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Evaluation of the efficacy of probiotics in the complex treatment for patients with hyperosmolar diarrhea

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ABSTRACT

The study aimed to examine the nature and characteristics of intestinal dysbiosis in patients with pancreatic and biliary system diseases, with the presence of hyperosmolar diarrhea, in the clinical setting, and to evaluate the efficacy of probiotics as part of the treatment regimen. A two-stage retrospective descriptive and prospective randomized controlled trial was performed at the NpJSC Astana Medical University. The first stage was the clinical assessment of the intestinal microbiocenosis of patients with diseases of the pancreato-biliary system with the presence of hyperosmolar diarrhea. In total, 284 medical records were analyzed. The second stage was a prospective randomized controlled trial conducted to compare the efficacy of probiotics Biovestin-Lacto (contains strains of *Bifidobacterium bifidum*, *B. adolescentis* and *Lactobacillus plantarum*) and Normobact Forte (contains the strain *L. rhamnosus*). The study found that in patients with diseases of the pancreato-biliary system with the presence of hyperosmolar diarrhea, dysbiotic changes of the intestinal microbiota are unidirectional. This probiotic stimulated the indigenous microflora and reduced the growth of opportunistic bacteria and yeast-like fungi.

Keywords: pancreato-biliary system, hyperosmolar diarrhea, intestinal microbiocenosis, dysbiosis, probiotics

INTRODUCTION

Pancreato-biliary disorders are among the most common diseases of the digestive system [1]. Thus, according to the literature, there are on average 2 times more patients with diseases of the biliary system than patients with peptic ulcer disease [2]. In recent decades, the number of patients with cholecystitis has increased significantly in many countries [3-5]. Due to the increase in cases of chronic calculous cholecystitis (CCC), the number of cholecystectomies is also increasing. As a result, the number of patients with postcholecystectomy syndrome (PCS) remains very significant [6, 7]. The anatomical and functional relationship of the pancreato-biliary system creates prerequisites for the frequent development of pancreatitis in cholelithiasis [8]. Pancreatitis of biliary origin accounts for from 26% to 60% of all acute pancreatic inflammation cases, while transition to chronic biliary pancreatitis is 43% [9, 10].

Diseases of the pancreato-biliary system typically entail hyperosmolar diarrhea and intestinal dysbiosis. This problem is exacerbated by external and internal factors that adversely affect the microbiocenosis of the gastrointestinal tract. These factors include unfavorable environmental conditions, the widespread and unsystematic use of antimicrobial drugs in medical practice, and suboptimal diet [11-13].

It is known that the normal human microbiota plays a significant role in the regulation of homeostasis. Currently, changes in intestinal microbiocenosis are considered a disruption in compensatory capabilities. This disruption occurs due to violations in the interaction between microorganisms and the host organism, leading to the development of a pathological process. If the body's adaptive mechanisms become exhausted, the problems with the microbiota can progress to a clinical stage, causing various symptoms [14, 15]. Given the multifunctional role of the human microbiota, research has paid considerable attention to the pathogenesis of gastrointestinal diseases, which are associated with dysbiotic changes. The peculiarities of intestinal microbiocenosis and the patterns of dysbiotic manifestations are among the most studied areas. There is an urgent need for more advanced strategies to correct dysbiotic imbalances and restore the intestinal microbiota [16-18].

Due to the high prevalence of dysbiosis among the human population, various schemes for its treatment have been developed in recent years. Most of these schemes recommend the use of probiotics, which typically include living microorganisms of the indigenous microflora, such as *Bifidobacterium* and *Lactobacillus* [19, 20]. The widespread use of probiotics as part of complex therapy has shown their positive effect on the treatment of various gastrointestinal diseases [21]. Therefore, it is important for practical medicine to identify dysbiotic imbalances, which often entail common variable immunodeficiency. The knowledge of these imbalances is the basis for an effective therapeutic probiotic treatment [22, 23]. The therapeutic effect of probiotics is

attributed to the bioavailability, species composition and quantitative content of the normal intestinal microbiota.

An example of probiotics is Biovestin-Lacto, a liquid probiotic developed by Bio-Vesta (Novosibirsk, Russia). This probiotic contains strains of *Bifidobacterium*, including p pharmacopoeial harmacopoeia *B. bifidum* and *B. adolescentis* with a live bacterial concentration of 10^9 colony-forming units (CFU)/ml, pharmacopoeial *Lactobacillus plantarum* with a live bacterial concentration of 10^8 CFU/ml, as well as metabolic products from the indigenous microflora [24, 25]. The efficacy of this drug has been investigated empirically: among children and adults with various pulmonary, skin, and endocrine conditions, as well as in children with chronic gastroduodenitis. Additionally, this biological preparation has been tested in the fields of oncohematology and gynecology [26]. Some studies have also examined the influence of biopreparation on microbiocenosis formation among newborns and the prevention of nosocomial infections [27, 28]. The efficiency of Biovestin-Lacto has also been studied in the complex treatment of helicobacter associated gastritis and gastroduodenitis in children [29]. However, there is a lack of literature data on the efficiency of this biopreparation in the treatment of dysbiotic imbalances for adult patients with pancreato-biliary disorders.

According to the statistics of the general morbidity level in Kazakhstan, gastrointestinal diseases occupy a leading position with a high incidence of pancreato-biliary system damage [30]. To date, there are no scientific papers discussing the findings from studies based on the microbiological evidence for the use of probiotics in the treatment of patients with pancreato-biliary disorders among the population of Kazakhstan.

Research Objective

To study the nature and specifics of intestinal dysbiosis in patients with pancreato-biliary disorders and hyperosmolar diarrhea and justify the effectiveness of probiotics Biovestin-Lacto (contains strains of *B. bifidum*, *B. adolescentis* and *L. plantarum*) and Normobact Forte (contains the strain *Lactobacillus rhamnosus* GG [LGG]) in the complex treatment.

