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Efficacy and Safety of Postbiotic (Metabiotic) Contained Inactivated Lactobacillus Reuteri (Limosilactobacillus Reuteri) DSM 17648 as Adjuvant Therapy in the Eradication of Helicobacter Pylori in Adults with Functional Dyspepsia: A Randomized Double-Blind Placebo Controlled Trial

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Abstract: Increasing the effectiveness of eradication therapy is an important task of gastroenterology. The aim was to evaluate the efficacy and safety of postbiotic (metabiotic) contained inactivated Limosilactobacillus reuteri DSM 17648 (Pylopass™; Helinorm™) as adjuvant treatment for Helicobacter pylori eradication in patients with functional dyspepsia (FD). This randomized, double-blind, placebo-controlled, multicenter, parallel study included H. pylori positive patients with FD. The postbiotic group received Pylopass 200 mg bid for 14 days in combination with the eradication therapy (esomeprazole 20 mg bid + amoxicillin 1000 mg bid + clarithromycin 500 mg bid for 14 days) and another 14 days after completion of eradication therapy. The eradication efficiency was 96.7% for the postbiotic group versus 86.0% for the placebo group (P=0.039). Both groups showed significant improvements in quality of life and reduction of most gastrointestinal symptoms with no significant differences between groups. The overall number of digestive adverse effects in the postbiotic group was lower than in the placebo group. Serious adverse effects were not registered. The postbiotic (metabiotic) contained inactivated L. reuteri DSM 17648 significantly improves the effectiveness of H. pylori eradication therapy in FD and decreases overall number of digestive adverse effects of this therapy.

Keywords: postbiotics; metabiotics; eradication; functional digestive disease; probiotics; microbiota

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1. Introduction

According to the Rome IV criteria, functional dyspepsia (FD) is a complex of symptoms (postprandial fullness, early satiation epigastric pain and epigastric burning) for the last 3 months with symptom onset at least 6 months before diagnosis with no evidence of organic, systemic, or metabolic disease that would explain the symptoms on routine investigations [1,2]. According to the results of large-scale studies, the prevalence of FD worldwide ranges from 10 to 30%. Patients with FD have a reduced quality of life, which is associated with the severity of symptoms and concomitant depression, the need for high medical costs and reduced ability to work [1,3]. The pathogenesis of FD development is not fully understood [1–4].

The prevalence of Helicobacter pylori among patients with FD ranges from 20 to 60%. [5]. Eradication therapy in these patients led to a decrease in the symptom severity in some patients but it is not always effective [1,6,7]. To increase eradication therapy efficacy, several methods have been proposed, including addition probiotics and mucosal protective agents such as rebamipide to standard eradication therapy. This complementation showed a positive effect [6–9].

The Lactobacillus reuteri (the new name is Limosilactobacillus reuteri) strain DSM 17648 was selected as a highly specific H. pylori antagonist binding these bacteria in artificial gastric juice, reducing their mobility and adhesion ability. L. reuteri strain DSM 17648 co-aggregates different types and species of Helicobacter (H. pylori type I and II, H. heilmannii type I and II, H. canis), but not bacterial representatives of normal oral or intestinal microbiota [10]. It has been suggested that not only live bacteria, but also inactivated bacteria, containing their metabolic products (postbiotics, also called metabiotics, ghostbiotics or paraprobiotics) can have a positive effect [11,12]. Postbiotics are safer for consumers, faster in action, and less demanding to storage conditions than probiotics because they do not contain live bacteria. Postbiotic based on L. reuteri DSM 17648 with a trade name Pylopass™ showed ability to reduce activity of H. pylori, and exerted a preventive effect of secondary diseases and related symptoms due to H. pylori infection. Moreover, the preventive effect was carried over well after the supplementation itself, at least for the six month period [10,13,14]. However, there was no placebo-controlled randomized study that directly showed its ability to increase the effectiveness of eradication therapy in adults. Evaluation of the efficacy and safety of postbiotic based on L. reuteri DSM 17648 as supplementation for the H. pylori eradication therapy in patients with functional dyspepsia is the aim of this study.

2. Materials and Methods

This randomized, double-blind placebo-controlled, multicenter, parallel study was approved by the Local ethics committee of Sechenov University (protocol № 10-19 dated 17.07.2019) in accordance with the Helsinki declaration and registered in the ISRCTN registry (ISRCTN20716052). The study was conducted in 6 independent centers in Russia from September 2019 to June 2023.

