Original article

Combined method of colon microbiota correction after colon x-ray in children with chronic colostasis

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Abstract

Objective: The problem of chronic colostasis is especially relevant in children. Numerous research studies have been devoted to this type of pathology, but until now, no final solution has been proposed both in traditional therapy and surgical treatment. The research aimed to study the state of intestinal microbiota in children with chronic colostasis in preparation for irrigation and to evaluate the combined method of its correction by oral and rectal probiotic administration. ***Materials and methods:*** The study enrolled 100 patients of both genders aged 5-15 years with the diagnosis of chronic colostasis. All patients were divided into main and control groups consisting of 50 people in each one. For the correction of intestine microbiota, the probiotics in the main group were administered orally and using enema with direct irrigation of the large intestine cavity after preparation for X-ray analysis, while patients of the control group received probiotics only orally. ***Results and Discussion*:**In the main group, the concentration of all types of useful microorganisms in the intestine increased to normal values after the correction, the number of useful microorganisms is statistically significantly higher, and the number of opportunistic enterobacteria is less in comparison with the those in the control group ($p<0.001$).***Conclusion*:**The developed method of combined application of probiotics orally and introduction into the lumen of the large intestine with an enema is an effective way to correct the quantitative composition of intestinal microbiota at chronic colostasis in children.

Keywords: chronic colostasis; large intestine;microbiota.

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Introduction

To date, chronic constipation remains a relevant and socially significant problem.[1] According to various authors, it affects from 0.3 to 28% of the population.[2] Chronic colostasis, accompanied by constant colonic intoxication and sensitization, manifests into a wide range of intestinal and extraintestinal symptoms, which could lead to diagnostic and treatment mistakes.[3]

The problem of chronic colostasis is especially relevant in children. Numerous research studies have been devoted to this type of pathology, but until now, no final solution has been proposed both in traditional therapy and surgical treatment. Thus,

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there is almost no data available on the impact of medical procedures on the microbiota of the colon, in particular, repeated cleansing enemas in preparing for X-ray examination. However, these aspects are of high importance since the disproportion in the normal ratio of microorganisms in the colon can exacerbate chronic colostasis.

There are many studies in the world literature on the role of intestinal microbiota in the development of this type of pathology, which indicates the importance of microbial substances in the development of colostasis.[4-7]

The intestinal microbiota is a set of microorganisms that live in the gastrointestinal tract with concentrations up to 1011–1012 cells/g of luminal contents.[4] The overwhelming majority of microbiota in the large intestine and up to 60% of the dry mass of feces are bacteria.[5,6] The total number of bacterial species living in the human intestine is 300–1000 according to data from various authors,[5,8] although 99% of the bacteria belong to 30–40 species.[8]Thus, a 1 ml content of the distal part of the large intestine contains around 109-1012 microorganisms. The highest density of semination is observed in the rectum. Feces micro-fauna is de facto the micro-fauna of the distal part of the large intestine. The normal microbiota of the colon is for 99% composed of anaerobic bacteria, which outnumbers the number of aerobic ones by 1000 times.[9] The predominant species are *Bifidobacterium* and *Bacteroides* being present in the approximately same amount.[10] Enterobacteria are a widespread component of the intestine, as well as *Enterococci*, *Lactobacilli*, and *Clostridia*, which are detected constantly according to some reports.[11]

The process of interaction between intestinal microbiota and the human body is a complex multi-level process. The relationship between intestine and microbiota are not pure commensalism but rather interact mutually.[4]Microorganisms perform a lot of useful functions for the host. Thus, *Bifidobacterium*, *Lactobacillus*, and *Enterobacterium* species participate in the processes of digestion and metabolism, for example. This is ensured by the possibility of their emergence from a large number of enzymes (proteases, lipases, amylases, cellulases, etc.), which are directly involved in the metabolism of proteins, fats, carbohydrates, bile acids, cholesterol, bilirubin, and estrogens, as well as participate in water-electrolyte metabolism, which promotes the absorption of ions through the intestinal wall of calcium, iron, vitamin D[1]. Depending on the conditions, various representatives of the intestinal flora can positively or negatively affect the host organism. *Bifidobacterium* and *Lactobacillus* species have a pronounced antagonistic activity with respect to pathogenic bacteria, regulate the quantitative and qualitative composition of the intestinal microflora in a normal way, and inhibit the growth and multiplication of pathogenic and opportunistic microorganisms in it.[12,13] Therefore, the balance of microorganisms in the intestine is important for its normal functioning.

