, restoration of the qualitative and quantitative composition of the gastric microbiota, relief of infectious process with the elimination of morphological and clinical signs of gastritis [31].

Both formulated mechanisms of restriction of the pathogen *H. pylori* need experimental confirmation using not only the causative agent of stomach infection, but also pathogens of intestinal infections, if only for the reason that most publications on dysbiosis and intestinal microbiota presented data previously all in terms of colonization resistance of the intestinal mucosa, and to a lesser extent of the stomach.

The aim of the study is to analyze the role of colonization resistance of the gastric and intestinal mucosa in the development of bacterial infections of the gastrointestinal tract in conventional white mice.

## Materials and Methods

The following strains of microorganisms were used in the experiments: strain *Y. enterocolitica* KM1407 serotype 0:9, containing a plasmid with a molecular weight of 42 MDa, was isolated from a patient with a manifest form of intestinal yersiniosis, severe symptoms of gastroenterocolitis and joint damage, the $LD_{50}$ value for white mice when administered orally is $7,4 \cdot 10^7$ live microbes [33]; strain *Y. pseudotuberculosis* KM07 serotype I, containing a calcium-dependence plasmid with a molecular weight of 47 MDa, was isolated from a patient with severe symptoms of pseudotuberculosis, the $LD_{50}$ value for white mice after oral administration is $8,5 \cdot 10^4$ live microbes [34,35]; strain *S. enteritidis* KM08 isolated from a patient with severe symptoms of gastroenterocolitis, the $LD_{50}$ value for white mice when administered orally is $8,7 \cdot 10^7$ live microbes; *E. coli* strain EG was isolated from a patient with severe symptoms of gastroenterocolitis and bloody diarrhea, the $LD_{50}$ value for white mice after oral administration is $7,1 \cdot 10^7$ live microbes.

We used conventional white mice weighing 18 - 20 g, acclimatized in a vivarium for experiments to study the composition of the gastric and intestinal microbiota. During the experiments, stomachs were taken from the animals, homogenized in isotonic sodium chloride solution and plated on a dense nutrient medium for growing at a temperature of 37 °C and counting the grown colonies.

Cultivation of cultures of test microorganisms was carried out under optimal conditions on nutrient media of the recommended composition [36]. The number of representatives of the intestinal and gastric microbiota (CFU) - bifidobacteria, lactobacilli and escherichia, isolated from homogenates of stomachs and faeces of animals, was determined by seeding the corresponding tenfold dilutions of the studied suspensions on solid nutrient media [36] in Petri dishes using systems for anaerobic cultivation Anaerobic system Mark III-LE003 (HiMedia Laboratories Pvt. LTD, Mumbai, India) with gas generator packages HiAnaero Gas Pacet and counting grown colonies after incubation time.

Identification of exogenous (from the oral cavity) invasive microorganisms was carried out by morphological characteristics and using a kit for biochemical identification of fungi of the genus *Candida* (manufactured by HiMedia, India), miniature biochemical series of API strips (bioMerieux, France), as well as using a test -systems AUXACOLOR2 (Bio-RAD Laboratories, USA) and test systems for identification of microorganisms MICRO-LA-TEST (ERBA Lachema, Croatia).

Metaprebiotic Stimbifid plus was used in the experiments, 1 tablet of which weighing 500 mg contains oligofructose 270 mg, inulin 116 mg, calcium 19 mg (manufactured by OOO V-MIN, Sergiev Posad, Russia).

For histological examination, fragments of the stomach and small intestine were taken, the tissues were fixed in a 10% solution of neutral formalin, dehydrated in isopropanol, and embedded in paraffin. Tissue section preparations were stained with Ehrlich's hematoxylin and eosin and examined on a Mikmed-2 microscope (LOMO LLC, Russia) at a magnification of 100-200 ×.

Statistical processing of the experimental results was carried out according to the Kerber method as modified by I.P. Ashmarin and A.A. Vorobyov [37].

## Results and Discussion

Due to the fact that quite often the state of the microbiocenosis of biotopes of the gastrointestinal tract is assessed by the number of so-called beneficiary microorganisms, which include bifidobacteria, lactobacilli and escherichia, at the first stage of research we obtained comparative data on the quantitative composition of the microbiota of the stomach of humans and conventional white mice with intact mucosa.

The number of bifidobacteria in 1 ml of gastric contents of humans ranges from 0 to $4{,}0 \cdot 10^2$ CFU, lactobacilli - reaches $(3{,}0 - 5{,}0) \cdot 10^3$ CFU, escherichia are absent; the number of bifidobacteria in 1 ml of gastric contents of conventional white mice reaches $(4{,}6 \pm 0{,}6) \cdot 10^4$ CFU, lactobacilli - $(3{,}9 \pm 0{,}6) \cdot 10^5$ CFU, escherichia - $(4{,}6 \pm 0{,}7) \cdot 10^3$ CFU; in addition to the beneficiaries, streptococci, candida, *Staphylococcus aureus*, saccharomyces, *Pseudomonas aeruginosa*, *Klebsiella* spp. were identified, the number of which reached a value of $(1{,}6 \pm 0{,}7) \cdot 10^4$ CFU.

Obviously, the lifestyle of rodents, in particular house mice (*Mus musculus* species), determined the natural, evolutionarily formed microbiota of the stomach, corresponding to the type of nutrition, as well as a more powerful level of colonization resistance, which coordinately counteract the adhesion and colonization of the gastric mucosa by *Helicobacter pylori* bacteria at the beginning of the infectious process, translating it into the category of anthroponoses [30,31].

These results allow us to conclude that the normobiota of the stomach in an adequate amount, as well as the colonization resistance of the mucosa, largely determine "health or disease" and this statement is consonant with the data on the importance of restoring the microbiota of the stomach and intestines given in [38].

Taking into account the results noted above, as well as experimental data obtained on laboratory animals and human volunteers [31], indicating the effectiveness of the metaprebiotic Stimbifid plus in correcting disorders and restoring colonization resistance of the gastric mucosa in helicobacteriosis and eradication of the pathogen, special experiments were performed to assess the possibility of using the potential of the metaprebiotic Stimbifid plus when administered orally to conventional white mice to create a microecological advantage not only for their gastric microbiota, but also intestinal microbiota, and increase colonization resistance, which would provide active resistance to intestinal pathogens that cause pseudotuberculosis, intestinal yersiniosis, salmonellosis and escherichiosis.