MATERIALS AND METHODS

Research Design

This study is a two-stage retrospective descriptive and prospective randomized controlled trial conducted at the NpJSC Astana Medical University.

Medical records of 398 patients with gastrointestinal pathology associated with diarrhea were analyzed. In the studied sample of patients, there were 284 (71.3%) cases of hyperosmolar diarrhea against the background of pancreato-biliary disorders. These patients were selected for further study.

The first stage was the examination of the intestinal microbiocenosis of patients with pancreato-biliary disorders. The presence of hyperosmolar diarrhea in the medical records of these patients was a symptom indicating the activity of the pathological process and intestinal dysbiosis. The research cohort consisted of patients with the following disorders of the pancreato-biliary system: chronic cholecystitis (CC) (acalculous and calculous), PCS, and chronic pancreatitis (CP). Based on the research goal, the groups included patients who underwent microbiological examination of the contents of the large intestine for dysbiosis. In 87 cases, patients underwent an ultrasound examination of the abdominal organs. In 43 cases, computed tomography of internal organs was performed for differential diagnosis. In addition, 111 medical charts of the studied patient population contain the results of endoscopic examinations of the stomach, duodenum, and large intestine (colonoscopy). These examinations were performed to clarify the diagnosis. The medical charts of 53 patients included the results of stool tests. Biochemical blood tests were administered to 161 patients and included the following parameters: total bilirubin and its fractions, transaminase levels, glucose levels, alkaline phosphatases, gamma-glutamyl transpeptidase, and amylase.

Based on the results of the first stage, patients requiring dysbiosis treatment were identified and included in the subsequent stage of the study. At this stage, a prospective controlled study was conducted to comparatively evaluate the effectiveness of probiotics in the complex treatment of pancreato-biliary disorders.

The inclusion criteria for this study were: the presence of diagnosed pancreato-biliary disorders, accompanied by hyperosmolar diarrhea (increased bowel movements up to 3 or more times per day); the 3rd degree of dysbiosis; the absence of pronounced organ dysfunction; age between 15 and 60 years; consent to participate in the study.

The exclusion criteria were acute intestinal infections; acute gastrointestinal pathologies; diseases that affect the gastrointestinal tract and cause constipation; patients with helminthiasis; the presence of concomitant pathology indicating endocrine pancreatic insufficiency (diabetes mellitus), and thyroid glands; simultaneous administration of other biopreparations; alcohol consumption and smoking during the study period; refusal to continue participating in the study.

The age of the patients ranged from 18 to 60, with an average of 41.5 ± 3.9. The pathology was more commonly diagnosed in women (61%) than in men (39%). For differential diagnosis, before the experiment, 58 patients underwent ultrasound examination of the abdominal organs, 16 underwent computed tomography, 89 underwent fibrogastroduodenoscopy, and 22 underwent colonoscopy. To exclude helminthiasis and parasitosis, fecal samples were examined using a formal-ether concentration technique. All patients were also administered microbiological analysis of feces for dysbiosis. This analysis was conducted again after the completion of the complex treatment program.

In complex treatment, patients with CC were prescribed to take myotropic cholespasmolytics (no-spa, spasmalgon, and spasmomen). The cholekinetic effect was mainly achieved through choleretic drugs of plant origin, such as flamin, chophytol, hepabene. Patients with CP were prescribed replacement therapy with pancreatic enzymes. Kreon was recommended in medium therapeutic doses. Analgesics such as analgin, baralgin, and ketoprofen were used to relieve pain.

To achieve the research goal, patients were divided into two groups by simple randomization. The randomization of patients (for each of the above diseases) was performed independently using a special computer program by a specialist who was not familiar with the study design. The groups were comparable in gender, age, and nosology. The

first experimental group (group 1) consisted of 49 patients who received the probiotic Biovestin-Lacto as part of their treatment (Bio-Vesta, Novosibirsk, Russia). Biovestin-Lacto is a liquid probiotic, which contains 2 strains of *Bifidobacterium*: pharmacopoeial *B. bifidum* and *B. adolescentis*, pharmacopoeial strain *L. plantarum*, as well as metabolic products from the indigenous microflora. In total, 1 ml of the probiotic contains 10^9 CFU of live *Bifidobacterium* and 10^8 CFU of *Lactobacillus* for the entire shelf life of the drug [24] The second experimental group (group 2) consisted of 47 patients who received Normobact Forte (Allianz Biosciences Private Limited, India). This biopreparation contains the strain LGG in a concentration of 6×10^9 CFU/g, which is similar to indigenous flora, as well as another form of delivery (capsules) [31]. In addition, the study included a control group of 30 patients. In this group, probiotics were not administered.

The patients took 12 mL of the liquid probiotic Biovestin-Lacto 30 minutes before meals for four weeks. The dose of Normobact Forte was one capsule 2 times a day with meals for 28 days. For the purity of the experiment, probiotics were prescribed to patients with inflammatory diseases of the pancreato-biliary system 10 days after antibiotic therapy. The effects of the therapy were evaluated 4 weeks after the start of the probiotic treatment.

The second microbiological examination of feces for dysbiosis was conducted after 4 weeks of complex therapy with the inclusion of a probiotic. In group 1 (49 patients), 3 patients failed to attend a follow-up analysis, therefore, the cohort of those examined was 46 individuals. In group 2 of 47 patients, 5 were not re-tested for dysbiosis, therefore, the sample included 42 individuals.

Study of Microbial Ecology in Intestinal Ecosystems

To assess intestinal microbiocenosis in patients with pancreato-biliary disorders and hyperosmolar diarrhea, the study employed a bacteriological research method. This method involves the use of various nutrient media for growing microbial populations (live bacteria) depending on their metabolic activity.