Based on the violation of microbiocenosis in children with chronic colostasis, the assumption has been made that these changes play a significant role in the development of chronic constipation.[1,4] Patients with chronic colostasis, especially with its decompensated form, must undergo an X-ray examination, and sometimes mandatory colon decompression procedures.[14,15] Studies of intestinal microbiota in the period after an enema showed an even more pronounced decrease in the number of beneficial intestinal microorganisms.[16] Besides, according to S. Salminenand E. Salminen, with a decrease in the number of beneficial microorganisms, pathogens take their place.[17] The Rome Consensus IV identifies chapters that address the role of intestinal microbiota in the pathogenesis of functional intestinal diseases, including irritable bowel syndrome with predominant constipation.[18] The pathogenetic role is played by products of microbiota metabolism, the interaction of microbes and host that is mediated by immune and metabolic factors, as well as changes in bile acids metabolism. From the host organism, an increase in intestinal permeability and associated changes in nervous and immune regulation are noted.

In this regard, the normalization of colon microbiocenosis is considered as one of the main tasks in the treatment of chronic colostasis. However, the methods of correcting the intestinal microbiota by oral administration of probiotics do not always achieve the desired result. Therefore, the research aimed to study the state of intestinal microbiota in children with chronic colostasis in preparation for irrigation and to evaluate the combined method of its correction by oral and rectal probiotic administration.

Materials and Methods

Object

The study included patients regardless of gender, aged 5 to 15 years, who received conservative

treatment in the surgical infections department at the City Children's Hospital No. 2 of the Nur-Sultan city from October 2018 to October 2019. The number of participants in the main group was 50 people, the control group consisted also of 50 people (total, 100 people).

The criteria of inclusion into the study were 5-15 years old children suffering from chronic constipation, who do not require surgical correction and do not have a concomitant pathology.

Exclusion criteria were children under the age of 5, as well as children suffering from constipation that requires surgical correction or with concomitant pathology.

The authors declare that the work is written with due consideration of ethical standards. The study was conducted in accordance with the ethical principles approved by the Human Experiments Ethics Committee of NSC Astana Medical University (Protocol No. 7 of 15.08.2019). Also the research was conducted according to the World Medical Association Declaration of Helsinki.All participants gave their written informed consent.

Research and Treatment Methods

Normalization of colon microbiocenosis through the method developed in the clinic for the prevention of dysbiosis by applying transrectal administration of the probiotic into the lumen of the colon (patent for the invention "Method for the prevention of Hirschsprung - associated enterocolitis in children" No. 32679 from January 29, 2018, Ministry of Justice of the Republic of Kazakhstan;[19]

• Bacteriological examination of feces;

• Biometric methods for data analysis.

Normalization of Colon Microbiocenosis

To enable the conditions for a special X-ray examination – irrigography, all patients in the main and control groups with chronic colostasis were prescribed with special preparation, which included not only a general correction of homeostasis disorders and intestinal decontamination against a background of chronic colostasis but also a special preparation of the colon with multiple cleansing enemas. For diagnostic purposes, all the children underwent an X-ray contrast study of the large intestine (irrigography).

To prepare the large intestine for irrigography, siphon enemas with 1% sodium chloride at room temperature were performed on all patients in the main and control groups to clean the intestine. The volume of enema fluid was determined by the age of the patients. Patients had indications for siphon enemas such as complaints of a stool absence or its retention for several days over a long period of time.

After preparation for X-ray examination, the intestinal microbiota was corrected by the proposed method in the main group. Probiotics were injected orally and by an enema with direct irrigation of the large intestine cavity in the age dosage specified in the instruction, namely, one pack 3-4 times per day.Control group patients received probiotics only enterally.Powder form medicines were used as probiotics:

• Bifidumbacterin Forte® containing live *Bifidobacterium sp.* in an amount of at least 50 million CFU / g that regulates the balance of intestinal microbiota. The action of the preparation is caused by a high concentration of *Bifidobacterium sp.*sorbed on activated carbon particles, which are antagonists of a wide range of pathogenic (including *Shigella spp*., *Salmonella spp*., *Staphylococcus aureus*) and opportunistic microorganisms (including *Proteus spp.,Klebsiella spp*.). Produced by Vitafarma PLC, Russia.