The randomization lists was created using the blockrand package (version 1.3) of the Microsoft R Open statistical software with the following parameters:

- two blinded groups with equal distribution into groups;
- six uniformly created randomization lists - one for each center
- randomization sequences (seed) were 4037 (Center 1), 3031 (Center 2), 3739 (Center 3), 3034 (Center 4), 3259 (Center 5), and 3125 (Center 6).
- variable block length (2, 4, 6, 8, 10, 12, 14, 16, 18 or 20), total number of blocks were 18 for Center 1, 17 for Center 2, 16 for Center 3, 16 for Center 4, 20 for Center 5, and 18 for Center 6.

2.1. Patients

All patients who visited the clinics of the centers with characteristic complaints of functional dyspepsia were screened for participation in the study.

The inclusion criteria were:

- male or female aged 18-65;
- for a woman of childbearing age: mandatory use of contraceptive methods;
- confirmed diagnosis of functional dyspepsia according to the Rome IV criteria [1];

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- presence of H. pylori infection according to 13C UBT [6,7];
- no history of previous eradication therapy at least a year before the screening;
- ability to understand and willingness to follow all protocol details;
- signed informed consent.

The exclusion criteria were:

- erosive, ulcerative, or cicatricial changes in the stomach and/or duodenum;
- history of eradication therapy less than 1 year prior to screening;
- use of antibiotics and/or bismuth trication dicitrate and/or H2 secretion blockers and/or proton pump inhibitors 30 days before and during the study;
- use of macrolide antibiotics less than 1 year prior to screening;
- any severe, decompensated, or unstable medical condition that could affect the clinical evaluation of the investigational product or put the patient at risk;
- pregnancy, lactation;
- known sensitivity to any components of the study product and any of the drugs prescribed in this study;
- history of surgical treatment of the stomach, resection of the small intestine or operations on the pancreas;
- a positive blood test result for HIV and/or syphilis and/or HbsAg and/or HCVAb;
- chronic diarrhea of various etiologies, except for functional diarrhea or irritable bowel syndrome with diarrhea;
- participation in another clinical study 30 days before and during the study;
- use of probiotics, symbiotics, prebiotics for the treatment of H. pylori infections and for other reasons within 30 days prior to study entry;
- refusal to continue the study, including the refusal of visits 3 and / or 4 and the investigations on these visits (other than taking blood for analyses);
- development of a clinical condition that is associated with safety and, in the opinion of the investigator, requires termination of participation in the clinical study;
- failure to comply with the minimum duration of eradication therapy (10 days, compliance less than 75%);
- use during the treatment and follow-up period of antibiotics, probiotics, prebiotics, postbiotics, antisecretory or bismuth drugs, sucralfate, drugs with a pronounced hepato- and nephrotoxic effect, with the exception of drugs that were used in the tested eradication therapy regimens.

Initially, based on study by Zagari et al. [15], it was assumed for sample size estimation that the eradication rate would be 75% in the control group and 90% in the postbiotic group, which at significance level of 95%, power of 80%, and randomization rate 1:1 gave the required number of patients included in 172 persons. However, the development of the COVID-19 pandemic has slowed patient enrollment and we have revised our calculations based on more recent work by De Francesco et al. [16], estimating that that the eradication rate would be 79% in the control group and 94% in the postbiotic group). Based on these data the STATISTICA 10 software (StatSoft Inc., Tulsa, Oklahoma, USA) calculated required number of patients as 64 for each group which corresponds to 128 included patients.

2.2. Intervention

Patients were randomized 1:1 to the postbiotic and placebo groups. Out-patients in the postbiotic group simultaneously with standard eradication therapy (esomeprazole 20 mg bid 15-30 min before meals + amoxicillin 1000 mg bid after meal+ clarithromycin 500 mg bid after meal for 14 days) received 1 capsule with 324 mg Helinorm™ containing 200 mg Pylopass™ (2 x 1010 spray-dried L. reuteri DSM 17648 cells) bid with meals for 14 days and another 14 days after completion of eradication therapy.

2.3. Controls

Out-patients in the placebo (control) group simultaneously with standard eradication therapy (esomeprazole 20 mg bid 15-30 min before meal + amoxicillin 1000 mg bid after meal+ clarithromycin

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500 mg bid after meal for 14 days) received the placebo at a dose of 324 mg bid with meals for 14 days and another 14 days after completion of eradication therapy. Visually, the placebo capsules and their jars did not differ from the postbiotic capsules and jars. Neither the patients nor the medical staff working with them knew whether it was postbiotic or placebo.