The study of the microbial population in the colon was conducted in accordance with established guidelines. Ten-fold serial dilutions were followed by inoculation on a variety of selective and differentially diagnostic nutrient media [16, 17]. The analysis of the growth pattern of microorganisms made it possible to identify their genus. Each type of microorganism in 1 g of feces was estimated according to the following formula: M = N × 10 n1, where M is the number of microbes per 1g, N is the number of colonies grown on a Petri dish, n is the degree of dilution. The number of microorganisms was presented in absolute values: the number of CFU per 1 g of stool in decimal logarithms (lg CFU/g). Microorganisms, after the isolation of pure bacteria and Gram staining, were identified on Vitek 2-Compact, a microbiological computer analyzer developed by bioMerieux. For bacteriological studies, all samples were numbered, and patient data was kept confidential from the researcher conducting the analysis.

All the subjects were on a diet 3 days before the test. Their diet excluded the intake of products that enhance the fermentation processes in the intestines, as well as alcohol and antimicrobials. The feces of patients were delivered to the microbiological laboratory at JSC National Scientific Medical Center in sterile vials in the amount of 2-3 g without preservative within 2 hours from the moment of collection. The vials were assigned a unique number and information about patients and study group allocation was unavailable to the laboratory technician performing the analysis.

The diagnosis of intestinal dysbiosis was based on the following microbiological criteria:

- the number of bifidobacteria of less than 10^8 CFU/g of stool,
- lactobacilli of less than 10^6 CFU/g,
- *Escherichia coli* with altered properties (lactose-negative forms or atypical enzymatic properties) is more than 10% of the total,
- hemolytic microflora,
- the presence of obligate pathogenic bacteria (*Salmonella*, *Shigella*, pathogenic serovars of *E. coli*),
- the presence of opportunistic enterobacteria (OE) (genera such as *Enterobacter*, *Proteus*, *Klebsiella*, *Citrobacter* etc.), as well as bacteria of the *Pseudomonas*, *Acinetobacter* and other genera. The concentration of these bacteria of above 10^5 CFU/g was etiologically significant,
- the presence of fungi of the genus *Candida* in a concentration of 10^5 CFU/g and higher,
- the presence of pathogenic *Staphylococcus*, and
- the presence of *Clostridium* at a concentration of more than 10^5 CFU/g [32].

To evaluate the degree of severity of intestinal dysbiosis, the clinical classification of Bondarenko was used [33]. According to this classification, there are degrees of dysbiosis:

1st degree (latent, compensated) is characterized by minor changes in the aerobic part of microbiocenosis (an increase or decrease in the number of *E. coli*), the content of bifidoflora and lactoflora is not changed;

2nd degree (subcompensated, localized) occurs against the background of a slight decrease in the content of *Bifidobacterium*. This causes quantitative and qualitative changes in *E. coli* and an increase in the population of opportunistic bacteria, Pseudomonadaceae, and fungi of the genus *Candida*;

3rd degree (subcompensated, generalized). The level of bifid flora is significantly reduced. This is accompanied by a decrease in the content of *Lactobacillus* and a sharp change in the amount of *E. coli*. Following a decrease in the level of bifidoflora, the composition of the intestinal microbiota is disrupted, creating conditions for the aggressive manifestation of opportunistic microorganisms;

4th degree (decompensated) is the absence of bifidoflora, a significant decrease in lactoflora, and a change in the content of *E. coli*. The 4th degree entails an increase in the number of obligatory, facultative, and untypical (for a healthy person) types of opportunistic microorganisms in associations.

Statistical Analysis

The data obtained in this study were processed using Statistica 6.1 (StatSoft, Inc., USA). The mean values (M) and the mean error (± m) were specified. The normality of the data distribution was checked using the Shapiro-Wilk test. With a normal distribution of data, the student's two-sided test (independent two-sample t-test) was used to compare differences between the groups. The student's paired test (dependent two-sample t-test) was utilized to compare

Table 1. Intestinal microbiocenosis of patients with pancreato-biliary disorders accompanied by hyperosmolar diarrhea

Microorganisms	Frequency of detection				CFU/g
	Patients with CAC (%): n = 94	Patients with CCC (%): n = 31	Patients with CP (%): n = 91	Patients with PCS (%): n = 68	
Bifidobacterium	38.0 ± 2.4	32.2 ± 3.1	32.5 ± 2.8	31.4 ± 2.6	10^8 and higher
Lactobacillus	21.4 ± 1.9	20.3 ± 1.7	18.3 ± 1.6	19.6 ± 1.7	10^6 and higher
Escherichia coli with normal enzymatic activity	47.4 ± 3.0	45.3 ± 2.8	50.2 ± 3.4	46.7 ± 2.7	10^7 and higher
E. coli with atypical enzymatic activity	12.1 ± 1.4	15.4 ± 1.9	12.8 ± 1.3	13.1 ± 1.7	-
Enterococcus	34.6 ± 3.1	30.2 ± 2.1	38.4 ± 3.4	41.1 ± 3.6	10^5 and higher
Clastridium	10.6 ± 1.9	10.8 ± 1.7	7.2 ± 1.5	6.9 ± 1.4	10^6 and higher
Opportunistic Enterobacteriaceae	34.4 ± 3.4	36.6 ± 3.2	37.5 ± 3.6	33.4 ± 3.3	10^5 and higher
Pseudomonas aeruginosa	11.2 ± 1.4	16.3 ± 1.6	12.1 ± 1.6	13.6 ± 1.2	-
Staphylococcus aureus	4.1 ± 1.1	8.6 ± 1.8	10.2 ± 1.7	9.3 ± 1.5	-
Yeast-like fungi of the genus *Candida*	8.3 ± 1.7	10.2 ± 2.0	6.2 ± 1.4	7.6 ± 1.6	10^5 and higher
Bifidobacterium	38.0 ± 2.4	32.2 ± 3.1	32.5 ± 2.8	31.4 ± 2.6	10^8 and higher

indicators within each group before and after treatment. The results were considered statistically significant at $p < 0.05$.