The conventional white mice used in the experiments were divided into 4 groups of 30 individuals per group, respectively, for each of the causative agents of intestinal infections. In each group of animals, 3 subgroups were identified (10 animals per subgroup). The first subgroup was the control one: the animals of this subgroup were infected orally with the culture of the causative agent of intestinal infection. Animals of the second (experimental) subgroup 3 days before infection and within 6 days after infection were orally administered metaprebiotic Stimbifid plus, and animals of the third experimental subgroup were orally administered metaprebiotic Stimbifid plus for 6 days 2 hours after oral infection with the pathogen culture intestinal infection. The administered daily dose of the metaprebiotic Stimbifid plus was 7,8 mg per animal (in a volume of 0,2 ml of distilled water), which corresponds to that for humans.

The infectious dose of pathogens of intestinal yersiniosis, pseudotuberculosis, salmonellosis and escherichiosis for animals of four groups was 8 $LD_{50}$, which ensured the development of the infectious process with oral administration of a bacterial suspension.

The number of dead and surviving animals, the timing of death, the content of bacteria - representatives of the gastric and intestinal microbiota, imported microflora were noted during the experiments, and the stomachs and intestines of animals were taken for pathohistological study. When isolating and identifying the introduced microbiota, we were guided by the previously obtained data [31] on its presence in the stomach of conventional white mice orally infected with *H. pylori* bacteria. Figure 1 shows the cultures of microorganisms of gastric contents grown on a solid nutrient medium on the second day after oral administration of *Helicobacter* bacteria to animals.

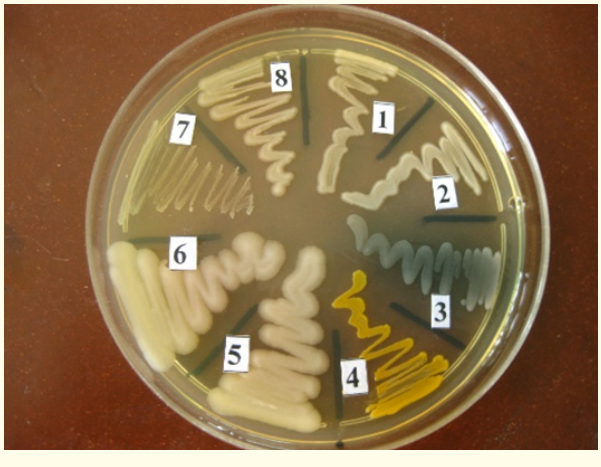



***Figure 1:*** *Growth of microorganisms isolated from the stomach contents of conventional white mice on day 2 after infection with H. pylori KM-11 (RifR), on a solid nutrient medium.*

*1 - H. pylori KM-11 (RifR), 2 - Escherichia coli, 3 - Pseudomonas aeruginosa, 4 - Staphylococcus aureus, 5 – Candida sp., 6 – Streptococcus sp., 7 – Lactobacillus sp., 8 – Bifidobacterium sp.*

The results of studies with causative agents of intestinal infections and the histopathological picture of lesions of the stomach and small intestine are shown in table 1 to 5 and in (Figure 2 and 3).

| Causative agent of infection | Group of animals | Bacterial content...in stomach homogenates, CFU·g$^{-1}$ (x ±$I_{95}$, n=10) | | | | |
|---|---|---|---|---|---|---|
| | | Causative agent of infection | Bifidobacteria | Lactobacilli | Escherichia | Alien microbiota |
| *Y.enterocolitica* KM1407 | Control | (3,5 ± 0,7)x10$^6$ | 130 ± 25 | 115 ± 15 | 89 ± 9 | - |
| | Experimental 1 | 0 | (4,8 ± 0,7) x10$^4$ | (3,9 ± 0,6) x10$^5$ | (4,0 ± 0,6) x10$^3$ | + |
| | Experimental 2 | 0 | (4,6 ± 0,6) x10$^4$ | (3,5 ± 0,7) x10$^5$ | (3,1 ± 0,7) x10$^3$ | + |
| *Y. pseudotuberculosis* KM07 | Control | (6,8 ± 0,7)x10$^6$ | 180 ± 35 | 160 ± 35 | 95 ± 16 | - |
| | Experimental 1 | 0 | (4,6 ± 0,5) x10$^4$ | (3,8 ± 0,6) x10$^5$ | (4,3 ± 0,6) x10$^3$ | + |
| | Experimental 2 | 0 | (4,5 ± 0,6) x10$^4$ | (3,7 ± 0,7) x10$^5$ | (4,1 ± 0,7) x10$^3$ | + |
| *S. enteritidis* KM08 | Control | (4,5 ± 0,6)x10$^6$ | 109 ± 28 | 85 ± 9 | 69 ± 9 | - |
| | Experimental 1 | 0 | (4,6 ± 0,7) x10$^4$ | (3,8 ± 0,6) x10$^5$ | (4,5 ± 0,7) x10$^3$ | + |
| | Experimental 2 | 0 | (4,7 ± 0,8) x10$^4$ | (3,7 ± 0,6) x10$^5$ | (4,2 ± 0,6) x10$^3$ | + |
| *E.coli* EG | Control | (3,9 ± 0,7)x10$^6$ | 89 ± 8 | 65 ± 9 | 69 ± 9 | - |
| | Experimental 1 | 0 | (4,6 ± 0,6) x10$^4$ | (3,7 ± 0,5) x10$^5$ | (4,6 ± 0,7) x10$^3$ | + |
| | Experimental 2 | 0 | (4,5 ± 0,6) x10$^4$ | (3,6 ± 0,7) x10$^5$ | (4,4 ± 0,7) x10$^3$ | + |
| Notes:<br>1) At the beginning of the experiment, the number of bifidobacteria in 1 ml of gastric contents (4,5±0,7)·10$^4$ CFU, lactobacilli – (3,6±0,6)·10$^5$ CFU, escherichia – (4,5±0,5)·10$^3$ CFU.<br>2) Experimental group 1: administration of the metaprebiotic Stimbifid plus per os 3 days before infection and within 6 days after infection; experimental group 2: administration of the metaprebiotic Stimbifid plus per os 2 hours after infection for 6 days.<br>3) "+" - the presence of the introduced microbiota, "-" - the absence of the introduced microbiota. | | | | | | |