2.4. Outcomes

The primary outcome was successful eradication of H. pylori, defined as a negative 13C- UBT 28 days after the end of the postbiotic/placebo administration (42 days after the end of eradication therapy). The period of eradication therapy (14+/-2 days) was defined as the treatment period, and the period after the end of eradication therapy until the repeated breath test for H. pylori (42+/-3 days) was defined as the follow-up period.

13C-UBT was carried out according to the same methodology at the inclusion of the study and at its end. It was performed in the morning on an empty stomach using the Helicarb kit (Isocarb Limited, Moscow, Russia). The patient drank 200 ml of orange juice included in this kit and 5 minutes after it he/she exhaled air into the Urease test bag 1. Then he/she was given a 50 ml aqueous solution containing 50 mg 13C-urea. During the next 30 minutes, the patient did not eat, rested and did not smoke, and subsequently a new sample of exhaled air was taken from him/her in the Urease test bag 2. Both bags were delivered to the laboratory on the same day or in the morning of the next day, where their contents were analyzed by infrared isotope analyzer IRIS-Doc (Kibion AB; Sweden) in accordance with the manufacturer's instructions. The presence of H. pylori infection was assessed by an increase in 13C-carbon dioxide between the samples UBT 2 and UBT 1, which these bacteria produce from the 13C-urea with the urease enzyme in the stomach .

Secondary outcomes included:

1) a change in the severity of digestive symptoms, defined as a change in the 7x7 [17] and Gastrointestinal Symptom Rating Scale (GSRS) [18] questionnaire scores ;

2) a change in the quality of life, defined as change in the SF-36 [19] questionnaire scores;

3) a change in the value of the main parameters of a complete blood count (hemoglobin, white blood cell and platelet count, erythrocyte sedimentation rate) and a biochemical blood test (the serum levels of total protein, total bilirubin, creatinine, amylase, alkaline phosphatase, ALT, and AST);

4) frequency and severity of adverse effects of therapy;

5) therapy compliance, defined as the ratio of the number of used drugs to those that should be used. The number of used drugs was estimated by the number of empty places in the drug blisters, which patients returned by the end of eradication therapy.

The study scheme is presented in Table 1.

Table 1. The study scheme.

	Screening	Eradication treatment		Post-eradication treatment	Follow-up
		14 days		14 days	28 days
	Visit 1 (Day -8 –[-1])	Visit 2 (Day 0)	Visit 3 (Day 14)		Visit 4 (Day 56 ± 3)
Postbiotic/Placebo taking					
Eradication treatment					
Demographics	+				
Medical history	+				
Complete blood count	+		+		
Serum chemistry	+		+		

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Anti-HIV Ab, RPR, HBsAg, HCV-Ab	+				
Gastroscopy	+				
C-13 urease breath test for H. pylori	+				+
Pregnancy test	+				
Signing informed consent	+				
Checking inclusion and exclusion criteria		+	+		+
Randomization		+			
Initiation of eradication therapy for 14 days + postbiotic/placebo for 28 days		+			
7x7 questionnaire		+	+		+
GSRS questionnaire		+	+		+
SF-36 questionnaire		+	+		+
Assessment of adverse effects			+		+
Compliance assessment			+		

2.5. Statistics

Quantitative parameters were presented as a median [interquartile range]. Comparison of categorical data was carried out by Fisher's exact test; comparison of quantitative and semi-quantitative data was evaluated by the Mann-Whitney method. Changes in the values of extended and semi-quantitative parameters were assessed by the Wilcoxon test. Significance criterion was $p<0.05$. Statistical data processing was carried out using the STATISTICA 10 software (StatSoft Inc., Tulsa, Oklahoma, USA). The effectiveness of therapy was assessed according to the per-protocol analysis; safety and compliance were assessed according to the intention-to-treat analysis.

3. Results

Of the 204 screened patients, 129 met the criteria and were included in the study. Among them, 66 were randomized to the postbiotic group and 63 did to the placebo group. Five patients in each group refused to visit the centers again (visits 3 and/or 4) to evaluate the results of therapy. One patient in each group developed adverse effects that led to the termination of eradication therapy before the minimum time (10 days), so they were also excluded from the analysis. In total, data from 60 patients in the postbiotic group and 57 patients in the placebo group were analyzed (Figure 1). There was no significant difference in baseline parameters between groups (Table 2). There was no significant difference between groups in the incidence of comorbidities and the use of medication to treat them (Supplementary Table S1).

