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Can probiotics play a role in Helicobacter pylori (*H. Pylori*) eradication?

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Abstract

Background H. Pylori is one of the commonest infectious diseases worldwide. In recent years, PPI-based triple therapy has been described to be losing its efficacy against *H. pylori* due to high rates of antibiotic resistance, antibiotics-associated side effects, and low compliance. Probiotics are suggested to improve the H. pylori eradication rate when added to H pylori therapy. Probiotics have anti-inflammatory and anti-oxidative mechanisms that may improve bowel microecology and interact with the microbial flora of the gastrointestinal tract to produce a beneficial effect in H Pylori eradication. Probiotics may be also responsible for the reduction of the adverse effects related to *H. pylori* therapy that may result in treatment failure.

Aim of the work In our study, we assessed the role of probiotics in improving the H. Pylori eradication rate and reducing side effects after antibiotic-based therapy.

Patients and method One hundred fifty-nine patients positive for H. pylori stool antigen and had never received previously H. pylori eradication therapy, were included in the study, 59 patients received triple therapy alone (Standard group) and 100 patients received triple therapy and probiotics (study group). One hundred fifty patients completed the treatment. Quantification of Lactobacilli and Bifidobacteria concentration in stool was done by PCR before and after therapy. Eradication of H. pylori was assessed in each group by H. pylori stool antigen after 4 weeks of finishing therapy.

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Results H Pylori eradication rate was higher in patients who received probiotics with standard therapy compared to those who received standard therapy alone. The improvement in the eradication rate was statistically significant when probiotics were received after standard therapy (81.04% versus 71.19%, P-Value 0.021). Short-term diarrhea after antibiotics therapy was significantly lower in patients who received probiotics especially when given before antibiotic therapy (7.50% versus 25.50%, P-value 0.0001). Probiotics may play a role to restore gut dysbiosis as evidenced by stool PCR for Lactobacilli and Bifidobacteria before and after therapy.

Conclusion Probiotics have a beneficial role to improve the eradication rate of H. pylori, particularly when given after standard therapy. Adding probiotics was associated as well with less diarrhea as a side effect of antibiotic therapy.

Trial registration The trial has been registered on the Pan African Clinical Trial Register website, No of registration, PACTR202304859303467. Registered 24 April 2023 - Retrospectively registered, https://pactr.samrc.ac.za/TrialDisplay.aspx?TrialID=25434.

Keywords Helicobacter pylori, Eradication therapy, Probiotics, Diarrhea after antibiotics therapy

Introduction

Helicobacter pylori (H. pylori), a microaerophilic, Gram-negative spiral bacterium, is colonizing approximately half of the world's population, 80% of whom are asymptomatic [1]. It has a significant role in the etiology and pathogenesis of peptic ulcer disease and increases the risk of developing gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma [2]. The prevalence of *H. pylori* infection differs widely among countries: Prevalence is higher in developing countries (20–90%) than in developed countries (10–60%) [3, 4]. Several Egyptian studies reported that the prevalence of *H. pylori* infection ranged from 60 to 90% [5–7].

Additionally, *H. pylori* has a critical role in other extra gastric diseases such as chronic urticaria [8], ischemic heart disease [8], increased risk of metabolic syndrome and diabetes [9], autoimmune thyroid diseases [8], iron deficiency anemia [10], idiopathic thrombocytopenic purpura [11], neurologic diseases [9], and hepatobiliary diseases [9].

Eradication of *H. pylori* does not only heal gastritis and peptic ulcer disease but may prevent the spread of infection and *H. pylori* recurrence. It may also decrease the risk of gastric cancer, thus reducing further costs required for the treatment of subsequent *H. pylori*-associated diseases [12].

PPI-based triple therapy has been described to be losing its efficacy against *H. pylori*, with eradication cure rates ranging as low as 50 to 70%, due to high rates of antibiotic resistance, high rates of antibiotic-associated side effects, and weak compliance [13].

Globally, *H. pylori* antibiotic resistance has increased and is the main factor affecting the efficacy of current therapeutic regimens. The prevalence of bacterial resistance varies by geography. It correlates with the widespread use of certain antibiotics in the general population [14]. A recent study from Egypt showed that antimicrobial resistance of *H. pylori* to metronidazole and amoxicillin was relatively high (25% and 18.3% respectively). The resistance rates of clarithromycin and tetracycline were low (6.7% and 1.7% respectively) [15].