***Table 1:*** *The results of determining the number of pathogens of intestinal infections, representatives of gastric microbiota and imported microbiota in homogenates of the stomachs of conventional white mice on the 21st day of the experiment (or on the day of death).*

| № | Group of animals | Infectious dose, CFU | Number of animals, individuals ... | | Dates of death, days, ` ($X_{min}$-$X_{max}$) | The content of live bacteria in 1 g of feces on ... day of the experiment, CFU·g$^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|
| | | | taken in the experiment | died from intestinal yersiniosis | | isolated microorganisms | start of experiment | 21 (or on the day of death) |
| 1 | Control | 8 $LD_{50}$ (5,9·10$^8$) | 10 | 10 | 10,2 (8-16) | Total amount | (6,8 ± 0,5)·10$^9$ | (1,8 ± 0,7)·10$^4$ |
| | | | | | | Bifidobacteria | (6,7 ± 0,6)·10$^6$ | (1,4 ± 0,6)·10$^2$ |
| | | | | | | Lactobacilli | (2,7 ± 0,7)·10$^8$ | (1,2 ± 0,6)·10$^2$ |
| | | | | | | Escherichia | (1,9 ± 0,5)·10$^4$ | (1,1 ± 0,5)·10$^1$ |
| 2 | Experimental 1 Administration of the metaprebiotic Stimbifid plus per os 3 days before infection and within 6 days after infection | | 10 | 0 | - | Total amount | (6,6 ± 0,6)·10$^9$ | (8,0 ± 0,6)·10$^9$ |
| | | | | | | Bifidobacteria | (6,5 ± 0,7)·10$^6$ | (5,2 ± 0,6)·10$^7$ |
| | | | | | | Lactobacilli | (2,2 ± 0,5)·10$^8$ | (4,2 ± 0,7)·10$^8$ |
| | | | | | | Escherichia | (1,6 ± 0,7)·10$^4$ | (3,9 ± 0,6)·10$^4$ |
| 3 | Experimental 2 Administration of the metaprebiotic Stimbifid plus per os 2 hours after infection within 6 days | | 10 | 0 | - | Total amount | (6,9 ± 0,6)·10$^9$ | (4,1 ± 0,7)·10$^9$ |
| | | | | | | Bifidobacteria | (6,4 ± 0,8)·10$^6$ | (5,8 ± 0,6)·10$^7$ |
| | | | | | | Lactobacilli | (2,7 ± 0,6)·10$^8$ | (4,4 ± 0,6)·10$^8$ |
| | | | | | | Escherichia | (1,2 ± 0,5)·10$^4$ | (3,0 ± 0,8)·10$^4$ |
| Note - Here and in Tables 3,4,5 are the results of determining the content of bacteria isolated from the intestinal contents of dead animals in group 1 (control); in groups 2-3 (experiment) on the 21st day of the experiment | | | | | | | | |

***Table 2:*** *Protective efficacy of the metaprebiotic Stimbifid plus at oral infection of conventional white mice with the causative agent of intestinal yersiniosis (±$I_{95}$, n = 10).*

| № | Group of animals | Infectious dose, CFU | Number of animals, individuals ... | | Dates of death, days, ` ($X_{min}$-$X_{max}$) | The content of live bacteria in 1 g of feces on ... day of the experiment, CFU·g$^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|
| | | | taken in the experiment | died from pseudotuberculosis | | isolated microorganisms | start of experiment | 21 (or on the day of death) |
| 1 | Control | 8 $LD_{50}$ (6,8 $10^5$) | 10 | 10 | 8,6 (6-14) | Total amount | (6,7 ± 0,6)·$10^9$ | (1,8 ± 0,6)·$10^4$ |
| | | | | | | Bifidobacteria | (6,4 ± 0,6)·$10^6$ | (1,2 ± 0,6)·$10^2$ |
| | | | | | | Lactobacilli | (2,6 ± 0,7)·$10^8$ | (1,4 ± 0,6)·$10^2$ |
| | | | | | | Escherichia | (1,3 ± 0,5)·$10^4$ | (1,1 ± 0,5)·$10^1$ |
| 2 | Experimental 1 Administration of the metaprebiotic Stimbifid plus per os 3 days before infection and within 6 days after infection | | 10 | 0 | - | Total amount | (6,5 ± 0,6)·$10^9$ | (5,6 ± 0,5)·$10^9$ |
| | | | | | | Bifidobacteria | (6,5 ± 0,7)·$10^6$ | (5,2 ± 0,6)·$10^7$ |
| | | | | | | Lactobacilli | (2,7 ± 0,8)·$10^8$ | (4,2 ± 0,7)·$10^8$ |
| | | | | | | Escherichia | (1,6 ± 0,7)·$10^4$ | (4,9 ± 0,6)·$10^4$ |
| 3 | Experimental 2 Administration of the metaprebiotic Stimbifid plus per os 2 hours after infection within 6 days | | 10 | 0 | - | Total amount | (6,6 ± 0,6)·$10^9$ | (5,1 ± 0,7)·$10^9$ |
| | | | | | | Bifidobacteria | (6,5 ± 0,8)·$10^6$ | (5,5 ± 0,6)·$10^7$ |
| | | | | | | Lactobacilli | (2,7 ± 0,5)·$10^8$ | (4,2 ± 0,6)·$10^8$ |
| | | | | | | Escherichia | (1,6 ± 0,7)·$10^4$ | (3,6 ± 0,8)·$10^4$ |