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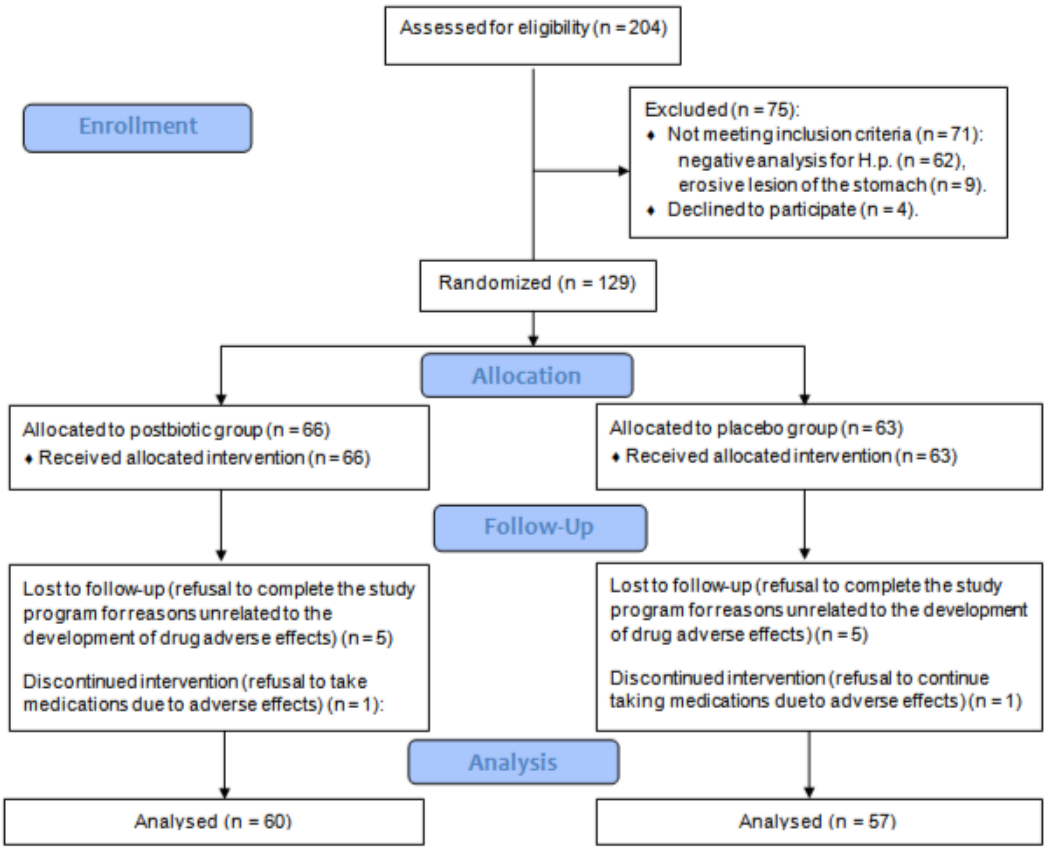

Figure 1. CONSORT 2010 Flow Diagram.

Table 2. Baseline characteristics of patients.

	Postbiotic group (n=60)	Placebo group (n=57)	p
Age, years	47[38-56]	47[36-53]	0.213
Male/Female	19/41	21/36	0.346
Body mass index, kg/m^2	24.8[22.9-27.4]	25.6[22.4-29.0]	0.343
Hemoglobin, 10^9/L	137[125-145]	138[127-147]	0.278
White blood cell, 10^9/L	6.1[5.4-6.7]	6.1[5.4-7.2]	0.332
Platelet, 10^9/L	239[213-283]	257[216-296]	0.271
Erythrocyte sedimentation rate, mm/h	8[6-12]	7[4-10]	0.097
Serum total protein, g/L	72[69-76]	72[68-76]	0.983
Serum total bilirubin, μmol/L	10.3[7.5-13.2]	10.6[7.0-16.4]	0.691
Serum creatinine, μmol/L	82[74-90]	79[74-93]	0.775
Serum amylase, U/L	59[42-113]	55[44-78]	0.325