Ethical Consent

The study was approved by the Ethics Committee of NpJSC Astana Medical University (protocol no. 46) in a meeting held on 12 December 2023. Each patient involved in the study provided their written informed consent.

RESULTS

Intestinal Microbiocenosis in Diseases Accompanied by Hyperosmolar Diarrhea

Among all diseases associated with hyperosmolar diarrhea, CC was diagnosed in 125 (44%) cases, including chronic acalculous cholecystitis (CAC) in 91 (32%) cases, and CCC in 31 (11%) cases. CP was diagnosed in 91 (32%) cases, and PCS was detected in 68 (24%). In the first stage of the study, the state of intestinal microecology was assessed and compared among patients with these diseases.

The analysis of microbiological studies of patients with CC indicates a low content of the main representatives of the indigenous flora (**Table 1**). Thus, the values of *Bifidobacterium* and *Lactobacillus*, which were within acceptable limits, were recorded only in 38 and 26.4% of cases, respectively. The content of *E. coli* with normal enzymatic activity did not reach the norm in 50% of the examined patients. At the same time, *E. coli* was found in the contents of the large intestine in patients with chronic inflammation of the gallbladder. The bacterium had atypical enzymatic activity, especially in patients with calculous cholecystitis.

In the considered population of patients, *Staphylococcus aureus* was found in the intestinal microbiota, which was significantly more common in patients with CCC ($p < 0.01$). Most likely, this indicates the involvement of large areas of the biliary tract in the pathological process, as confirmed by clinical and laboratory data on patients with cholelithiasis. Thus, the general blood test of patients with CAC, in comparison with those suffering from CAC, revealed signs of inflammation in the form of moderate leukocytosis and increased ESR. In liver samples of patients with cholelithiasis, signs of cholestasis, cytolysis, and mesenchymal inflammation were detected in 26% of cases.

A comparative analysis of the species composition of the microflora showed that each third patient suffering from CC had opportunistic bacteria in the contents of the large intestine. Among patients with CCC, *Enterobacter* bacteria were found more often than in the group of individuals with CAC. Of the four identified species of this bacteria, *En. aerogenes* and *En. cloace* were the most common. In patients with cholecystitis, the *Citrobacter* bacteria were represented by two species and were detected in 5% of cases.

Klebsiella and protea were more frequently identified in the feces of patients with cholelithiasis, whereas the *Kluyvera* bacteria were less frequently recorded than in individuals with CAC. *Pseudomonas aeruginosa* was detected in 16.3% of feces among patients with CCC and in 11.2% of feces among patients with CAC. This indicates the immunodeficiency state of the macroorganism. The endotoxin produced by *P. aeruginosa* is significantly inferior in its activity to the endotoxins of other gram-negative bacteria, but it is actively involved in diarrhea development. Among patients with CC, the 1st degree of intestinal dysbiosis was detected in 24% of cases, the 2nd degree of dysbiosis was reported in 35% of cases, and the 3rd degree of dysbiosis was found intestinal dysbiosis in 41% of cases.

The microbial analysis of individuals suffering from chronic pancreatitis revealed a significant deficiency of *Bifidobacterium* and *Lactobacillus*. Their values did not reach the norm in 67.5% and 81.3% of cases, respectively. However, half of the examined patients had a level of *E. coli* with normal enzymatic activity, which corresponded to acceptable values. At the same time, *E. coli* with atypical enzymatic activity was detected in 12.8% of cases, which certainly indicates a limited compensatory capability of the autochthonous microflora. This condition leads to the activation of opportunistic bacteria, mainly those of the Enterobacteriaceae family, such as *Enterobacter*, *Klebsiella*, *Kluyvera*. *P. aeruginosa*, and *S. aureus*, as well as fungi of the genus *Candida*, were found in the contents of the large intestines of individuals suffering from CP. In most cases, these bacteria were in association with various representatives of the opportunistic flora, causing significant dysbiotic changes.

In patients with PCS, the contents of *Bifidobacterium* and *Lactobacillus* were acceptable only in 31.4 and 19.6% of cases, respectively. More than half of the examined patients had a reduced level of *E. coli* with normal enzymatic activity. Additionally, there was *E. coli* with atypical enzymatic activity in 13.1% of cases. Enterococci and bacteria of the Enterobacteriaceae family prevailed in the microbial contents of the intestines of patients with PCS. The species composition was mainly represented by *En. aerogenes*, *Klu. ascorbata*, *Proteus mirabilis*, and *Kle. pneumonia*. *P. aeruginosa* and fungi

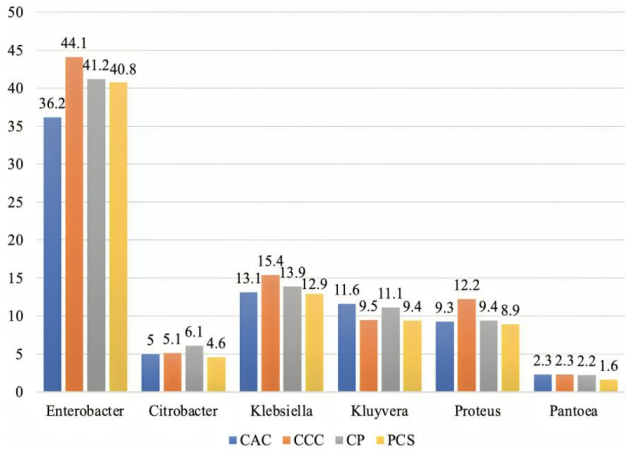

Figure 1. Composition of genera of opportunistic bacteria in patients with pancreato-biliary disorders (%) (Source: Authors' own elaboration)

of the genus *Candida* were detected in the contents of the large intestine of patients with PCS (in 13.6% and 7.6% of cases, respectively). Typically, *P. aeruginosa* and fungi of the genus *Candida* were in association with opportunistic bacteria. At the same time, 9.3% of the examined patients had *S. aureus* in monoculture.