Antibiotic therapy for *H. pylori* eradication can impair the healthy gut microbiota, leading to short-term clinical consequences [16]. It also may select antibiotic-resistant components of gut microbiota [16].

Probiotics refer to a group of beneficial bacteria that can improve the eradication rate of *H. pylori* infections. This is achieved through several mechanisms like competitive inhibition, co-aggregation potential, boosting mucus production, generation of bacteriocins, and regulation of the immune system [17].

Aim of the proposed research

To study the impact of adding probiotics to standard triple therapy for achieving better *H. pylori* eradication rates compared to standard triple therapy alone. Study the incidence of short-term diarrhea after eradication therapy.

Patients and methods

Subjects

Patients coming to the outpatient clinics of the Gastroenterology and Hepatology Department, Theodor Bilharz Research Institute (TBRI), Egypt complaining of dyspepsia or other symptoms suggestive of *H. pylori* infection were tested for *H. pylori* stool antigen. Those who tested positive and had never been treated for *H. pylori* were invited to participate in the study. Informed consent was signed by each patient who agreed to participate. Stool specimens were obtained initially from each patient and processed for *H. pylori* stool antigen and real-time PCR to assess the concentration of Lactobacillus and Bifidobacterium strains.

Patients were defined as positive for *H. pylori* infection if *H. pylori* stool antigen was positive and were randomized to receive the eradication therapy either in group 1 or group 2.

Group 1 (standard therapy group)

Patients were treated with standard triple antibiotic therapy for 2 weeks, followed by 2 weeks of placebo (yogurt not containing probiotics).

Group 2 (study group)

Patients were treated with standard triple antibiotic therapy for 2 weeks and given probiotics supplementation for another 2 weeks. In this group, some of the patients were given yogurt-containing probiotics supplementation for 2 weeks just before the antibiotic therapy and others were given yogurt-containing probiotics supplementation for 2 weeks just after the antibiotic therapy (please see below for details of dosing).

Sample size

The sample size for this study was calculated using the G*power program 3.1.9 (G power program version 3.1, Heinrich-Heine-University, Düsseldorf, Germany) for a two-tailed test. A priori: compute the required sample size based on *t* tests (means: difference from constant, one sample case), type I error (alpha = 0.05), type II error (1-βeta = 90%), and the effect size $d = 0.2666667$. Considering a 15% dropout rate, this study's appropriate minimum sample size will be 150 patients.

Inclusion criteria

Symptomatic patients who were positive *H. pylori* stool antigen and had never been treated for *H. pylori*.

Exclusion criteria

patients below 18 years or above 80 years of age, previously received *H. pylori* eradication therapy, use of antimicrobials or medications that might interfere with *H. pylori* stool antigen detection (like PPI or H2 blockers use within the previous 2 weeks of stool testing), previous incidents of gastric or duodenal bleeding, gastric surgery, gastric malignancy or other life-threatening conditions, and unwilling to give consent.

Written informed consent was obtained from all patients. Patients have the right to withdraw at any time.

Method

All patients were subjected to full medical history with special stress on alarm symptoms like hematemesis or melena, weight loss, anemia, family history of gastric malignancy, and previous treatment for *H. pylori*. Clinical examination, with a particular focus on the presence of abdominal scars and alarm signs.

Laboratory investigations included:

H. pylori stool antigen, before treatment, to confirm *H. pylori* infection and after treatment by 4 weeks to assess eradication.

Real-time PCR to measure the concentration of Lactobacillus and Bifidobacterium strains, which were present in the stool before and after treatment, to investigate the ability of probiotics to restore the gastrointestinal microbiota and correct gut dysbiosis after probiotic therapy.

According to the recommendations of the International Biological Program, some anthropometric measurements were taken using standardized instruments to assess obesity as a variant factor: Body weight using a seca scale, body height using a stadiometer, and body composition. This assessment was done as obesity might affect the patient acquisition, response to standard therapy, and the impact of adding probiotics to treatment. Patients were randomly assigned to:

Group 1 (standard therapy group)

Two weeks of triple therapy (standard therapy) comprising proton pump inhibitor (Omeprazole 40 mg bid), amoxicillin (1000 mg bid), and clarithromycin (500 mg bid), followed by 2 weeks of placebo in the form of yogurt not containing probiotic.

Group 2 (study group)

The same triple therapy was given to the patients plus 2 weeks of probiotics (yogurt containing probiotics). Patients were randomly selected to receive probiotics for 2 weeks either just before or just after the standard triple therapy.