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Serum alkaline phosphatase, U/L	110[62-158]	106[67-146]	0.907
Serum ALT, U/L	19[13-25]	19[15-28]	0.258
Serum AST, U/L	20[16-26]	20[17-24]	0.883
SF-36 questionnaire			
Physical functioning	90[78-95]	85[70-95]	0.347
Physical role functioning	75[25-100]	75[25-100]	0.633
Emotional role functioning	67[0-100]	33[33-100]	0.593
Vitality	55[45-65]	55[40-70]	0.972
Mental health	56[44-68]	60[48-72]	0.804
Social role functioning	75[63-88]	75[63-88]	0.699
Bodily pain	68[45-78]	95[45-78]	0.789
General health perceptions	55[45-70]	50[40-60]	0.198
7x7 questionnaire			
Pain in the stomach area	4[3-5]	4[2-5]	0.872
A feeling of burning in the stomach area	3[0-4]	2[0-4]	0.531
Fullness in the stomach after a meal	2[1-3]	2[1-3]	0.624
Early satiety	2[0-3]	2[0-3]	0.610
Abdominal pain decreases after a bowel movement	0[0-2]	0[0-3]	0.762
Bloating	4[0-5]	4[0-5]	0.485
Violation of the consistency or stool frequency	0[0-2]	0[0-2]	0.825
Total	15[11-19]	15[11-20]	0.721
Gastrointestinal Symptom Rating Scale (GSRS)			
Pain or discomfort in your upper abdomen	4[3-4]	4[2-5]	0.543
Heartburn	2[1-4]	2[1-4]	0.777
Acid reflux	2[1-4]	3[1-4]	0.324
Hunger pains	2[1-4]	2[1-3]	0.627
Nausea	1[1-3]	1[1-3]	0.663
Rumbling	3[2-4]	3[2-5]	0.130
Bloating	4[2-4]	4[3-5]	0.103
Burping	3[2-5]	3[2-4]	0.379
Passing gas or flatus	4[2-5]	3[1-5]	0.612
Constipation	1[1-3]	1[1-3]	0.709
Diarrhea	1[1-2]	1[1-3]	0.612
Loose stools	1[1-2]	1[1-3]	0.281
Hard stools	1[1-3]	1[1-3]	0.693
Urgent need to have a bowel movement	1[1-2]	1[1-2]	0.563
Sensation of not completely emptying the bowels	2[1-3]	1[1-3]	0.403
Total	34[26-47]	33[27-53]	0.576

At the end of the follow-up period, 2 (3.3%) patients in the postbiotic group and 8 (14.0%) patients in the placebo group had a positive 13C-UBT for H. pylori. The eradication efficiency was 96.7% for the postbiotic group and 86.0% for the placebo group ($p = 0.039$; Table 3).

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Table 3. Efficiency of H. pylori eradication in patients receiving the postbiotic or placebo as an adjuvant to eradication therapy.

	Eradication is effective	Eradication is not effective	Eradication rate	p
Postbiotic	58	2	97.3%	0.039
Placebo	49	8	86.0%	

By the end of the follow-up period, both groups showed significant improvements in quality of life according to all sections of the SF-36 scale with no significant differences between groups (Table 4).

Table 4. Changes in digestive symptoms (decreases in the scores of the 7x7 and Gastrointestinal Symptom Rating Scales) and quality of life (increases in the scores of the SF-36 scale) in the patients at the end of eradication therapy (day 14) and at the end of the follow-up period (day 56±3) compared with baseline.

	At the end of eradication therapy			At the end of the follow-up period		
	Postbiotic group	Placebo group	P	Postbiotic group	Placebo group	p
SF-36 questionnaire						
Physical functioning	5[0-10]*	0[0-10]*	0.898	0[0-10]*/**	5[0-15]*/**	0.344
Physical role functioning	0[0-25]	0[0-25]	0.529	0[0-50]*/**	0[0-50]*/**	0.583
Emotional role functioning	0[0-33]	0[0-34]	0.293	0[0-67]*/**	33[0-67]*/**	0.414
Vitality	5[0-10]*	5[0-20]*	0.655	10[0-20]*/**	10[0-25]*/**	0.583
Mental health	4[0-8]*	4[0-12]*	0.915	8[0-20]*/**	8[4-16]*/**	0.894
Social role functioning	0[0-25]*	0[0-13]*	0.497	13[0-25]*/**	13[0-25]*/**	0.938
Bodily pain	10[0-23]*	10[0-22]*	0.943	22[2-32]*/**	22[0-42]*/**	0.940
General health perceptions	0[-3-10]*	5[-5-10]*	0.910	5[-5-10]*	5[0-15]*	0.400
7x7 questionnaire						
Pain in the stomach area	2[1-3]*	2[0-3]*	0.413	3[2-4]*/**	3[2-4]*/**	0.791
A feeling of burning in the stomach area	1[0-3]*	0[0-2]*	0.879	2[0-4]*/**	0[0-3]*/**	0.436
Fullness in the stomach after a meal	1[0-2]*	1[0-2]*	0.711	1[0-2]*/**	2[0-3]*/**	0.629
Early satiety	0[0-2]*	0[0-2]*	0.541	1[0-3]*/**	1[0-3]*/**	0.934
Abdominal pain decreases after a bowel movement	0[0-1]	0[0-0]	0.378	0[0-2]*	0[0-2]*/**	0.954
Bloating	1[0-2]*	2[0-3]*	0.436	1[0-3]*/**	2[0-4]*/**	0.476
Violation of the consistency or stool frequency	0[0-0]	0[0-0]	0.943	0[0-1]**	0[0-1]**	0.380