• Lactobacterin that includes live lactobacilli with antagonistic activity against a wide range of pathogenic and opportunistic bacteria (including *Staphylococcus*, *Proteus*, enteropathogenic *Escherichiacoli*, etc.). Produced by Microgen SPA, Russia.

Normalization of Colon Microbiocenosis Procedure

The enema is performed in a specially equipped room. Later, 30 minutes after the siphon enema, the child was placed in the position on the left side, pulling the legs bent at the knees to the stomach. At the same time, probiotic was mixed with water in age-related dosage and heated up to + 37-38° C. The Janet syringe was used to introduce this solution in the amount of up to 50 ml. A single-use sterile catheter, lubricated with sterile paraffin oil, was slowly inserted through the anus into the colon until free passage and connected to the Janet syringe injecting the drug into the intestinal lumen by slow strokes of the syringe plunger. Afterward, the syringe was disconnected clamping the catheter with a metal instrument followed by a small amount of air drawn into the syringe, the syringe was then reconnected with the catheter and, releasing air from

the syringe, drug residues were pushed from the tube into the intestine. With caution, the catheter is slowly removed from the intestinal lumen.

The Method of Bacteriological Research

The quantitative intestinal microbiota composition for bacteriological examination of feces was evaluated in accordance with the guidelines "Bacteriological diagnosis of intestinal dysbiosis" approved by Order of the Ministry of Health in the Republic of Kazakhstan (No. 60 dated 09/12/2003). Feces for research were delivered to the laboratory without preservative in a sterile glass flacon in the amount of 2-3 g within 2 hours from the moment of collection.

In the laboratory, feces were weighed in the amount of 1 g in a previously weighed sterile vial with the subsequent addition of sterile physiological saline in the amount of 9 ml. The result was the main breeding of feces 10^{-1}. The contents of the vial were thoroughly mixed with a glass rod and left at room temperature for 10-15 minutes. Afterward, a series of dilutions were prepared from the supernatant according to the scheme from 10^{-1} to 10^{-8}. Eventually, the material was sown from the dilutions on nutrient media for the cultivation of various groups of microorganisms.

The determination of the total bacteria count (TBC) is not quite informative, which, therefore, defines the necessity to identify and count specific types of microorganisms.To isolate pathogenic enterobacteria, the starting liquid material or dilution of dense stools with 10^{-1} loops was seeded on Endo agar, Ploskireva agar, and bismuth-sulfite agar (BSA) medium with addition of enrichment medium (magnesium and selenite spilled in test tubes of 5-7 ml) in the amount of 1-2 ml.

To detect candida fungi, 0.1 ml was inoculated from 10-3 dilution onto Sabouraud agar (SDA) with the addition of antibiotics.To identify staphylococci and study their lecithinase activity, 0.1 ml of 10-1 and 10-3 breeding was inoculated on vitelline salt agar (VSA).To detect creeping protea growth, 0.1 ml was inoculated from 10-3 dilution on meat-peptone agar (MPA) condensate according to the Shukevich method.To isolate opportunistic enterobacteria and some non-fermenting gram-negative bacteria 0.1 ml of 10^{-1} and 10^{-3} dilutions was inoculated on Endo agar. Of these dilutions, 1 ml was inoculated in 9 ml of Wilson-Blair medium for detecting the growth of sulfite-reducing clostridia.

To determine the hemolytic activity of microorganisms and to count them, 0.1 ml was sown from a dilution of 10^{-5} on 5% blood agar (BA).For quantitative determination of lactobacilli and enterococci, 0.1 ml of 10^{-3} dilution was inoculated onto Rogosa agar or other media for culturing lactobacilli and vitelline salt agar (VSA) with triphenyltetrazolium chloride (TTX).From 10^{-6} and 10^{-7} dilutions, 0.1 ml was inoculated on blood agar with kanamycin to detect growth and quantify Bacteroides.And, 1 ml of 10^{-7}, 10^{-8}, and 10^{-9} dilutions was inoculated into the regenerated Blaurock medium or other media recommended for the cultivation of *Bifidobacterium* species.