Among patients suffering from CP and PCS, the 1st degree of intestinal dysbiosis was detected in 25% of cases, the 2nd degree of intestinal dysbiosis was found in 29% of cases, and the 3rd degree of intestinal dysbiosis was found in 46% of cases. In the general group of patients, the 1st degree intestinal dysbiosis led to a quantitative decrease in obligate flora, primarily the level of *Bifidobacterium*. Intestinal dysbiosis of the 2nd degree was caused by an increase in the level of various opportunistic bacteria to a concentration of 10^5-10^7 CFU/g, as well as a quantitative decrease in obligate flora, primarily *E. coli*. In the contents of the large intestine, there was an increase in the amount of *E. coli* with atypical enzymatic activity, and, against this background, *S. aureus* was detected. At the same time, among 21% of the examined patients, changes in the intestinal microbiota were due to the association of opportunistic bacteria. Intestinal dysbiosis of the 3rd degree entailed a decrease in the frequency of detection of *Bifidobacterium* and *Lactobacillus*, which led to the activation of opportunistic flora in associations, including with fungi of the genus *Candida*. Thus, the analysis showed that all patients with pancreatic and biliary disorders had dysbiotic changes in intestinal microbiocenosis of varying severity, regardless of the clinical form of the disease (**Table 1** and **Figure 1**). These conditions require corrective therapy.

Treatment of Microbiocenosis in Patients With Hyperosmolar Diarrhea

Table 2 shows the frequency of detection of the main representatives of the intestinal microbiota in patients before and after the administration of probiotics Biovestin-Lacto (group 1) and Normobact Forte (group 2) as part of the complex treatment for CC and CP. The analysis revealed that in the intestinal microbiota of patients from both groups, there was a reduced frequency of detection of obligate flora representatives, as evidenced by the deficiency of bacteria from the genera *Bifidobacterium* and *Lactobacillus*. Thus, in the group of patients receiving the probiotic Biovestin-Lacto, only 32.5% had a level of *Bifidobacterium* of 10^8 CFU/g or higher, and only 20.4% had a level of *Lactobacillus* of 10^6 CFU/g or greater. In the group of patients receiving the probiotic Normobact Forte, the content of these bacteria in stool samples did not reach the acceptable level in 63.8% and 81% of cases, respectively.

In both groups, only about one-third of patients had the number of *Enterococcus* bacteria within normal limits. The level of *E. coli* with normal enzymatic activity was observed in less than 50% of cases and was within acceptable ranges. At the same time, 10.2% of patients in group 1 and 8.5% of patients in group 2 had *E. coli* with atypical enzymatic activity (lactose-negative and hemolytic *E. coli*) in the contents of the large intestines.

The above indicates pronounced dysbiotic changes in intestinal microbiocenosis. Against this background, 38.7% of patients in group 1 and 36.2% in group 2 were infected with opportunistic enterobacteriaceae, represented mainly by the genus *Enterobacter* of more than 10^5 CFU/g. This genus of bacteria was predominantly represented by species such as *En. aerogenes* and *En. gergovia*.

In group 1, microorganisms of the genera *Klebsiella* (8.2%), *Citrobacter* (6.1%), *Kluyvera* (4%), and *Proteus* (3.6%) were found in the feces of patients somewhat less frequently than

Table 2. Quantitative (CFU/g) and qualitative (M ± m [%]) composition of the microbiota in the large intestines of patients with pancreato-biliary disorders before and after complex therapy with probiotics

Microorganisms	Frequency in group 1 (amount of CFU/g is normal)		Frequency in group 2 (amount of CFU/g is normal)		Frequency in control group (amount of CFU/g is normal)	
	Before treatment (n = 49)	After treatment (n = 46)	Before treatment (n = 47)	After treatment (n = 42)	Before treatment (n = 30)	After treatment (n = 30)
Bifidobacterium	32.5 ± 3.9	73.9 ± 6.1*	36.2 ± 3.2	41.8 ± 3.8	37.2 ± 3.2	36.2 ± 3.2
Lactobacillus	20.4 ± 2.2	52.2 ± 4.5 *	19.0 ± 2.1	36.2 ± 2.4 *	19.3 ± 2.0	18.7 ± 1.9
Escherichia coli with normal enzymatic activity	51.0 ± 4.5	67.3 ± 5.7	49.3 ± 3.7	60.8 ± 4.3	48.6 ± 3.3	49.6 ± 3.1
E. coli with atypical enzymatic activity	10.2 ± 1.5	4.4 ± 0.8*	8.5 ± 1.4	7.1 ± 0.9	8.2 ± 1.4	8.4 ± 1.3
Lactose-negative *E. coli*	6.3 ± 3.5	3.2 ± 0.8*	6.3 ± 3.5	4.7 ± 1.0	6.3 ± 3.5	5.9 ± 3.2
Hemolytic *E. coli*	8.5 ± 4.0	4.3 ± 0.8*	8.5 ± 4.0	7.1 ± 0.8	8.2 ± 3.7	8.7 ± 4.0
Enterococcus	36.8 ± 3.6	56.5 ± 5.1	32.6 ± 3.0	47.3 ± 4.1	31.9 ± 2.8	33.7 ± 2.7
Clostridium	10.2 ± 1.7	6.5 ± 0.9	10.6 ± 1.9	6.2 ± 1.1	10.8 ± 1.7	10.3 ± 1.8
Enterobacteriaceae	38.7 ± 3.1	15.2 ± 1.5*	36.2 ± 5.9	28.5 ± 2.7	35.9 ± 5.9	35.1 ± 5.7
Pseudomonas aeruginosa	8.2 ± 1.1	4.4 ± 0.6*	8.5 ± 1.3	4.3 ± 0.7*	8.2 ± 1.2	8.3 ± 1.3
Staphylococcus aureus	8.1 ± 1.0	4.3 ± 0.8*	6.3 ± 0.9	4.8 ± 0.8	5.9 ± 0.7	6.1 ± 0.6
Yeast-like fungi of the genus *Candida*	10.2 ± 0.8	4.3 ± 0.8*	10.1 ± 1.1	8.5 ± 1.3	9.9 ± 1.0	10.3 ± 1.2