***Table 3:*** *Protective efficacy of the metaprebiotic Stimbifid plus at oral infection of conventional white mice with the causative agent of intestinal pseudotuberculosis (±$I_{95}$, n = 10).*

| № | Group of animals | Infectious dose, CFU | Number of animals, individuals ... | | Dates of death, days, ` ($X_{min}$-$X_{max}$) | The content of live bacteria in 1 g of feces on ... day of the experiment, CFU·g$^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|
| | | | taken in the experiment | died from intestinal infection | | isolated microorganisms | start of experiment | 21 (or on the day of death) |
| 1 | Control | 8 $LD_{50}$ (5,7·$10^8$) | 10 | 10 | 10,2 (8-16) | Total amount | (6,8 ± 0,5)·$10^9$ | (1,4 ± 0,7)·$10^4$ |
| | | | | | | Bifidobacteria | (6,7 ± 0,6)·$10^6$ | (1,3 ± 0,6)·$10^2$ |
| | | | | | | Lactobacilli | (2,7 ± 0,7)·$10^8$ | (1,2 ± 0,6)·$10^2$ |
| | | | | | | Escherichia | (1,9 ± 0,5)·$10^4$ | (1,1 ± 0,5)·$10^1$ |
| 2 | Experimental 1 Administration of the metaprebiotic Stimbifid plus per os 3 days before infection and within 6 days after infection | | 10 | 0 | - | Total amount | (6,6 ± 0,6)·$10^9$ | (8,0 ± 0,6)·$10^9$ |
| | | | | | | Bifidobacteria | (6,5 ± 0,7)·$10^6$ | (5,2 ± 0,6)·$10^7$ |
| | | | | | | Lactobacilli | (2,2 ± 0,5)·$10^8$ | (4,2 ± 0,7)·$10^8$ |
| | | | | | | Escherichia | (1,6 ± 0,7)·$10^4$ | (3,9 ± 0,6)·$10^4$ |
| 3 | Experimental 2 Administration of the metaprebiotic Stimbifid plus per os 2 hours after infection within 6 days | | 10 | 0 | - | Total amount | (6,9 ± 0,6)·$10^9$ | (4,1 ± 0,7)·$10^9$ |
| | | | | | | Bifidobacteria | (6,4 ± 0,8)·$10^6$ | (5,8 ± 0,6)·$10^7$ |
| | | | | | | Lactobacilli | (2,7 ± 0,6)·$10^8$ | (4,4 ± 0,6)·$10^8$ |
| | | | | | | Escherichia | (1,2 ± 0,5)·$10^4$ | (3,0 ± 0,8)·$10^4$ |

***Table 4:*** *Protective efficacy of the metaprebiotic Stimbifid plus at oral infection of conventional white mice with enterotoxigenic E. coli (±$I_{95}$, n = 10).*

| № | Group of animals | Infectious dose, CFU | Number of animals, individuals ... | | Dates of death, days, ` ($X_{min}$-$X_{max}$) | The content of live bacteria in 1 g of feces on ... day of the experiment, CFU·g$^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|
| | | | taken in the experiment | died from salmonellosis | | isolated microorganisms | start of experiment | 21 (or on the day of death) |
| 1 | Control | 8 $LD_{50}$ ($6,9·10^8$) | 10 | 10 | 9,4 (6-15) | Total amount | $(6,3 ± 0,5)·10^9$ | $(1,4 ± 0,7)·10^4$ |
| | | | | | | Bifidobacteria | $(6,1 ± 0,6)·10^6$ | $(1,3 ± 0,6)·10^2$ |
| | | | | | | Lactobacilli | $(2,5 ± 0,7)·10^8$ | $(1,1 ± 0,6)·10^2$ |
| | | | | | | Escherichia | $(1,2 ± 0,5)·10^4$ | $(1,1 ± 0,5)·10^1$ |
| 2 | Experimental 1 Administration of the metaprebiotic Stimbifid plus per os 3 days before infection and within 6 days after infection | | 10 | 0 | - | Total amount | $(6,6 ± 0,6)·10^9$ | $(7,6 ± 0,6)·10^9$ |
| | | | | | | Bifidobacteria | $(6,5 ± 0,7)·10^6$ | $(5,8 ± 0,6)·10^7$ |
| | | | | | | Lactobacilli | $(2,2 ± 0,5)·10^8$ | $(4,1 ± 0,7)·10^8$ |
| | | | | | | Escherichia | $(1,6 ± 0,7)·10^4$ | $(2,9 ± 0,6)·10^4$ |
| 3 | Experimental 2 Administration of the metaprebiotic Stimbifid plus per os 2 hours after infection within 6 days | | 10 | 0 | - | Total amount | $(6,9 ± 0,6)·10^9$ | $(5,1 ± 0,7)·10^9$ |
| | | | | | | Bifidobacteria | $(6,4 ± 0,8)·10^6$ | $(5,2 ± 0,6)·10^7$ |
| | | | | | | Lactobacilli | $(2,7 ± 0,6)·10^8$ | $(4,4 ± 0,6)·10^8$ |
| | | | | | | Escherichia | $(1,2 ± 0,5)·10^4$ | $(3,1 ± 0,8)·10^4$ |

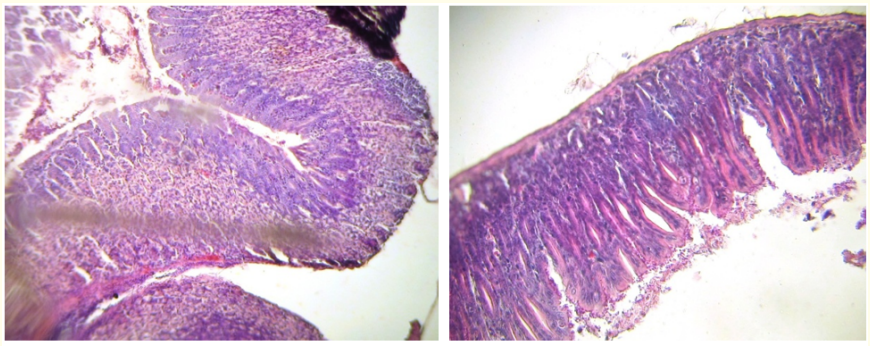
***Table 5:*** *Protective efficacy of the metaprebiotic Stimbifid plus at oral infection of conventional white mice with Salmonella ($±I_{95}$, n = 10).*

As follows from the data presented in table 1, in animals of the control groups, orally infected with pathogens of intestinal infections, the corresponding infectious disease actually developed, as evidenced by the isolation of pure cultures of pathogenic *Yersinia*, *Salmonella* and *Escherichia* from the organs of dead animals.

The data of bacteriological studies of homogenates of stomachs of dead animals give grounds to assert the development of dysbiotic changes in the microbial community with a sharp decrease in bifidobacteria, lactobacilli and escherichia in comparison with their initial amount (moreover, bifidobacteria showed a higher resistance to pathogenicity factors of infectious agents), with the disappearance of invasive microbial bacteria and with a significant content in the microbial communities of cells of pathogens of intestinal yersiniosis, pseudotuberculosis, salmonellosis and escherichiosis.