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Total	5[2-9]*	7[3-11]*	0.432	9[6-13]*/**	10[6-15]*/**	0.536
Gastrointestinal Symptom Rating Scale (GSRS)						
Pain or discomfort in your upper abdomen	1[0-2]*	1[0-2]*	0.250	2[0-3]*/**	2[1-3]*/**	0.817
Heartburn	0[0-2]*	0[0-2]*	0.357	1[0-2]*/**	1[0-2]*	0.795
Acid reflux	0[0-2]*	0[0-1]*	0.501	0[0-2]*/**	1[0-2]*/**	0.260
Hunger pains	0[0-2]*	0[0-2]*	0.155	1[0-2]*/**	0[0-2]*	0.866
Nausea	0[0-1]*	0[0-1]*	0.460	0[0-2]*/**	0[0-2]*/**	0.978
Rumbling	1[0-2]*	1[0-2]*	0.078	1[0-2]*/**	2[0-3]*/**	0.141
Bloating	1[0-2]*	2[0-2]*	0.108	1[0-3]*/**	2[1-4]*/**	0.059
Burping	1[0-2]*	1[0-2]*	0.301	1[0-2]*/**	2[0-3]*/**	0.068
Passing gas or flatus	0[0-2]*	1[0-2]*	0.567	1[0-2]*/**	1[0-3]*/**	0.834
Constipation	0[0-1]*	0[0-0]	0.343	0[0-1]*	0[0-1]*	0.851
Diarrhea	0[-1-1]	0[0-1]	0.351	0[0-1]*/**	0[0-1]*/**	0.657
Loose stools	0[0-1]	0[0-1]	0.249	0[0-1]*/**	0[0-2]*/**	0.105
Hard stools	0[0-1]*	0[0-1]*	0.806	0[0-1]*	0[0-1]*	0.965
Urgent need to have a bowel movement	0[0-0]	0[0-1]	0.406	0[0-0]	0[0-1]	0.151
Sensation of not completely emptying the bowels	0[0-1]*	0[0-1]*	0.494	0[0-2]*	0[0-1]*	0.271
Total	7[1-15]*	7[1-18]*	0.257	11[4-21]*/**	16[5-25]*/**	0.349

* - significant changes compared to the baseline (the Wilcoxon test); ** - significant changes at the end of the follow-up period compared with the end of eradication therapy (the Wilcoxon test).

At the end of eradication therapy (Day 14) in both groups, there were significant improvements compared to baseline in most digestive symptoms, including symptoms of functional dyspepsia (pain in the stomach area, a feeling of burning in the stomach area, fullness in the stomach after a meal, early satiety, and bloating), which became even more significant by the end of the follow-up period (Table 4). There was no significant difference in the change in the severity of digestive symptoms neither at the end of eradication therapy nor at the end of the follow-up period between the groups (Table 4).

There was no significant change in any of the tested laboratory parameters from baseline to the end of eradication therapy and there were also no differences in these parameters between the groups (Supplementary Table S2).

Compliance with the postbiotic was 100% in 98.5% of patients and did not significantly differ from the compliance with placebo. Compliance with standard eradication therapy was 100% in 97.0% of patients in the postbiotic group and did not differ significantly from that in the placebo group (Table 5).

Table 5. Compliance with intervention.