Note. *$p < 0.05$

isolates of the genus *Enterobacter*. *Clostridium* in the amount of 10^6 or more was detected in every fifth patient. *P. aeruginosa* and *S. aureus* were identified in 8% of cases, and yeast-like fungi of the genus *Candida* were found in the colon contents of every fifth individual. Isolates of nonfermenting gram-negative bacteria in most cases were found in association with representatives of the genera *Proteus* and *Klebsiella*. In 25.5% of cases, dysbiotic shifts in the intestinal microbiota of group 1 patients were due to the associations of 2 or more opportunistic microorganisms. Colonies of *S. aureus* and yeast-like fungi of the genus *Candida* in the feces of patients were rarely isolated.

In group 2, isolates of microorganisms of the genera *Kluyvera* and *Klebsiella* were found in the feces of patients somewhat less frequently than representatives of the genus *Enterobacter* (12% and 10%, respectively). Strains of bacteria of the genus *Citrobacter* accounted for 6.3% of all isolates of opportunistic enterobacteriaceae, in most cases represented by *C. freundii*. The microorganisms of the genus *Proteus* were reported in 3.2% of cases, and the genus *Patonteae* was found in only 1.7% of the cases. In the structure of the intestinal microbiocenosis of group 2 patients, *P. aeruginosa* (a nonfermenting gram-negative bacterium) was found in 8.5% of cases. *Clostridium* in an amount of 10^6 or more was found in 10.6% of the examined individuals. In general, the above-mentioned types of opportunistic bacteria, including with *S. aureus* and yeast-like fungi of the genus *Candida*, were identified in the form of associations in the contents of the large intestine in every fifth patient.

The second microbiological examination of feces for dysbiosis was conducted after 4 weeks of complex therapy, which included the use of the probiotic Biovestin-Lacto in group 1. The examination showed positive dynamics in the composition of intestinal microbiocenosis. This is evidenced by a significant increase in representatives of the indigenous flora, primarily *Bifidobacterium* and *Lactobacillus*. In 73.9% of cases, the concentration of *Bifidobacterium* was 10^8 CFU/g and higher; in 52.2% of cases, the concentration of *Lactobacillus* was 10^6 CFU/g and higher ($p < 0.05$). A comparative analysis showed that the probiotic therapy contributed to a significant increase in the number of patients with activated obligate flora. Biovestin-Lacto exerted a strong effect on the restoration of *E. coli* isolates with normal enzymatic activity (reduction of *E. coli* with atypical enzymatic activity to 4.4%, $p < 0.05$).

An important criterion for the effectiveness of the studied probiotics is a significant decrease in lactose-negative and hemolytic *E. coli* in the content of the large intestine, up to 3.2% and 4.3% ($p < 0.05$). The presence of bifidobacteria and lactobacilli in the composition of Biovestin-Lacto significantly increased isolates of obligate flora. As a result, the number of OE examined in the large intestine decreased by up to 15.2%. The number of *P. aeruginosa* also decreased by up to 4.4% ($p < 0.05$). In addition, the associations of opportunistic bacteria included no more than 2 microorganisms, including *S. aureus* and yeast-like fungi of the genus *Candida*. The frequency of their detection decreased by 1.9 and 2.4 times, respectively ($p < 0.05$).

After 4 weeks of complex therapy with the inclusion of the biopreparation Normobact Forte, a control analysis of feces for dysbiosis revealed positive changes in the quantitative and qualitative composition of intestinal microbiocenosis among patients of group 2. Thus, due to the therapy with Normobact Forte, there was an increase in the number of patients who demonstrated normal indicators of the indigenous flora. In particular, *Lactobacillus* at a concentration of 10^6 CFU/g or higher amounted to 36.2% ($p < 0.05$) of cases. At the same time, the number of *E. coli* isolated with atypical enzymatic activity, including hemolytic and lactose-negative *E. coli*, decreased by 1.2 times. The therapy, based on the use of the studied probiotics, led to an increase in the rate of *Enterococcus*. OE became less frequent by 15%, primarily due to a decrease in the number of bacteria of the genera *Enterobacter*, *Klebsiella*, and *Citrobacter*.

The number of nonfermenting gram-negative bacteria in the contents of the large intestine decreased by 2 times. The number of isolates of fungi of the genus *Candida* also decreased. Accordingly, associations of opportunistic bacteria, including yeast-like fungi of the genus *Candida* and *S. aureus*, began to appear in a smaller number of the examined patients. In the control group, there were no significant differences in the composition of the intestinal microbiota before and after treatment.

Thus, the conducted studies confirmed the positive effects exerted by probiotics such as Biovestin-Lacto and Normobact Forte within the complex therapy of patients with hyperosmolar diarrhea. Although the probiotics were not able to completely restore the microbiocenosis in all patients examined, they did positively affect the intestinal microbiota. A comparative analysis of the studied probiotics revealed their effectiveness in terms of correcting the identified dysbiotic shifts in the microbiota. The results of the analysis showed that against the background of using the probiotic Biovestin-lacto in the complex therapy of patients with hyperosmolar diarrhea, the content of bifidoflora isolates in the large intestines increased significantly ($p < 0.05$). In addition, this increase was more substantial compared to that in the group receiving Normobact Forte. The most likely reason for this result is the presence of 2 types of *Bifidobacterium* in this biopreparation (*B. bifidum*, *B. adolescentis*). In addition, the rapid growth rate of *B. adolescentis* allows for the quick establishment of an artificial microbiocenosis in the intestines. At the same time, metabolic products with prebiotic activity enhance the growth of indigenous microflora.