Sowings were incubated at 37 ° C:

1) on enrichment media – for 18 hours;

2) on the media Endo, Ploskirev, 5% BA, and MPA with sowing according to Shukevich – for up to 24 hours;

3) on the BSA, VSA, Blaurock media, and vitelline medium of Turchinsky – for up to 48 hours;

4) on the of Rogoz media, BA with kanamycin, Wilson-Blair medium - up to - 72 hours;

5) on Saburo medium are incubated for 48 hours at 37 ° C and another 3 days at room temperature or five days at 22° C.

Methods of Statistical Analysis

In this work, the Wilcoxon test was used with $p<0.05$ null hypothesis to compare the indices by means of average values between dependent groups (before and after). No significant differences in group deviations were recorded.

Ethical approval and consent to participate: The authors declare that the work is written with due consideration of ethical standards. The study was conducted in accordance with the ethical principles approved by the Human Experiments Ethics Committee of NSC Astana Medical University (Protocol No. 7 of 15.08.2019). Also the research was conducted according to the World Medical Association Declaration of Helsinki. All participants gave their written informed consent.

Results

During the bacteriological analysis of the quantitative composition of intestinal microflora in feces before its correction it was found that in patients with signs of chronic colonic in the large intestine of the main (50 people) and control (50 people) groups, the number of intestinal microorganisms

(*Bifidobacterium*, *Lactobacillus*, and *Escherichia coli* with normal enzymatic activity) was below the minimum reference value with more statistically significant difference in the control group ($p<0.001$).

That is, compared to normal microbiota in both groups, a decrease of beneficial intestinal microorganisms was statistically more expressed in the control group ($p<0.001$). Also, opportunistic microorganisms such as hemolyzing E. coli, Staphylococcus aureus, lactose-negative *Escherichia coli*, *Enterococci*, and yeast-like fungi of the genus *Candida* were sown. Table 1 presents data of intestinal microbiota species and its number in patients with chronic constipation before the correction.

Table 1. Intestinal microbiota species and its number in patients of the main and control groups before treatment.

Microorganisms	Standard values in children (CFU / g)	Main group (n=50)	Abs (%)*	Control group (n=50)	Abs (%)*	P-value
	>1	central trends (dispersion measures)		central trends (dispersion measures)		
1	2	3	4	5	6	7
Bifidobacterium	10^9-10^{10}	10^7 CFU / g ### (10^6; 10^9)	12 (24%)	10^7 CFU / g ### (10^6; 10^9)	13 (26%)	p<0.001
Lactobacillus	10^6-10^7	10^4 CFU / g ### (10^4; 10^7)	23 (46%)	10^5 CFU / g ### (10^4; 10^7)	21 (42%)	p<0.05
E. coli with normal enzymatic activity	10^7-10^8	10^8 CFU / g ## (10^4; 10^8)	24 (48%)	10^6 CFU / g ## (10^4; 10^8)	22 (44%)	p<0.01
E. coli with reduced enzymatic activity	10^5-10^7	10^8 CFU / g ### (10^5; 10^8)		10^7 CFU / g ### (10^6; 10^8)		p<0.05
Lactose-negative *E. coli*	$\leq 10^4$	10^8 CFU / g ### (10^4; 10^8)		10^7 CFU / g ### (10^4; 10^7)		p<0.05
Hemolytically active *E. coli*	$<10^4$	10^6 CFU / g ### (10^5; 10^7)		10^7 CFU / g ### (10^5; 10^7)		p>0.05
Proteus	$<10^4$	10^5 CFU / g ## (10^3; 10^5)		10^5 CFU / g ## (10^4; 10^5)		p>0.05
Opportunistic *Enterobacterium sp.*	$\leq 10^4$	10^8 CFU / g ### (10^5; 10^8)		10^7 CFU / g ### (10^5; 10^8)		p<0.05
Pathogenic *Staphylococcus*	$\leq 10^1$	10^4 CFU / g ### (10^2; 10^4)		10^3 CFU / g ### (10^2; 10^5)		p<0.05
Other staphylococci	$\leq 10^5$	10^3 CFU / g ### (10^3; 10^7)		10^3 CFU / g ### (10^3; 10^5)		p<0.05
Enterococci	10^6-10^7	10^6 CFU / g ### (10^4; 10^8)		10^7 CFU / g ### (10^4; 10^8)		p<0.05
Clostridia	$\leq 10^5$	10^6 CFU / g ## (10^5; 10^6)		10^5 CFU / g ## (10^5; 10^6)		p<0.05
Candida	$\leq 10^3$	10^5 CFU / g ### (10^4; 10^6)		10^6 CFU / g ### (10^4; 10^6)		p<0.05
Non-fermenting Gram-negative bacteria	$\leq 10^3$					p<0.05