According to published data [39], manifest forms of intestinal infections, especially in young children, are often complicated by the development of aspiration syndrome (pneumonia, acute respiratory failure, myocarditis) in the early days of the disease due to translocation of the pathogen to other biotopes, which confirms the results of these studies about the non-accidental nature of the detection of pathogens in the stomach and other organs of experimental animals.

Changes in the gastric mucosa are indicated by the results of a histopathological examination of organ fragments in a healthy white mouse infected with *S. enteritidis* KM08 pathogen (Figure 2). Microscopic examination of the histoarchitectonics of the stomach of a healthy mouse is preserved, the blood filling is moderate, the thickness of the mucous membrane is moderate, the glands are deep, there are many parietal cells, the epithelium is without signs of dystrophy, and there are no focal changes and infiltration.



A B

***Figure 2:*** *Section of the stomach of a healthy mouse (A) and a mouse infected with the pathogen S. enteritidis KM08 (B).*

Macroscopically, the stomach of an infected animal is swollen, the walls are thinned, a hyperemic area is determined in the region of the stomach body. In an experimental (infected) animal, the mucous membrane of the affected area is thinned, the glands are atrophic, in some places there are shallow erosions, blood circulation is reduced, in the submucosa there is moderately expressed small-cell infiltration. In general, the histopathological picture corresponds to atrophic gastritis.

Pathomorphological changes similar to those described were revealed in the stomachs of animals infected with other pathogens, with the only difference that with yersiniosis, a picture of capillaritis with secondary changes in vascular genesis is observed.

Metaprebiotic Stimbifid plus administered orally to infected animals in appropriate doses completely prevented the colonization and multiplication of intestinal infections bacteria and the generalization of the infectious process in animals of the experimental groups. Bacteriological study of homogenates of the stomachs of these groups of animals indicates that they did not reveal dysbiotic changes in the composition of the gastric microbiota, and by the 21st day of the experiment, the introduced microbiota was released.

Thus, the totality of stomach microorganisms of different species, with the assistance of the metaprebiotic Stimbifid plus, formed a kind of microecological consortium that resists intestinal pathogens.

From the results presented in tables 2, 3, 4 and 5, it can be seen that the animals of the control subgroups died 6-16 days after oral administration of microbial suspensions as a result of infection with pathogens of yersiniosis, salmonellosis and escherichiosis. A pure culture of the pathogens with which they were infected was isolated from all the dead animals, which is evidence of the generalization of the infectious process.

A bacteriological study of the contents of the colon of conventional white mice that died from intestinal yersiniosis, pseudotuberculosis, salmonellosis and escherichiosis revealed the growth of bacteria of these infections. At the same time, there was a decrease in the total number of bifidobacteria - by four orders of magnitude, lactobacilli - by six orders of magnitude, escherichia - by three orders of magnitude.

The noted imbalance of the intestinal microbiota is evidence of the developed dysbiosis, against the background of which the pathogens that caused the infectious process, possessing a solid set of pathogenicity factors [40], are able to initiate an inflammatory response of the mucosa of the digestive tract, which facilitates their translocation into the blood capillaries and further spread through the circulatory system throughout the body.

Metaprebiotic Stimbifid plus, administered orally to animals of the second and third experimental subgroups, respectively, 3 days before infection and within 6 days after infection (experimental subgroup 1) and 2 hours after oral infection with a culture of the pathogen of intestinal infection within 6 days after infection (experimental subgroup 2), completely prevented the multiplication of pathogens *Y. enterocolitica* KM1407, *Y. pseudotuberculosis* KM07, *S. enteritidis* KM08, *E. coli* EG in the intestine, generalization of the process, and the death of animals. In the animals of the second and third experimental subgroups that survived after infection, no causative agents of intestinal infections were identified in the feces. In addition, bacteriological examination of animal feces did not reveal dysbiotic changes in the intestinal microbiota, as was the case in the study of the contents of the large intestine of animals in the control group.

It is important to emphasize that the protective effect of the metaprebiotic Stimbifid plus against the causative agents of intestinal infections is manifested both against the background of its "prophylactic" administration (that is, 3 days before infection of animals of the second subgroup), and 2 hours after infection of animals (third subgroup).

Histological examination of sections of the small intestine of a healthy animal and infected with the pathogen *S. enteritidis* KM08 revealed significant differences (Figure 3). In animals of the control group, the blood filling of the wall of the small intestine is moderate, there is no hemorrhage, the thickness of the mucosa is moderate, the villi are pronounced and well contoured, the epithelium of the villi and crypts is without signs of dystrophy, the submucosa without infiltration.

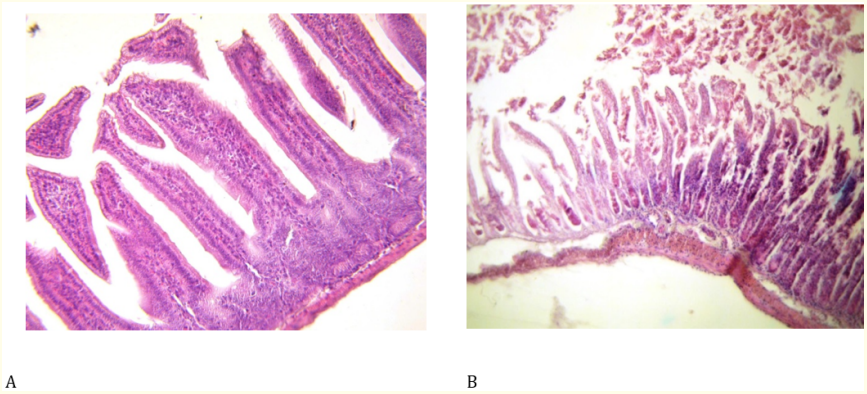



***Figure 3:*** *Section of the small intestine of a healthy mouse (A) and a mouse infected with the pathogen S. enteritidis KM08 (B).*

Histological examination of the small intestine of an infected animal shows that the intestinal wall is sharply thinned, the blood supply is reduced, the villi are fragmented, and some villi are necrotic, the submucosa with small focal infiltration, the muscular membrane is thinned, myocytes are edematous, dystrophic.

Pathohistological changes in the small intestine in animals infected orally with pathogenic Yersinia and Escherichia are generally similar to changes in the small intestine in animals infected with Salmonella, and these changes affect almost the entire thickness of the intestinal wall.