A 4-week course of therapy with Biovestin-Lacto activated the entire obligate flora of the contents of the large intestine in patients with chronic inflammatory diseases of the biliary tract and pancreas. Thus, *Lactobacillus* and *Enterococcus* were detected 1.4 and 1.2 times more frequently, respectively, compared to the group of patients receiving the probiotic Normobact Forte. The activation of the indigenous flora contributed to a decrease in the number of hemolytic and lactose-negative *E. coli*. These bacteria were statistically significantly less frequently detected in the feces of patients receiving the Biovestin-Lacto probiotic within the complex therapy ($p < 0.05$).

In group 1, OE were found in 15.2% of patients. At the same time, OE were detected in the large intestines of every third patient receiving the biopreparation Normobact Forte. It is necessary to note that yeast-like fungi of the genus *Candida* were detected 2 times less frequently in the intestinal microbial landscape of patients with hyperosmolar diarrhea who received treatment with the Biovestin-Lacto probiotic ($p < 0.05$).

An analysis of changes in intestinal microbiocenosis among patients with hyperosmolar diarrhea showed that the use of probiotics altered the distribution of patients according to the

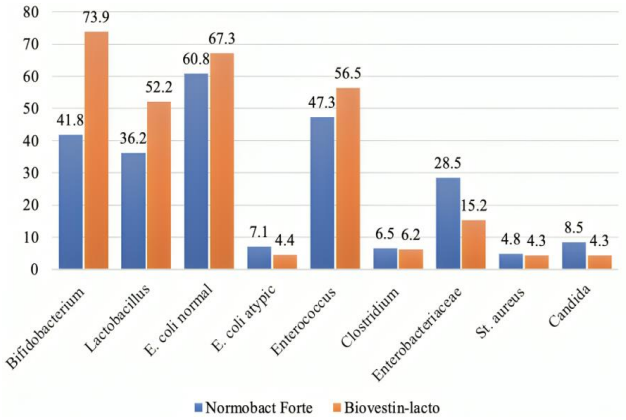

Figure 2. Comparative analysis of the intestinal microflora of patients with pancreato-biliary disorders after the complex therapy with probiotic (Source: Authors' own elaboration)

severity of intestinal dysbiosis. Against the background of complex therapy with the use of Normobact Forte, 16.7% of cases were dysbiosis of the 1st degree, 47.6% were the 2nd degree, and 35.7% of patients had dysbiosis of the 3rd degree. Among patients who received treatment for intestinal dysbiosis with Biovestin-Lacto, 30.4% of cases were the 1st degree of dysbiosis, 52.2% were the 2nd degree, and 17.4% of the examined patients had dysbiosis of the 3rd degree.

Thus, a comparative analysis showed a higher bacteriological efficacy of Biovestin-Lacto. This probiotic has a positive effect on the intestinal microflora of patients with gastrointestinal diseases. Biovestin-Lacto stimulates the indigenous flora and replenishes the deficiency of obligate microorganisms, thereby reducing the growth of opportunistic bacteria and yeast-like fungi (**Figure 2**).

DISCUSSION

This study assesses the nature and features of dysbiotic shifts in the microflora of patients with pancreato-biliary disorders accompanied by hyperosmolar diarrhea. In addition, the study aims to substantiate the effectiveness of probiotics in correcting dysbiotic shifts in intestinal microbiocenosis. The microbiological analysis revealed dysbiotic shifts in the microbiocenosis of all individuals suffering from chronic inflammatory diseases of the pancreato-biliary system and hyperosmolar diarrhea. These shifts were characterized by a decrease in content of obligate flora and the activation of opportunistic microorganisms, including *E. coli* with atypical enzymatic activity, *S. aureus*, and yeast-like fungi of the genus *Candida*. These microorganisms were mainly found in associations with bacteria of the Enterobacteriaceae family.

The obtained data are consistent with the results of other studies that have investigated intestinal microbiocenosis in patients with chronic diseases of the pancreato-biliary system. Previous studies have found that 100% of these patients had certain disorders of intestinal microbiocenosis [34]. According to research data, the main patterns of the intestinal microflora imbalance are a decrease in bacteria of the normoflora (primarily *Bifidobacterium* and *Lactobacillus*) and an increase in the number of *E. coli* with atypical properties, enterococci, fungi of genus *Candida* and bacteria of the Enterobacteriaceae family [35, 36]. The deficiency of *Bifidobacterium* is exacerbated by the worsening of the pathological process.

The microbiocenosis of the large intestine plays a direct role in maintaining the internal balance of the human body [14]. The most notable task of microbiocenosis is the creation of colonization resistance. Microbiocenosis prevents colonization of the intestine by pathogenic microorganisms through the synthesis of substances that inhibit their growth, as well as competition for nutrients and adhesion sites [15]. The intestinal microbiota is also involved in the synthesis of vitamins (vitamin K, biotin, and folic acid), which are absorbed through the intestinal wall and play an important role in metabolism [37]. Short-chain fatty acids, which are synthesized by bacteria of normal microflora, are absorbed in the intestine and serve as an additional source of energy. These acids also participate in the stimulation, proliferation, and differentiation of the intestinal epithelium, being participants in a complex system of interaction between the microflora and the immune system [15].

The problem of studying intestinal microbiocenosis is reduced to attempts to correct it with the help of microbiological preparations [38]. Lactic acid-producing microorganisms, such as *Bifidobacterium* and *Lactobacillus*, are part of the normal human microflora. Therefore, these microorganisms are the most commonly used for this type of treatment [18, 20]. In addition to lactic acid, some strains produce substances known as bacteriocins. For example, in vitro studies have shown that the *L. acidophilus* La1 strain produces a compound with antimicrobial activity that is effective in the treatment of diseases associated with *Helicobacter pylori* [39].