Patients were randomized through a sealed envelope system where participating clinicians were given treatment allocations randomly generated within sealed opaque envelopes. After obtaining the patient's consent to enter the trial, the corresponding envelope was opened and the allocated treatment regimen was offered to the patient.

Yogurt containing probiotics preparation

The probiotics include a combination of 12 strains from Bifidobacterium and Lactobacillus strains (B/L). The 12 strains included are *B. lactis*, *B. bifidum*, *B. breve*, *B. longum*, *L. acidophilius* (CUL 60), *L. acidophilius* (CUL21) *L. casei*, *L. planatrum*, *L. paracasei*, *L. salivarius*, *L. rhamnosus*, and *L. bulgaricus*.

The buffalo milk was sterilized at 15 lb pressure (1.05 kPa) for 10 min and cooled to 37 °C. The sterilized milk was inoculated with yogurt starter culture and activated probiotic bacteria mixture in a 2/100 ratio at 37 °C. Then, the inoculated milk was divided into

Table 1 Group-specific primers based on 16 S rDNA sequences

Target bacteria	Primer	Sequence (5′–3′)	PCR product size
Bifidobacterium PCR assay	F-bifido	CGCGTCYGGTGTGAAAG	
	R-bifido	CCCCACATCCAGCATCCA	244 pb
	MGB-bifido	AACAGGATTAGATACCC	
Lactobacillus PCR assay	F-lacto	GAGGCAGCAGTAGGGAATCTTC	
	R-lacto	GGCCAGTTACTACCTCTATCCTTCTTC	126 pb
	MGB-lacto	ATGGAGCAACGCCGC	

Strain (Bifidobacterium longum and Lactobacillus acidophilus) Quantitative PCR assay based on genus-specific 16S rDNA primers and 3′ minor groove binder (MGB) probes for accurate detection and quantification of a wide range of Bifidobacterium spp. (30 species) and Lactobacillus spp. (15 species) in fecal samples

100 mL volumes in plastic cups covered with plastic lids and incubated for 6 h at 42 °C to form the curd. Finally, yogurt was transferred to the refrigerator and stored at 4 °C till consumption.

The dose was 100 million CFU/gm, administered via yogurt as per the recommendation of the Food and Drug Organization (FAO). The yogurt contains 100 g, to be consumed twice daily for 14 days.

Detection of *H. pylori* stool antigen

The *H. pylori* antigen rapid test cassette (feces) is a qualitative, lateral flow immunoassay for the detection of *H. pylori* antigens in human feces specimens. In this test, the membrane is pre-coated with anti-*H. pylori* antibodies on the test line region of the test. During testing, the specimen reacts with the particle coated with anti-*H. pylori* antibodies. The mixture migrates upward on the membrane by capillary action to react with anti-*H. pylori* antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that the proper volume of specimen has been added and membrane wicking has occurred [18].

Stool specimens were kept at − 70 °C until assaying. The investigators were blinded to the results of other *H. pylori* tests. A commercial H. pylori Ag rapid test (ALL TEST; Hangzhou AllTest Biotech Co., Ltd., Hangzhou, China) was used to detect H. pylori, according to the manufacturer's instructions.

H. Pylori stool antigen test was used for both diagnosing and confirming eradication after the end of therapy by 4 weeks.

Quantification of Bifidobacterium spp. and Lactobacillus spp. in fecal samples by Real-time PCR before and after treatment

Real-time PCR was used for quantifying Bifidobacterium spp. and Lactobacillus spp. in fecal samples before and after the treatment for all participants. The investigators remained blinded to the treatment regimen and the eradication status of the patients.

DNA extraction

The QIAamp DNA Stool Minikit (Qiagen) was used to extract DNA from one gram of fresh or frozen stool sample according to the manufacturer's instructions. Briefly, the procedure involved lysis of the bacterial cells within the fecal material in ASL buffer, adsorption of impurities to InhibitEX reagent, and purification of the DNA on a spin column. ASL buffer has been specially developed to remove inhibitory substances from stool samples. The DNA was eluted in a final volume of 200 µl and stored at − 20 °C. The method used to isolate DNA from the various reference strains was similar to that applied to fecal samples.