Despite the fact that the pathogens used in the experiments cause diseases that differ clinically, morphologically and epidemiologically, they have a common enteral route of entry into the body of humans and animals, and the first barrier to their further spread, in accordance with individual characteristics of manifestation of *In vivo* activity of pathogenic factors, is the stomach. It is this clinically significant barrier that falls out of sight of scientists and physicians who are not familiar with the results of experimental studies on the role of gastric

microbiota and colonization resistance in the development of intestinal infections. This is of fundamental importance, since even in the recommendations of the Maastricht V/Florentine Consensus for the treatment of *Helicobacter pylori* infection in paragraph 2 of subsection "*H. pylori* and gastric microflora "explicitly states that the composition of a healthy stomach microbiota and how *H. pylori* bacteria affect the microbiota have not yet been determined [41,42].

It can be assumed that this statement is also applicable to the gastric microbiota of laboratory animals, since experimental infectious diseases have not yet become widespread in clinical practice. That is why the concept of the benefits of adding certain probiotics to antibiotic regimens for the treatment of *H. pylori* in order to increase the rate of eradication of the pathogen *H. pylori* did not find its experimental confirmation, just as the concept of the effectiveness of mono-prescription of a probiotic without concomitant antibiotic therapy was not confirmed [43].

Currently, the arsenal of antibacterial drugs, including for the eradication of *H. pylori*, is quite sufficient [41], but the problem is that the doctor needs to choose the right treatment regimens and apply them consistently. It was to help the practitioner that internationally agreed documents were developed [41,42], which prescribe the choice of eradication treatment, taking into account the population antibiotic resistance of the pathogen and the individual history of antibiotic use, and in case of ineffectiveness of treatment, successively switching from one line of therapy to the next, and so on to the last 16 line (triple therapy with rifabutin) [41,44].

The ineffectiveness of the treatment of both helicobacteriosis and intestinal infections cannot simply be reduced to the resistance of the causative agent of intestinal infection to one or another antibiotic [45], the side effect of which may be dysbiosis as a violation of the qualitative and quantitative composition of the microbiota of the digestive tract, which greatly complicates the course of the disease itself.

The results of both current and previous experimental studies [28,31,33,35] indicate the need to take into account the state of the gastric and intestinal microbiota, as well as the colonization resistance of the gastrointestinal mucosa in the treatment of gastrointestinal infections. In this regard, it should be emphasized that the epithelial surface of the stomach and intestines is phylogenetically the most ancient defense system of the whole organism. Acting as a primary protective barrier, it prevents the translocation of pathogenic bacteria to other biotopes.

The results of a histopathological study of sections of the stomach and small intestine of conventional white mice (Figures 2 and 3), infected with the causative agent of salmonellosis, are visible evidence of the depth of destruction of their mucous membrane under the influence of pathogenic factors of bacteria *S. enteritidis* KM08. The destruction of the mucosa itself is nothing more than the dismemberment of the microbial-tissue complex, which, with all its structural elements, is considered in the scientific literature as the "cradle of the immune system", since evolutionarily the complex was in constant contact with environmental antigens, including antigens of microorganisms [46].

However, the microbiota as a constituent element of the microbial-tissue complex is not a kind of solidified formation, it is constantly renewed [47], being in dynamic equilibrium with microorganisms coming from outside. It should be especially noted that the formation of the microbiota of the gastrointestinal tract begins even from the prenatal period of life and is a long, complex, multifactorial process, the violation of which is associated with the development of various pathological conditions in the child's body [48]. By the end of the first year of life, the formation of the microbiocenosis of the digestive tract is practically completed, but its differences from the microbiocenosis of an adult persist up to 7 years. And all the following years, from birth to old age, the macroorganism reacts sharply to the intake of microbiota from the oral cavity and controls its number using the means at its disposal: mechanical, chemical, immune (specific and nonspecific).

It is this set of response means that the macroorganism uses in the fight against pathogens of the gastrointestinal tract, which, in turn, fully implements the evolutionarily established set of pathogenicity factors [40,49], emerging victorious in this struggle. In the current

situation, when antibiotic therapy does not promote recovery, but inhibits the natural microbiota of the digestive tract with the subsequent development of dysbiosis, it remains to rely only on a local increase in the colonization resistance of the gastric mucosa and, in general, the gastrointestinal tract and on the creation of a microecological advantage for the residues of normobiota in conditions for the development of the infectious process.

At the same time, the state of colonization resistance directly depends on the biological properties of microorganisms of the entire community of the gastrointestinal tract, as well as opportunistic (pathogenic) and probiotic microorganisms prescribed for prophylactic or therapeutic purposes [50]. In addition, in the mechanisms of colonization resistance, an important role is played by the blockade of adhesion of opportunistic microorganisms to the surface of the gastric mucosa with the participation of immunoglobulins, as well as non-specific immune defense, phagocytic protection of macrophages and leukocytes, the complement and lysozyme system, factors of cellular immunity [48].

The positive results of the present studies, which used the metaprebiotic Stimbiefid plus, give reason for some optimism. And as in the case of the complete cleansing of their stomachs from the pathogen of helicobacteriosis using the metaprebiotic Stimbifid plus, proved in experimental animals, and in the course of an experimental study on self-infection of volunteers [31], we are close to understanding that the dream of clinicians to obtain an "ideal antimicrobial agent" can be implemented in practice.

In both cases, both in experiments on conventional white mice and in the experiment of self-infection of volunteers, the therapeutic efficacy of the metaprebiotic Stimbifid plus was tested. To this should be added the results of a clinical assessment of the effectiveness of the metaprebiotic Stimbifid plus in the eradication of *H. pylori* in 30 patients with chronic gastritis associated with persistence of *H. pylori*, which was confirmed by the achievement of eradication of the pathogen reaching 80% [51].

This drug, used as an effective means of monotherapy for patients with persistent helicobacteriosis, as well as in experiments on animals orally infected with cultures of pathogens of intestinal yersiniosis, pseudotuberculosis, salmonellosis and escherichiosis, is not a classic antibiotic, resistance cannot be developed to it, the drug inhibits the growth of pathogenic bacteria and at the same time stimulates the growth of indigenous microbiota, being a source of exclusive nutrition for it; the drug is able to penetrate the mucus layer, reach the target on the surface of pathogenic bacteria without losing antimicrobial activity, as well as increase the colonization resistance of the gastric mucosa and promote its decontamination from pathogenic bacteria.