Typically, probiotics are used as prophylactic preparations and for the purpose of correcting dysbiotic disorders [23]. The development of dysbiosis goes beyond the deficiency of obligate and facultative microflora. Dysbiosis indicates an impairment of the microecosystem. Accordingly, the administration of probiotics is clearly insufficient to correct microbiocenosis. The main goal is not to introduce bacteria from the normal microbiota into the intestinal mucosa of patients, but rather to restore the balance of microorganisms in the gastrointestinal tract and increase the density of indigenous microflora. At the present stage, molecular genetic research and molecular biological studies have identified more than 60 species of *Bifidobacterium* and *Lactobacillus* [40]. Therefore, a probiotic must contain those species of the genera *Bifidobacterium* and *Lactobacillius* that have the following properties: rapid reproduction, high rates of survival and antagonistic activity, and the ability to colonize natural habitats. Experimental studies have shown that not all strains of normoflora microorganisms are applicable for the treatment of various diseases. Currently, strong antagonists with a wide range of antimicrobial activity against pathogenic and opportunistic microorganisms are of interest. In this regard, lactobacilli, which persist in the gastrointestinal tract for a long time, have selective advantages and are capable of survival in a macroorganism. Previous research has shown that orally administered exogenous probiotic lactobacilli (including *L. plantarum* that produce bacteriocins) are preserved for a longer time in the intestine [41].

Thus, probiotics containing *Bifidobacterium* and *Lactobacillus* have a proven clinical significance and effective mechanism of action. Nevertheless, their effectiveness in the prevention and treatment of gastrointestinal pathologies is contradictory in some cases and remains understudied.

However, the study of the frequency and structure of dysbiosis, as well as the results of probiotic correction, confirm that the use of bifido- and lactose-containing probiotics is clinically significant for patients. The use of biological preparations containing indigenous flora has mainly shown a clinical and microbiological advantage, as well as a clear morphological effect. Apparently, the most promising are the development, production, and testing of new polyvalent or combined biopreparations containing autochthonous bacteria of various taxonomic groups for therapeutic correction.

One of the probiotics that is currently used in medical practice to correct dysbiotic disorders is Biovestin-Lacto, produced by Bio-Vesta (Novosibirsk, Russia). The basis of this probiotic includes two strains of *Bifidobacterium* (*B. bifidum*, *B. adolescentis*) and a specific strain of *Lactobacillus plantarum*, as well as metabolic products of indigenous flora. For the entire shelf life, 1 ml of the probiotic contains 10^9 live *Bifidobacterium* and 10^8 *Lactobacillus* [24]. This combination of *Bifidobacterium* allows for using this preparation for the population of all ages–from newborns to the elderly. Due to the high antagonistic activity of *Bifidobacterium* and *Lactobacillus*, Biovestin-Lacto can be used for dysbiosis caused both by a decrease in the indigenous microflora and by opportunistic microorganisms. The rapid growth rate of *B. adolescentis* allows for the quick establishment of artificial microbiocenosis in the intestines. Metabolic products with prebiotic activity enhance the growth of indigenous microflora.

The comparative analysis performed in this study showed that Biovestin-Lacto has a higher bacteriological effectiveness, which leveled dysbiotic shifts in the intestinal microbiocenosis of patients with hyperosmolar diarrhea. This probiotic stimulated the indigenous flora and replenished the deficiency of obligate microorganisms, thereby reducing the growth of opportunistic bacteria and yeast-like fungi. In turn, Normobact Forte turned out to be less effective.

The literature and the presented data suggest a systemic mechanism of action for the probiotic under study. However, its potential use in treating various diseases, particularly those affecting the gastrointestinal system in adults, has not been sufficiently explored. This gap highlights the need for further research.

The limitation of this study is that patients with various diseases of the pancreato-biliary system were grouped together. It is known that the pancreas and gallbladder interact anatomically and functionally. A dysfunction in one of these organs can lead to changes in the activity of the digestive triad, which includes the duodenum, biliary system, and pancreas. The resulting pathological process in one of the organs sooner or later causes the spread of the disease to neighboring organs. An analysis of morbidity in a group of patients with hyperosmolar diarrhea revealed that in 76% of cases, there was a combined chronic inflammation of the pancreas and gallbladder [2]. In this research, the goal was not to consider microbiocenosis in terms of nosology, but to study diarrhea development mechanisms and the effectiveness of probiotic administration. Therefore, patients with CC, pancreatitis, and PCS were grouped into one cohort, given a single hyperosmolar mechanism of diarrhea.

CONCLUSIONS

The intestinal microbiocenosis of patients with pancreato-biliary disorders accompanied by hyperosmolar diarrhea entails dysbiotic changes of varying severity. These changes were recorded in the entire group of examined patients. The dysbiotic shifts in the intestinal microflora are unidirectional and lead to the activation of opportunistic flora, including associations with *S. aureus*, *P. aeruginosa* and yeast-like fungi of the genus *Candida*, against the background of deficiency of *Bifidobacterium* and *Lactobacillus*.

The therapeutic effect of probiotics is attributed to the bioavailability, species composition and quantitative content of the normal intestinal microbiota. The use of Biovestin-lacto, a liquid probiotic containing strains of *B. bifidum* and *B. adolescentis* at a concentration of 10^9 CFU/ml and *L. plantarum* at a concentration of 10^8 CFU/ml, in the complex therapy of patients with hyperosmolar diarrhea was effective. This probiotic contributed to the positive dynamics of dysbiotic shifts in intestinal microbiocenosis. In turn, the administration of the biopreparation Normobact Forte (capsules) containing the strain LGG at a concentration of 6×10^9 CFU/G was less effective.

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Declaration of interest: No conflict of interest is declared by the authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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