Design of primers and probes

The primers and probes used to detect *Bifidobacterium* spp. and *Lactobacillus* spp. were based on 16S rRNA gene sequences retrieved from the National Center for Biotechnology Information databases using the Entrez program [19]. The *Bifidobacterium* spp. or *Lactobacillus* spp. sequences were aligned with sequences from closely related species using the ClustalW program from the European Bioinformatics Institute (http://www.ebi.ac.uk/clustalw.htm). The alignments revealed specific sequences for either *Bifidobacterium* spp. or *Lactobacillus* spp. Our primers and probes do not cover the entire *Bifidobacterium* and *Lactobacillus* genera and can thus detect only part of the species believed to be present in the fecal microbiota (Table 1). Forward primers, reverse primers, and TaqMan MGB probes were designed with the help of the Primer Express 2.0 software (Applied Biosystems, Foster City, CA) (Table 1). These oligonucleotide sequences were then checked for their specificity, using the Check-Probe function of the Ribosomal Database Project software package [20] and the BLAST database search program [19].

Table 2 Clinical information of subjects in the standard and study groups

Items	Standard therapy group (*N* = 59)	Study therapy group (*N* = 100)	*P* value
Age (year)	42.15 ± 13.28	41.23 ± 11.72	0.660
Weight (kg)	80.49 ± 15.06	76.34 ± 17.40	0.116
Height (cm)	163.59 ± 7.58	162.02 ± 9.87	0.262
BMI (kg/m^2)	30.20 ± 6.17	29.27 ± 7.03	0.390
Gender (males: females)	18 (30.50%): 41 (69.50%)	34 (34.00%): 66 (66.00%)	0.650
Diabetic (yes: no)	8 (13.60%): 51 (86.40%)	21 (21.00%): 79 (79.00%)	0.241
Diarrhea after antibiotic (yes: no)	14 (25.50%): 41 (74.50%)	9 (9.50%): 86 (90.50%)	0.009*

Quantitative data are expressed as mean ± standard deviation and compared by *t*-independent test

Qualitative data are expressed as numbers (percentages) and compared by the chi-square test

P value probability value, *P value* > *0.05* non-significant

*reflect significant *P* value

Real-time PCR assay conditions and cycle threshold

The amplification reactions were carried out in a total volume of 25 µl containing 1×TaqMan Universal PCR Master Mix (Applied Biosystems), both primers (each at 300 nM concentration), 200 nM TaqMan MGB probe, 60 ng purified target DNA, and BSA at the final concentration of 0.1 µg/µl (New England Biolabs). Amplification (2 min at 50 C, 10 min at 95 C, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C) and detection were carried out on an ABI Prism 7900 sequence detection system (Applied Biosystems). The *Bifidobacterium* fluorescent probe was labeled at its 5′ ends with the reporter dye 6-carboxyfluorescein (FAM) and the *Lactobacillus* probe with 6-carboxyrhodamine (VIC). MGB fluorescent probes with nonfluorescent quencher dyes (also called dark quenchers) were used (Applied Biosystems). The amount of genomic DNA extracted was determined by ultraviolet spectrophotometry at 260 nm. In our PCR assay, we compared different quantities of bacterial DNA extracted from fecal samples to overcome the bias due to the presence of inhibitory compounds such as bilirubin and bile salts [21]. We never found any interference in the assays when 60 ng genomic DNA was used per PCR reaction. A no-template control (NTC) and a positive control were included on each plate. Each assay was performed in duplicate in the same run.

Statistical analysis

The statistical analysis was conducted by using the statistical SPSS Package program version 25 for Windows (SPSS, Inc., Chicago, IL, USA). Quantitative descriptive statistics for basic information of subjects expressed as mean ± standard deviation and compared between study probiotic therapy groups and standard therapy group by *t*-independent test. Qualitative data are expressed as numbers (percentages) and compared between study probiotic therapy groups and standard therapy groups by chi-square test. Intention to treat analysis (ITTA) and per-protocol analysis (PPT) were used to compare between study probiotic therapy groups and standard therapy groups. Analysis of variance test (ANOVA-test) was used to compare standard therapy, probiotic before standard therapy, and probiotic after standard therapy groups for bacteria concentrations and paired *t*-test to compare between before- and after-standard therapies. All statistical analyses were significant at the 0.05 level of probability ($P \leq 0.05$).

Results

One hundred fifty-nine patients who were positive for *H. pylori* stool Ag and who had never received eradication therapy for *H. pylori* were included in the study. One hundred fifty completed the treatment. Fifty-nine patients received triple therapy alone (Standard group) and 100 patients received triple therapy and probiotics (study group). The mean age of the standard group was 42.15 ± 13.28 and the mean age in the study group was 41.23 ± 11.72. The baseline features of subjects were similar (Table 2).