Compliance	Postbiotic group (n=66)	Placebo group (n=63)	p
Compliance with postbiotic/placebo			
100%, n(%)	65 (98.5%)	62(98.4%)	0.736
76-99%, n(%)	-	1 (1.6%)	
50-75%, n(%)	1 (1.5%)	-	

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<50%, n(%)	-	-	
Compliance with esomeprazole			
100%, n(%)	65 (98.5%)	62 (98.4%)	0.736
76-99%, n(%)	-	1 (1.6%)	
50-75%, n(%)	1 (1.5%)	-	
<50%, n(%)	-	-	
Compliance with amoxicillin			
100%, n(%)	64 (97.0%)	60 (95.2%)	0.478
76-99%, n(%)	1 (1.5%)	2 (3.2%)	
50-75%, n(%)	-	1(1.6%)	
<50%, n(%)	1 (1.5%)	-	
Compliance with clarithromycin			
100%, n(%)	64 (97.0%)	60 (95.2%)	0.478
76-99%, n(%)	1 (1.5%)	2 (3.2%)	
50-75%, n(%)	-	1(1.6%)	
<50%, n(%)	1 (1.5%)	-	

Adverse effects developed in 30.8% of patients in the postbiotic group and in 38.0% of patients in the placebo group. Most of them were mild. Only in 1.5% of patients in the postbiotic group and in 3.2% of patients in the placebo group, the development of adverse effects required discontinuation of drugs (pain in the lumbar region in one patient in the postbiotic group; severe nausea with bitter taste in the mouth in one patient in the placebo group; moderate diarrhea in another patient in the placebo group). Serious adverse effects were not registered. The postbiotic regimen tended to cause diarrhea less frequently than regime without it, but this difference did not reach the level of significance (10.6% vs 15.9%; P=0.267). The overall number of digestive adverse effects in the postbiotic group was lower than in the placebo group. The incidence of non-digestive and specific digestive adverse effects also did not significantly differ between the groups (Table 6).

Table 6. Adverse effects of the therapy.

	Postbiotic group (n=66)	Placebo group (n=63)	p
Patients with any adverse effects, n(%)	20 (30.3%)	24 (38.0%)	0.228
Patients with mild adverse effects, n(%)	14 (21.2%)	19 (30.2%)	0.168
Patients with moderate adverse effects, n(%)	6 (9.1%)	5 (7.9%)	0.533
Patients with severe adverse effects, n(%)	-	-	
Patients with adverse effects not requiring intervention, n(%)	18 (27.3%)	21 (33.3%)	0.289
Patients with adverse effects leading to drug withdrawal, n(%)	1 (1.5%)	2 (3.2%)	0.482
Patients with adverse effects leading to the prescription of corrective drugs, n(%)	2 (3.0%)	3 (4.8%)	0.478
Specific adverse effects			
Bitterness in the mouth, n(%)	12 (18.2%)	14 (22.2%)	0.362
Diarrhea, n(%)	7 (10.6%)	10 (15.9%)	0.267

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Constipation, n(%)	-	1(1.6%)	0.488
Nausea, n(%)	3 (4.5%)	5 (7.9%)	0.333
Abdominal pain, n(%)	1 (1.5%)	3 (4.8%)	0.478
Gurgling in the stomach, n(%)	-	1 (1.6%)	0.488
Flatulence, n(%)	1 (1.5%)	-	0.512
Belching, n(%)	1 (1.5%)	-	0.512
Total cases of digestive symptoms, n	**25**	**34**	**0.049**
Headache, n(%)	-	1 (1.6%)	0.488
Dizziness, n(%)	-	1 (1.6%)	0.488
Pain in the lumbar region, n(%)	1 (1.5%)	-	0.512
Dry mouth, n(%)	1 (1.5%)	2 (3.2%)	0.482
Burning tongue, n(%)	1 (1.5%)	-	0.512
Multiple aphthae on the tongue and gums, n(%)	1 (1.5%)	-	0.512
Total cases of non-digestive symptoms, n	**4**	**4**	**0.615**
Increased serum creatinine level, n(%)	1 (1.5%)	-	0.512
Increased serum ALT level, n(%)	2 (3.0%)	2 (3.2%)	0.673
Total cases of laboratory disorders, n	**3**	**2**	**0.803**

4. Discussion

H. pylori infection is one of the most common human infections in the world. This bacterium is recognized as the etiological agent of most forms of chronic gastritis and peptic ulcer, and is also involved in the pathogenesis of gastric cancer and MALT-lymphoma [6]. It has also been suggested that it is responsible for the development of some cases of functional dyspepsia. The eradication therapy in such patients can lead to a regression of symptoms [6], which coincides with the results of our study.