* - the degree of reliability of the difference indicator between the groups, expressed in % ($p<0.05$);
\# - degree of reliability to normal at $p<0.05$;
- degree of reliability to normal at $p<0.01$;
- degree of reliability to normal at $p<0.001$

After applying the developed method for correcting biocenosis of the colon in patients of the main group and after traditional treatment of the control group, particular changes in the number of beneficial and opportunistic pathogens were observed based on bacteriological analysis of feces (Table 2).

Table 2. Investigation results of intestinal microbiota after using the developed method for the correction of colon biocenosis in patients of the main group and traditional treatment in patients of the control group.

Microorganisms	Standard values in children (CFU / g)	Main group (n=50)	Abs (%)*	Control group (n=50)	Abs (%)*	P-value
	>1	central trends (dispersion measures)		central trends (dispersion measures)		
1	2	3	4	5	6	7
Bifidobacterium	10^9-10^{10}	10^9 CFU / g$^{###}$ (10^7; 10^{10})	31 (62%)	10^6 CFU / g$^{###}$ (10^4; 10^9)	2 (4%)	p<0.001
Lactobacillus	10^6-10^7	10^7 CFU / g$^{###}$ (10^4; 10^7)	39 (78%)	10^4 CFU / g$^{###}$ (10^4; 10^7)	8 (16%)	p<0.05
E. coli with normal enzymatic activity	10^7-10^8	10^7 CFU / g$^{##}$ (10^1; 10^8)	38 (76%)	10^4 CFU / g$^{##}$ (10^3; 10^8)	9 (18%)	p<0.01
E. coli with reduced enzymatic activity	10^5-10^7	10^5 CFU / g$^{###}$ (10^5; 10^5)		10^8 CFU / g$^{###}$ (10^7; 10^8)		p<0.05
Lactose-negative *E. coli*	≤10^4	10^7 CFU / g$^{###}$ (10^2; 10^8)		10^7 CFU / g$^{###}$ (10^5; 10^8)		p<0.05
Hemolytically active *E. coli*	<10^4	10^4CFU / g$^{###}$ (10^4; 10^8)		10^7 CFU / g$^{###}$ (10^5; 10^8)		p>0.05
Proteus	<10^4	10^4 CFU / g$^{##}$ (10^3; 10^4)		10^6 CFU / g$^{##}$ (10^4; 10^7)		p>0.05
Opportunistic *Enterobacterium sp.*	≤10^4	10^4 CFU / g$^{###}$ (10^4; 10^7)		10^6 CFU / g$^{###}$ (10^5; 10^8)		p<0.05
Pathogenic Staphylococcus	≤10^1	10^2 CFU / g$^{###}$ (10^1; 10^4)		10^3 CFU / g$^{###}$ (10^3; 10^4)		p<0.05
Other staphylococci	≤10^5	10^3 CFU / g$^{###}$ (10^3; 10^4)		10^4 CFU / g$^{###}$ (10^3; 10^6)		p<0.05
Enterococci	10^6-10^7	10^4 CFU / g$^{###}$ (10^4; 10^7)		10^7 CFU / g$^{###}$ (10^4; 10^8)		p<0.05
Clostridia	≤10^5			10^7 CFU / g$^{##}$ (10^6; 10^8)		p<0.05
Candida	≤10^3	10^1 CFU / g$^{###}$ (10^1; 10^4)		10^7 CFU / g$^{###}$ (10^4; 10^7)		p<0.001
Non-fermenting Gram-negative bacteria	≤10^3	10^3 CFU / g$^{###}$				p<0.001

* - the degree of reliability of the difference indicator between the groups, expressed in% (p<0.05);

- degree of reliability to normal at p<0.05;

- degree of reliability to normal at p<0.01;

- degree of reliability to normal at p<0.001.