The study group was further divided into two subgroups. The first received probiotics just after antibiotic therapy (58 from 100) whereas the other group received probiotics just before antibiotic therapy (42 from 100). Among the 159 subjects, 9 lost follow-up: 4 in the standard therapy group and 5 in the study group. Of the 5 in the study group, three received probiotics after therapy while two received probiotics before therapy. The study's design is shown in Fig. 1.

After therapy, of the 59 patients in the standard group, 38 (64.40%) patients responded to the eradication therapy, 17 (28.80%) patients resisted the treatment, and 4 (6.80%) patients lost to follow-up. Of the 58 patients in

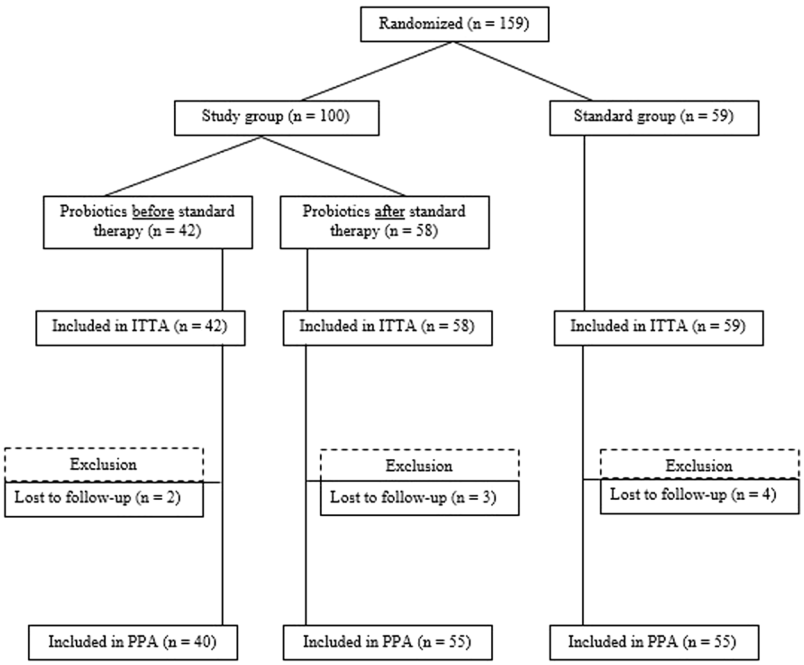

Fig. 1 Study design

Table 3 Patient distribution

Item	Negative	Positive	Lost follow up	Total
Standard therapy	38 (64.40%)	17 (28.80%)	4 (6.80%)	59 (100%)
Probiotic after standard therapy	44 (75.90%)	11 (19.00%)	3 (5.20%)	58 (100%)
Probiotics before standard therapy	29 (69.00%)	11 (26.20%)	2 (4.80%)	42 (100%)

Data are expressed as number (percentage). *Negative* = eradicated *H. Pylori*, *Positive* = failed to eradicate *H. pylori*

the group that received probiotics after antibiotic therapy, 44 (75.90%) patients responded to the eradication therapy, 11 (19.00%) patients resisted the treatment, and 3 (5.20%) lost to follow-up. In the group that received probiotics before antibiotic therapy (48 patients), 29 (69.00%) patients responded to eradication therapy, 11 (26.20%) patients resisted the treatment and 2 (4.80%) lost to follow-up. Patient distribution is demonstrated in Table 3.

The eradication rate was higher in patients who received probiotics with standard therapy than those treated with standard therapy alone (78% vs 71.19% by intention-to-treat analysis and 76.84% Vs 69.09% by per protocol analysis). The improvement in the eradication rate became statistically significant when probiotics were received after standard therapy (81.04% vs 71.19%, *P* value 0.021 by intention to treat analysis and 80.00% vs 69.09%, *P* value 0.018 by per protocol analysis). Per protocol and Intention to treat analysis for treatment groups is shown in Table 4.

No significant difference was detected between pretreatment mean lactobacillus strains concentration in different groups (*P* value, 0.998). Additionally, no significant difference was detected between the pretreatment mean Bifidobacterium strains concentration in different groups (*P* value, 0.999).