## Conclusion

In the structure of infectious pathology, diarrheal diseases occupy one of the leading places, second only to acute respiratory infections [49]. Since the beginning of the 50s, for the prevention and treatment of diseases associated with an imbalance of the symbiotic microbiota, and in particular diarrheal diseases, more than 150 different microecological agents have been developed in the world, including probiotics - living microorganisms that, when administered in adequate amounts, should have a positive effect on the host organism [38,52]. However, until now it has not been possible to clearly define the optimal number of bacteria to provide probiotic beneficial effects on human health. In addition, there is no exact knowledge about the mechanisms and targets of probitics, and experimental and clinical studies in recent years have shown the difficulty or even impossibility of designing industrially produced probiotics for the targeted maintenance of the indigenous microbiota at an optimal level [52].

Against this background, experiments were carried out to study the role of colonization resistance in the development of intestinal yersiniosis, pseudotuberculosis, salmonellosis and escherichiosis, which were a continuation of studies to analyze the possibility of using the metaprebiotic Stimbifid plus for eradication therapy of acute experimental helicobacteriosis.

In the experiments carried out, all orally infected experimental animals of the control group died within 6 to 16 days: the specificity of the lesion was established by isolating bacteriological cultures of bacteria with which the animals were infected. In animals that died

as a result of infection, significant dysbiotic changes in the microbiocenosis of the digestive tract, the absence of introduced microbiota, and pronounced pathohistological changes in the mucous membrane and submucous layer of the stomach and intestines were revealed.

All animals of the experimental groups infected with 4 types of intestinal pathogens and receiving the metaprebiotic Stimbifid plus with "prophylactic" and "therapeutic" courses survived. There were no dysbiotic changes in the gastrointestinal tract of the surviving animals on the 21st day of observation; histological studies confirmed the absence of pathomorphological changes in the mucosa and submucosa of the stomach and intestines; there was no isolation of the culture of the causative agent of an infectious disease from animals treated with the metaprebiotic Stimbifid plus.

Thus, in the course of experiments on the infection of experimental animals, evidence was obtained of the non-random effect of the metaprebiotic Stimbifid plus on the restoration of the colonization resistance of the gastric and intestinal mucosa and the preservation of the indigenous microbiota of the digestive tract of animals.

The conducted studies have shown the dominant role of colonization resistance and normal microbiota of the digestive tract in ensuring the state of protection of a sensitive organism from pathogens of intestinal infections and creating a selective microecological advantage of a normal microbiota that resists intestinal pathogens as a result of host versus pathogen biocompatibility.

And one more remark as a result of the experiments performed: for the targeted maintenance of the indigenous microbiota and the colonization resistance of the gastrointestinal mucosa at the optimal level, the metaprebiotic Stimbifid plus is industrially produced, supplied to the pharmacy network.

## Conflict of Interest

The authors declare no conflict on interests.

## Bibliography

1. Liu L., *et al*. "Global, regional and national causes of child mortality: an updated systematic analyses for 2010 with him trends since 2000". *Lancet* 379 (2012): 2151-2161.

2. Ruzhentsova TA., *et al*. "Optimization of pathogenetic therapy of acute intestinal infections in children: results of a randomized trial". *Infectious Diseases* 16. 2 (2018): 70-76.

3. Milyutina LN., *et al*. "Clinical and epidemiological aspects of the evolution of rotavirus infection in children". *Infectious Diseases* 16. 2 (2018): 6-10.

4. Gracheva NM., *et al*. "Fructooligosaccharides and fructopolysaccharides in the complex treatment of patients with gastrointestinal diseases with symptoms of intestinal dysbiosis". *Infectious Diseases* 8.1 (2010): 107-111.

5. Chicherin IYu., *et al*. "Study of the effect of large doses of the probiotic Bifidumbacterin and microorganisms of intestinal autoflora on the body of white mice and indicators of cellular immunity". *Medical Advisor of the Volga Region* 1 (2013): 85-90.

6. Zakharenko SM., *et al*. "Infections, human intestinal microbiota and metabolic syndrome". Effective pharmacotherapy". *Gastroenterology* 3 (2012): 14-20.

7. Somov GP., *et al*. "Pseudotuberculosis". 2nd edition. revised and add. M.: Medicine (2001): 256.

8. Yushchuk ND., *et al*. "Yersiniosis". M.: Medicine (2003): 208.

9. Yushchuk ND., *et al*. "Yersiniosis. In the book: Lectures on infectious diseases. In 2 volumes. Volume 1". -2nd edition. revised and add. Moscow: VUNMTs (1999): 454.

10. Yushchuk ND., *et al*. "Yersiniosis as a surgical problem". *Surgery* 12 (1999): 50-52.

11. Sedelnikova SM., *et al*. "Yersiniosis as a therapeutic problem". *Therapeutic Archive* 11 (2000): 27-30.

12. Sorrells KM., *et al*. "Pathogenicity of Salmonella gallinarum after metabolic injury by freezing". *Applied and Environmental Microbiology* 19.1 (1970): 39-43.

13. Guide to Infectious Diseases/Edition. Yu.V. Lobzin. SPb.: Foliant (2000): 932.

14. Medical Microbiology / Edition. A.M. Korolyuk, V.B. Sboychakova. SPb.: ZAO ELBI (1999): 272.

15. Pepe JC., *et al*. "Yersinia enterocolitica invasion: a primary role in the initiation of infection". *Proceedings of the National Academy of Sciences of the United States of America* 90 (1993): 6473-6477.

16. Darwin A., *et al*. "Identification of Yersinia enterocolitica genes affecting survival in an animal host using signature tagget transposon mutagenesis". *Molecular Microbiology* 32 (1999): 51-62.

17. Tseneva GYa., *et al*. "The choice of models for determining the pathogenic properties of the causative agent of pseudotuberculosis". *Journal of Microbiology* 11 (1982): 68-71.

18. Tseneva GYa., *et al*. "Characteristics of the invasiveness of the causative agent of pseudotuberculosis". *Journal of Microbiology* 5 (1984): 26-30.