The analysis of the data in Table 2 showed that after the preparation, the developed method for normalizing the biocenosis of the colon (combined oral and rectal introduction of probiotics) in the main group (n=50) increased the concentration of all types of beneficial microorganisms in the intestine to normal values (*Bifidobacterium*, *Lactobacillus*, and *E. coli* with normal enzymatic activity) with a statistically significant difference (p <0.001, p <0.001, and p <0.01, respectively).

When analyzing the amounts of opportunistic enterobacteria, no significant difference in the decrease of their number by types of microorganisms (hemolytically active *E. coli*, lactose-negative *E. coli*,

Proteus mirabilis) ($p>0.05$) was detected. However, these indicators had a statistical difference in the form of a decrease in the number of opportunistic bacteria against the background of using the developed method for correcting biocenosis ($p<0.05$).

In patients of the control group (n=50), during the bacteriological study of feces, which was obtained after preparation for an X-ray examination and with using only standard treatment regimens, i.e., only oral probiotics administration, the number of opportunistic microorganisms increased with the emergence of new types of pathogenic microorganisms despitea slight improvement in the ratio of the content of beneficial microorganisms in the intestine. Analysis of the data in Table 2 showed that despite the preparation and treatment measures taken at different stages of the examination using only the traditional preparation scheme, a decrease in the number of *Bifidobacterium* species can be observed with obvious statistical significance ($p<0.001$). Other types of beneficial microorganisms (*Lactobacillus* and *Escherichia coli* with normal enzymatic activity) increased in number but did not reach the level of normal indicators, and no significant difference ($p>0.05$) was revealed after statistical analysis. Also, analyzing the number of opportunistic enterobacteria, it is clearly seen that in the control analysis of feces, the number of pathogenic bacteria, as well as their concentration in the intestine increased above the norm with a clear statistically significant difference ($p<0.05$).

Discussion

Investigation of intestinal microbiota in children showed that chronic colonic stasis is characterized by a violation of intestinal microbiocenosis with a moderate decrease in number of beneficial microorganisms (*Bifidobacterium* and *E. coli* with normal enzymatic activity) with the presence of certain opportunistic microorganisms like hemolyzing *E. coli* and other ($p<0.05$).

According to relatively recent studies, it is noteworthy, that besides the diversity reduction of intestinal microbiota at chronic colostasis, the content of methanogenic bacteria utilizing hydrogen in the lumen decreased as well. This is particularly exhibited for the main methane-producing species *Methanobrevibactersmithii*. In addition to the deceleration of intestinal motility,[20] excessive accumulation of hydrogen may lead to abdominal distention, i.e., development of meteorism. At the same time, the deficiency of methane, which is an important regulator of peristalsis, may be accompanied by a secondary deceleration of intestinal transit.[21]

The Rome Consensus IV,[18] emphasized the prescription of pre- and probiotics for the correction of intestinal microbiota. The application of these preparations is a promising direction in the treatment of chronic constipation and is probably most pathogenetically justified and safe for functional intestinal diseases.[22] A meta-analysis of the results of various pro- and prebiotics application at functional constipation showed that, in general, the drugs are effective in eliminating abdominal pain, distention, and increased stool frequency at constipation.[23] It can be predicted that the most pronounced effect can provide drugs that include *Bifidobacterium.*[24] The *Bifidobacterium* species are known to exhibit pronounced saccharolytic and reparative activity, regulate peristalsis due to the production of short-chain fatty acids and the influence of components of its own cytoplasm, and provide anti-inflammatory and anxiolytic effects. Promising results were also obtained with the use of some *Lactobacillus* strains.[22,25,26]