The mean Lactobacillus strains and Bifidobacterium strains concentrations were significantly increased after treatment containing probiotics (*P* value 0.0001) which were not shown in the standard therapy alone (*P* value, 0.602 and 0.894). Comparison among 3 groups for probiotics concentrations at pre- and post-treatment is demonstrated in Table 5 and Fig. 2.

Short-term diarrhea was significantly lower in patients who received probiotics with standard therapy 9/95 (9.47%) compared to those patients who received standard therapy alone 14/55 (25.5%), particularly among those who received probiotics before standard therapy 3/40 (7.50%). Comparisons across the 3 groups regarding

Table 4 Per protocol and intention to treat analysis for the treatment groups

Item	Standard therapy	Probiotic therapy	Odd ratio (95% CI)	*P* value
Patients randomized	59	100		
Patients lost to follow-up	4	5		
Patients followed up	55	95		
Patients with *H. pylori* eradication	42	78		
Patients resist *H. pylori* eradication	17	22		
Intention to treat analysis	71.19% (42/59)	78.00% (78/100)	0.69 (0.33–1.45)	0.335
Per-protocol analysis	69.09% (38/55)	76.84% (73/95)	0.79 (0.51–1.22)	0.297
	Standard therapy	Probiotics *before* standard therapy	Odd ratio (95% CI)	*P* value
Patients randomized	59	42		
Patients lost to follow-up	4	2		
Patients followed up	55	40		
Patients with *H. pylori* eradication	42	31		
Patients resist *H. pylori* eradication	17	11		
Intention to treat analysis	71.19% (42/59)	73.81% (31/42)	0.87 (0.36–2.13)	0.772
Per-protocol analysis	69.09% (38/55)	72.50% (29/40)	0.85 (0.34–2.08)	0.719
	Standard therapy	Probiotic *after* standard therapy	Odd ratio (95% CI)	*P* value
Patients randomized	59	58		
Patients lost to follow-up	4	3		
Patients followed up	55	55		
Patients with *H. pylori* eradication	42	47		
Patients resist *H. pylori* eradication	17	11		
Intention to treat analysis	71.19% (42/59)	81.04% (47/58)	0.58 (0.24–1.37)	0.021*
Per-protocol analysis	69.09% (38/55)	80.00% (44/55)	0.53 (0.27–1.30)	0.018*
	Probiotics *before* standard therapy	Probiotic *after* standard therapy	Odd ratio (95% CI)	*P* value
Patients randomized	42	58		
Patients lost to follow-up	2	3		
Patients followed up	40	55		
Patients with *H. pylori* eradication	31	47		
Patients resist *H. pylori* eradication	11	11		
Intention to treat analysis	73.81% (31/42)	81.04% (47/58)	0.66 (0.26 – 1.70)	0.389
Per-protocol analysis	72.50% (29/40)	80.00% (44/55)	1.52 (0.58 – 3.95)	0.392

P value probability value, *P value* > *0.05* non-significant

*reflect significant *P* value

the incidence of short-term diarrhea after antibiotic therapy are demonstrated in Table 6.

Patients with high BMI showed a significantly lower eradication rate in comparison to those with normal or low BMI, whether they were treated by standard therapy alone or standard therapy with probiotics. The eradication rate was 60% with standard therapy and 70% with probiotics-containing therapy. These results are summarized in Table 7.

Patients with diabetes mellitus showed significantly lower eradication rates in comparison to non-diabetic patients either treated by standard therapy alone or standard therapy with probiotics. The eradication rate was 13% with standard therapy and 15% with probiotics-containing therapy. These are demonstrated in Table 7.

Discussion

Probiotic supplementations are gaining acceptance in improving the eradication rate of *H. pylori* by restricting its growth (anti-microbial activity) and inhibiting subsequent inflammatory processes related to H. pylori infection. Antimicrobial activity is mainly through inhibition of H. pylori adhesion and invasion of gastric epithelial cells and anti-inflammatory role through decreasing interleukin-8 production [22].