19. Glushanova NA., *et al*. "The relationship of probiotic and indigenous lactobacilli of the host in conditions of co-cultivation in vitro". *Journal of Microbiology* 2 (2005): 56-61.

20. Minushkin ON., *et al*. "Microflora imbalance and its correction". *Effective Pharmacotherapy. Gastroenterology* 4 (2013): 4-8.

21. Savina VE., *et al*. "Colonization resistance of the intestine". Society, Science, Innovation (NPK-2013). Collection of materials. Kirov: Publishing house of Vyatka State University (2013): 76-78.

22. Zvyagintseva TD., *et al*. "Correction of violations of intestinal colonization resistance". URL (2021).

23. Shenderov BA. "Medical microbial ecology and functional nutrition: in 3 volumes. Vol. 1: Human and animal microflora and its functions". M.: GRANT. 1998. 288 c.

24. Shenderov BA. "Normal microflora and its role in maintaining human health". *Journal of Gastroenterology, Hepatology, Coloproctology* 7.1 (1998):61-65.

25. Lammers KM., *et al*. "Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells". *FEMS Immunology & Medical Microbiology* 38.2 (2003):165-172.

26. Veckman V., *et al*. "Lactobacilli and streptococci induce inflammatory chemokine production in human macrophages that stimulates Th 1 cell chemotaxis". *Journal of Leukocyte Biology* 74.3 (2003): 395-402.

27. Maraculin IV., *et al*. "Design of pseudotuberculosis microbial strain that is promising for obtaining a polyvalent vaccine". *Biotechnology in Russia* 4 (2004):83-89.

28. Darmov IV., *et al*. "Prevention of experimental pseudotuberculosis infection by immunization with porin from Yersinia pseudotuberculosis". *Bulletin of Experimental Biology and Medicine* 127.2 (1999): 221-223.

29. Harseeva GG., *et al*. "Suppression of bacterial adhesion: current problems, approaches, prospects". *Advances in Modern Biology* 139.5 (2019): 506-515.

30. Pogorelsky IP., *et al*. "A method for modeling helicobacteriosis". RF patent No. 2690943 dated 06.07.2019, publ. 07.06.2019. bul. No. 16. Number of the title of protection G09B 23/28 (2019).

31. Chicherin IYu., *et al*. "Evaluation of the eradication efficiency of the metaprebiotic Stimbifid plus in acute experimental helicobacteriosis in laboratory animals and human volunteers". *Infectious Diseases* 16.4 (2018):62-74.

32. Cremniter J., *et al*. "Th-17 response and antimicrobial peptide expression in gastric mucosa of Helicobacter pylori – infected patients independently of their clinical outcomes". 23.3.

33. Chicherin Yu., *et al*. "Colonization resistance of the intestinal mucosa in experimental yersiniosis". *Journal of Infectology* 5.1 (2013):75-82.

34. Marakulin IV., *et al*. "Experimental model of pseudotuberculosis infection in monkeys". *Bulletin of Experimental Biology and Medicine* 118.7 (1994): 59-62.

35. Chicherin IYu., *et al*. "Experimental pseudotuberculosis: assessment of the possibility of prevention, treatment and correction of dysbiotic disorders of the intestinal microflora". *Journal of Infectology* 4.4 (2012): 71-79.

36. Bondarenko VM., *et al*. Methodical recommendations "Microbiological diagnosis of intestinal dysbiosis". M.: GU NIIEM im. N.F. Gamalei RAMS (2007): 72.

37. Ashmarin IP., *et al*. "Statistical methods in microbiological research. L.: Medgiz (1962): 280.

38. Reid G., *et al*. "Microbiota restoration: natural and supplement recovery of human microbial communities". *Nature Reviews Microbiology* 9.1 (2011): 27-38.

39. Podkolzin AT., *et al*. "Analysis of the structure of deaths in young children with acute intestinal infections". *Infectious Diseases* 11.2 (2013): 38-44.

40. Mavzyutov AR., *et al*. "Factors of pathogenicity of opportunistic enterobacteria and their role in the development of diarrhea". *Journal of Microbiology, Epidemiology and Immunobiology* 1 (2007):89-96.

41. Malfertheiner P., *et al*. "Management of Helicobacter pylori infection – the Maastricht V". Florence Cobsrnsus Report Gut (2016).

42. Pimanov SI., *et al*. "Recommendations of Maastricht V / Florentine Consensus for the treatment of Helicobacter pylori infection". *Consilium Medicum* 8.1 (2017): 8-21.

43. Dang Y., *et al*. "The effect of probiotics supplementation on Helicobacter pylori eradication rates and side effects during eradication therapy: a mata-analysis". *PloS One* 9.11 (2014): e111030.

44. Pimanov SI., *et al*. "Optimized eradication protocols: recommendations of the American College of Gastroenterologists, Maastricht V/ Florence and Toronto Consensus". *Medical Advice* 5 (2017): 10-17.

45. Bondareva TA., *et al*. "Current state and prospects of solving the problem of increasing the effectiveness of emergency prevention and treatment of systemic bacterial infections". *Molecular Medicine* 5 (2009): 21-25.

46. Gasbarrini G., *et al*. "The role of bacterial flora and its products in the functioning of the small intestine". *Digestive and Liver Disease Supplement* 1.1 (2007): 3-6.

47. Eckburg PB., *et al*. "Diversity of the human intestinal microbial flora". *Science* 308.5728 (2005): 1635-1638.

48. Nikolaeva IV., *et al*. "Formation of a child's intestinal microbiota and factors affecting this process". *Russian Bulletin of Perinatology and Pediatrics* 63.3 (2018): 13-18.

49. Murzabaeva RT., *et al*. "Clinical and immunological parallels in acute intestinal infections caused by opportunistic enterobacteria". *Infectious Diseases* 16.4 (2018):79-85.

50. Khavkin AI. "Experience of using the probiotic L. rhamnosus GG strain in pediatrics". *Journal of International Medicine* 6.11 (2014):101-106.

51. Minushkin OV., *et al*. "The first experience of using metaprebiotic therapy in the eradication of Helicobacter pylori". *Kremlin Medicine Clinical Bulletin* 2 (2019): 36-38.

52. Shenderov BA., *et al*. "Metabiotics: yesterday, today, tomorrow". SPb.: Kraft (2017): 80.

**Volume 18 Issue 9 September 2022**