Nevertheless, according to the coloproctologists Huang and Hu, the systematic review and meta-analysis of six randomized and controlled investigations showed that oral administration does not significantly affect the results of chronic colostasis treatment.[27]Attempts to correct microbiocenosis with oral probiotic administration were insignificant. This aspect also was noted in patients after cleansing enemas ($p<0.05$). This is because hydrochloric acid and pepsin are rather a serious obstacle to the advancement of microorganisms. In total, from 20 to 40% of *Bifidobacterium* and *Lactobacillus* pass through the entire digestive tract ($p<0.05$) when applying oral administration. Even though after ingestion, bacteria are found in the stool for 1 to 3 weeks, the colonizing ability of *Bifidobacterium* and *Lactobacillus* in the intestine is low. Therefore, to ensure the constant presence of microorganisms in the intestinal microbiota and achieve a stable positive effect from the use of *Bifidobacterium*, the preparations containing this species should be taken continuously for a long time and in significant doses.[28]

In this regard, the method for correcting disorders of the microbiocenosis in the large intestine has been developed using the intrarectal administration

of probiotic agents against the background of the regular use of a different type of enema. At that, of hydrochloric acid of the gastrointestinal tract and pepsin had no negative effect on the preparation (patent for invention "Promotes the treatment of Hirschsprung - associated enterocolitis in children" No. 32679 dated January 29, 2018, Ministry of Justice of the Republic of Kazakhstan),[18] which was used to treat children with chronic constipation. The purpose of the development of this method was to level the negative effect of multiple enemas on the colon microbiocenosis due to the effect of mechanical "leaching" of microorganisms from the lumen of the intestinal tube, which further aggravates the disturbed balance of microorganisms in chronic colostasis.

Comparative analysis of bacteriological studies on the intestinal microbiota of main and control groups revealed that in the period before preparation for the examination and treatment, no statistically significant difference was recorded in the number of useful and pathogenic microorganisms in the intestine between the representatives of the two groups ($p>0.05$). However, in the period after preparation for X-ray examination and treatment, the number of microorganisms in the stool cultures essentially differed from each other with a statistically significant difference in both groups. It is clearly seen that the number of beneficial microorganisms is statistically significant higher in the main group in comparison with the control group, along with a decrease in the number of opportunistic enterobacteria ($p<0.001$).

The results of this study allow stating that the deficiency of beneficial intestinal microorganisms was compensated by new types of pathogens with an increase in their frequency in the control group, with a clear statistically significant difference compared with the patients of the main group ($p<0.001$). In the main group, against the background of treatment using the method developed in this study, the opposite state of the quantitative composition of the intestinal microbiota was observed, namely, an increase in beneficial microorganisms with a smaller number of pathogenic bacteria ($p<0.001$).

The results of the study prove that the developed method, i.e., the use of a probiotic through the mouth and by introducing probiotic into the lumen of the colon by enema, is an effective way of correcting the quantitative composition of intestinal microbiota in chronic stool retention in obligatory conditions of cleansing enemas use. The number of beneficial microorganisms of the intestinal microbiota (*Bifidobacterium*, *Lactobacillus*, and *E. coli* with normal enzymatic activity) increased to normal values in the main group with clear statistical significance ($p<0.001$) (Table 2).

Conclusions

1. Disorders of intestinal microbiocenosis, i.e., a decrease in the number of obligate flora representatives and activation of opportunistic microbiota in the intestine ($p<0.001$), is a fundamental factor in exacerbating chronic colostasis.
2. The execution of multiple cleansing enemas before an X-ray contrast study exacerbates the existing shortage of beneficial microorganisms of the obligate flora due to mechanical leaching ($p<0.05$), which leads to their replacement by pathogenic enterobacteria ($p<0.001$).
3. The method of intestinal microorganisms restoring by introducing probiotics rectally has undeniable advantages over traditional methods of treating chronic colostasis, and it allows for adequate restoration of the natural spectrum of colon microorganisms.

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Availability of data and material: All data will be available on request.

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Authors' contribution: DK, AD designed research (project conception, development of overall research plan, and study oversight). VL,GB conducted research (hands-on conduct of the experiments and data collection). MK, YL analyzed data or performed statistical analysis. AS, AG wrote paper (only authors who made a major contribution). DShad primary responsibility for final content.All authors have read and approved the final manuscript.

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