The majority of probiotics are of the genera, Lactobacillus and Bifidobacterium [23]. Both strains mostly

Table 5 Comparing 3 groups for bacteria concentrations at pre- and post-treatment

Item	*Lactobacillus* concentration			*Bifidobacterium* concentration		*P* value
	Pre-treatment	Post-treatment	*P* value	Pre-treatment	Post-treatment	
Standard therapy	2,417,746 ± 2,012,295	1,601,763 ± 1,361,622	0.602	8,111,017 ± 5,497,298	7,287,636 ± 4,946,382	0.894
Probiotic before	2,428,500 ± 1,888,786	26,585,000 ± 15,566,805	0.0001*	8,105,143 ± 5,357,438	88,282,500 ± 52,174,878	0.0001*
Probiotic after	2,443,569 ± 2,230,328	32,065,455 ± 14,206,417	0.0001*	8,062,586 ± 5,685,795	113,201,818 ± 63,369,652	0.0001*
P value (between)	0.998	0.0001*		0.999	0.0001*	

Data are expressed as mean ± standard deviation and compared by analysis of variance test (ANOVA-test) among groups and paired *t*-test within each group

P value probability value, *P value* > 0.05 non-significant

* Significant (*P* < 0.05)

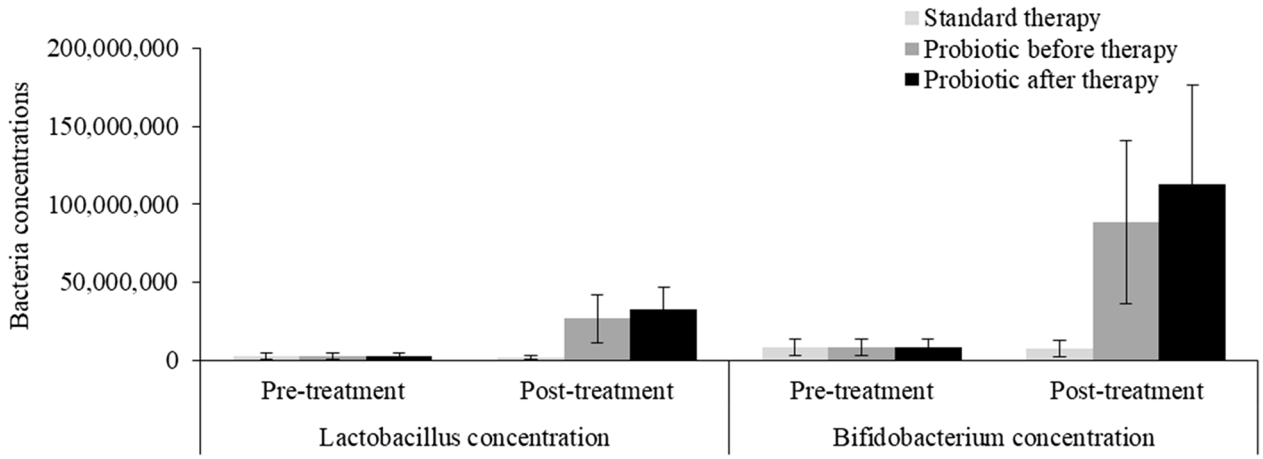

Fig. 2 Bacteria concentrations at pre-and post-treatment in 3 groups

Table 6 Comparing 3 groups regards the incidence of short-term diarrhea after antibiotic therapy

Item	No patients with diarrhea after antibiotic therapy	*P* value
Standard therapy	14/55 (25.5%)	0.009*
Patient received probiotics	9/95 (9.47%)	
Standard therapy	14/55 (25.50%)	0.0001*
Probiotics before standard therapy	3/40 (7.50%)	
Probiotic after standard therapy	6/55 (10.90%)	

Data are expressed as numbers (percentages) and compared by chi-square test

P value probability value, *P value* > 0.05 non-significant

* Significant (*P* < 0.05)

possess properties of acid tolerance and antimicrobial activity [24, 25].

In our study, we assessed the eradication rate of *H. pylori* after probiotic supplementation. In our study, the eradication rate was higher in patients who received probiotics with standard therapy compared to those patients treated with standard therapy alone (78% vs 71.19% by intention to treat analysis and 76.84% vs 69.09% by per protocol analysis). However, this difference was statistically not significant. Sub-analysis showed the eradication rate to be statistically significant when probiotics were received after standard therapy (81.04% vs 71.19%, *P* value 0.021 by intention to treat analysis and 80.00% vs 69.09%, *P* value 0.018 by per protocol analysis). This is in agreement with a metanalysis done by Yu et al. 2019, in which the eradication rates in the Lactobacillus supplementation group were 80.3% versus 69.1% in the control group by intention-to-treat analysis [26]. Other studies proved probiotics supplementation could significantly improve the eradication rate of *H. pylori* eradication rate via triple therapy [27–29]. On the other hand, some studies did not show a significant change in the eradication rate with probiotics supplementation [30].