Therefore, the eradication of H. pylori becomes an important target. Several drug regimens have been proposed, among which those with proton pump inhibitor (PPI) + amoxicillin + clarithromycin is one of the first line regimen in countries with low resistance of H. pylori to claritromycin (Russia is one of them) [6]. Recent studies have shown the benefit of longer therapy (14 days versus 10 and 7 days [6]). However, even in this case, the effectiveness of eradication therapy was about 80-90% [6,15,16,20,21]. To further increase it, several methods have been proposed, including the use of probiotics [22]. Among several microorganisms tested for this purpose, one of the most interesting is L. reuteri that survives in the gastric acid environment colonizes the gastric mucosa and inhibits the growth of several pathogenic bacteria, including H. pylori [23]. Various live strains of this bacterium have shown the ability to reduce the load of H. pylori (decrease in 13C-UBT values) [24,25]. The use of this probiotic together with PPI led to the eradication of H. pylori in a number of patients [24,25]. However, despite the fact that the efficacy of 10-14-day first-line eradication therapy combined with this probiotic in many studies tended to be higher than without it [27–30]., this difference reached significance only in the most recent study from Malaysia [31].

The efficacy of non-viable L. reuteri (postbiotic/metabiotic) for this purpose was also analyzed. After long-term screening, the DSM 17648, a strain with maximum anti-Helicobacter activity in artificial gastric juice, was selected. Its mechanism of action is to bind to H. pylori that leads to reducing mobility and adhesion ability of these pathogenic bacteria [10]. Postbiotic based on this strain has been marketed under the trade name Pylopass™. It showed the ability to reduce H. pylori load in the stomach according to the urease breath test [10,13,14] and its efficacy for H. pylori eradication in combination with IPP was as effective as standard eradication therapy [32]. Our study is the first multicenter placebo-controlled study to confirm the efficacy of non-viable L. reuteri DSM

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17648 (Pylopass™) as an enhancer of standard 14-day first-line bismuth-free eradication therapy in adults.

However, our study conflicts with a recently published work from China that did not show this effect. [33]. The difference between our and the Chinese therapy regimens was that the Chinese colleagues used co-treatment (eradication therapy + L. reuteri DSM 17648) with a 14-day pre-treatment with L. reuteri DSM 17648, but we used a 14-day post-treatment with this co-treatment. In addition, our Chinese colleagues used a half our dose of drug (1 x 1010 dead L. reuteri DSM 17648 cells vs. 2 x 1010 ones). All this could lead to an insufficient effect of this therapy. Further RCTs comparing these regimens are required to verify our hypothesis. Another reason for these differences may be the ethnic genetic specifics of patients and H. pylori strain differences. Studies in other countries are required to verify this hypothesis.

Although we did not obtain a significant reduction in the frequency of specific adverse effects of therapy as a result of the addition of L. reuteri DSM 17648 to the eradication therapy, the overall number of digestive adverse effects in the group of patients receiving it was significantly lower ($p=0.049$). Yang et al. [33] observed a significant effect from L. reuteri supplementation on the reduction of the incidence of eradication therapy-induced diarrhea ($p=0.022$) and abdominal distention ($p=0.022$), which may be due to the larger number of patients ($n=200$) included in that study or ethnic genetic specifics of these patients.

The strength of the present study is its design (multicenter, randomized, double-blind, placebo-controlled), which is gold standard of clinical research. It is the first study showing that addition of inactivated L. reuteri DSM 17648-to 14-day first-line eradication therapy significantly increases eradication efficacy.

One limitation of this study is the lack of a complete placebo group, that should have consisted of patients who received placebo instead of eradication therapy. This does not allow a correct assessment of the effect of the eradication therapy itself on the symptoms of FD. A further large study comparing live and non-viable L. reuteri DSM 17648 would answer the question of what is more effective in H. pylori eradication, a probiotic or a postbiotic. It would also be promising to evaluate how the addition of L. reuteri affects the efficacy of bismuth eradication quadruple therapy, which is another aim for future research.

5. Conclusions

The postbiotic (metabiotic) based on Lactobacillus reuteri DSM17648 significantly improves the efficacy of H. pylori eradication therapy in functional dyspepsia and decreases overall number of digestive adverse effects of this therapy.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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