In our study, short-term diarrhea, post-antibiotic therapy, was significantly lower in patients who received probiotics with standard therapy 9/95 (9.47%) in comparison to those who received standard therapy alone 14/55 (25.5%). This was particularly true for those who received probiotics before standard therapy 3/40 (7.50%). Probiotics supplementation before standard therapy tended

Table 7 Association between BMI and diabetes and *H. Pylori* Ag in stool after 4 weeks of therapy in standard and probiotic therapy groups

Categories		Standard therapy (*N* = 55)					Probiotic therapy (*N* = 95)				
		Total	Positive (*n* = 17)	Negative (*n* = 38)	Eradication rate (%)	*P* value	Total	Positive (*n* = 22)	Negative (*n* = 73)	Eradication rate	*P* value
BMI	< 25	12	0	12	100%	0.009*	26	1	25	96%	0.006*
	≥ 25	43	17	26	60%		69	21	48	70%	
	Total	55	17	38	69%		95	22	73	77%	
Diabetes mellitus	No	47	10	37	79%	0.0001*	75	5	70	93%	0.0001*
	Yes	8	7	1	13%		20	17	3	15%	
	Total	55	17	38	69%		95	22	73	77	

Data are expressed as numbers (percentages) and compared by chi-square test

P value probability value, *P value* > *0.05* non-significant, *Negative* = Eradicated H. Pylori, *Positive* = Failed to eradicate *H. Pylori*

* Significant (*P* < 0.05)

to increase the eradication rate (from 69.09 to 72.50% by PPA and from 71.19% to 73.81% by ITTA). However, the improvement in the eradication rate was not statistically significant. Nevertheless, its main role was in the correction of gut dysbiosis before antibiotic therapy may help to decrease the antibiotic's side effects and improve antibiotic tolerability. Our results revealed Lactobacillus and Bifidobacterium supplementation showed a significant reduction in the incidence of diarrhea which was in accordance with Viazis et al. [31]. On the other hand, Zheng et al. showed no significant changes in the antibiotics-related side effects of probiotic supplementations [32]. A possible explanation for inconsistencies with some other studies might be that the age distribution of the patients involved in the study was significantly different, and the duration and species of probiotics supplementation might be different.

H. pylori eradication therapy resulted in the enrichment of some detrimental bacteria taxa such as *Shigella*, *Klebsiella*, and *Streptococcus*, while probiotics supplementation could rapidly restore these taxa levels after eradication and increase the taxa of *Bacillus* and Lactobacillales [30]. In our study, there was a significant increment in Lactobacillus and Bifidobacterium concentration after probiotics supplementation was given, in comparison to standard therapy alone. Therefore, probiotics supplementation might help to construct a beneficial profile of gut microbiota after eradication therapy and these findings may be of value in the rational use of probiotics during *H. pylori* eradication.

Both Diabetes mellitus and obesity are factors predisposing to gut dysbiosis [33] using probiotics supplementation with standard therapy improves the eradication rate in patients with diabetes mellitus from 13 to 15% and in patients with high BMI from 60 to 70%. Despite the results not being statistically significant, the eradication rate tended to increase in such patients with probiotics supplementations. This finding may necessitate extending durations of probiotics supplementations in these patients with predisposing factors for gut dysbiosis.

Limitations

Limitations to this work include the duration of Lactobacillus and Bifidobacterium supplementation may lead to clinical heterogeneity and remain a point of interest to be investigated. Different strains were not studied separately, which may have effects on choosing more appropriate strains to supplement standard therapy.

Future studies should conduct further evaluations to investigate the impact of *H. pylori* eradication on the primary symptoms that are believed to be caused by *H. pylori*. This is an important area that was not covered in our study and could provide valuable insights into the effects of *H. pylori* eradication therapy on patient outcomes.

Conclusion

Probiotic supplementation might help to restore a beneficial profile of gut microbiota and improve H. pylori eradication rates, particularly when given after eradication therapy.

Recommendations

Future studies are needed to clarify the optimal duration of probiotics supplementations for enhancing the curative efficacy of antibiotic-based therapies for *H. pylori* infection and how to reduce the adverse effects.

Authors' contributions
All authors have seen and approved the final version of the manuscript being submitted. All authors confirm that this work is original and that all data, tables, etc. used in the manuscript are prepared originally by the authors and have not been published elsewhere nor is it currently under consideration elsewhere.

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Declarations

Competing interests
The authors declare that they have no conflict of interest.

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