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ORIGINAL ARTICLE

# Efficacy of Pyloshot in combination with standard four-drug antimicrobial treatment for *Helicobacter pylori* eradication: A randomized clinical trial

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**Key words**
four-drug treatment, *Helicobacter pylori*, probiotics, Pyloshot, standard diet.

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**Declaration of conflict of interest:** None.

## Abstract

**Background and Aim:** The treatment for *Helicobacter pylori* is considered to be a combination of several strict regimens, with patients' dissatisfaction and poor compliance. Probiotics are effective in patients' antibiotic regimens. This study investigated the efficacy of the Pyloshot probiotic in combination with standard four-drug therapy in patients with *H. pylori* infection.

**Methods:** This is an interventional study. The study population consisted of patients with *H. pylori* infection who were selected using the convenience sampling method and were randomly divided into two groups: (i) A or standard four-drug therapy (amoxicillin-clarithromycin-bismuth-pantoprazole) plus two placebo capsules (80 cases). (ii) B or Pyloshot group (standard four-drug therapy plus 2 Pyloshot probiotic capsules) (90 cases). Patients were followed up for 1 month after treatment completion. Eradication of *H. pylori* was also assessed using the stool antigen test. Data were analyzed using SPSS (v.26) statistical software.

**Results:** Based on the results from the present study, the number of negative stool antigen tests in group A was less than that in group B, and no statistically significant relationship was found between *H. pylori* eradication (based on stool antigen test) and the studied groups ($P = 0.20$). There was a statistically significant relationship between vomiting ($P = 0.03$), diarrhea ($P = 0.04$), constipation ($P = 0.01$), and headache ($P = 0.04$); vomiting and constipation were most frequent in the control group.

**Conclusion:** Based on the results of the present study, there was no significant difference between the effectiveness of treatment with Pyloshot probiotic and the standard four-drug therapy in patients with *H. pylori* infection. However, the incidence of side effects was lower in the group treated with Pyloshot.

## Introduction

*Helicobacter pylori* infection is one of the major causes of chronic gastritis and gastrointestinal diseases such as gastric ulcers, which significantly increases the risk of gastric cancer by stimulating the proliferation of gastric cells.[1,2] Today, the prevalence of *H. pylori* in developed countries has decreased significantly, owing to improvements in health and living conditions; however, in some developing countries, such as African nations, its prevalence is still high and estimated at 70.1%.[3,4] Nevertheless, despite the decrease in *H. pylori* infection, the incidence of gastric adenocarcinoma has increased.[5,6]

*H. pylori* infection increases the risk of several diseases of the upper gastrointestinal tract, such as chronic gastritis, gastric ulcer, gastric cancer, and/or lymphoma.[7] Therefore, the Kyoto Global Consensus report on *H. pylori* has recommended that in cases of *H. pylori* gastritis (especially those living in areas with a high prevalence of *H. pylori* infection), appropriate diagnostic assessment of *H. pylori* gastritis be carried out, aiming to prevent neoplastic changes and plan eradication treatment of the infection.[8,9] According to the protocols of the European Helicobacter and Microbiota Study Group (EHMSG), the standard eradication treatment regimen for *H. pylori* is triple therapy or concomitant therapy with four drugs for 1–2 weeks.[10]

Based on previous studies, the prevalence of this infection in Iran has been reported to be 36–90% in different regions, which is very significant and higher than all over the world.[11]

Also, it is revealed that the rate of drug resistance in people with *Helicobacter* worldwide is less than 10%, while in Iran, it is more than 60%.[11] In the same way, in many studies, it has been attempted to add treatments and investigate their effectiveness. For example, the results of one study showed the promising potential of new-generation probiotics in providing targeted interventions that overcome the limitations of existing approaches.[12] Another study showed that the use of standard treatment to eradicate *H. pylori* infection along with probiotics reduces drug side effects and increases treatment tolerance.[13] In another study, it has been shown that the combination of two antibiotics, tetracycline plus levofloxacin, is more effective in eradicating *H. pylori* compared with clarithromycin plus amoxicillin, despite more complications; thus, in their study, it is recommended that this treatment regimen is an ideal treatment regimen for areas It has resistance to clarithromycin.[14]

Probiotics are a group of living microorganisms that generally colonize the gastrointestinal tract[15] and have various therapeutic applications, such as controlling diarrhea, antibiotic-induced diarrhea, functional disorders of the gastrointestinal tract, inflammatory bowel disease (IBD), cardiovascular diseases, allergic reactions, and cancer (Figs. 1–4).[16]

According to the European Helicobacter and Microbiota Study Group (EHMSG), adjunct therapy with probiotics can play a significant role in increasing the likelihood of recovery from infections by different mechanisms such as inhibition of *H. pylori* colonization (overcoming gastric epithelium receptors or their co-accumulation), anti-*H. pylori* activity throughout the production of bacteriocins, and supporting role in intestinal tissues (increasing mucin synthesis).[17]

Currently, some studies report that the addition of probiotics to the treatment regimen of *H. pylori* infection increases the rate of infection eradication during the treatment of patients as first and second lines[18] and sometimes significantly reduces the incidence of side effects.[19] The administration of probiotics in *H. pylori* infection alone is not effective; however, they can only be prescribed as an auxiliary treatment in the treatment and improvement of the clinical condition of patients.[20]

Currently, one of the most common types of probiotics is *Lactobacillus* species, including *Lactobacillus reuteri*, which has been used alone or in combination with other probiotics and has yielded different results. The purpose of this study was to investigate the effectiveness of using probiotics with the brand name "Pyloshot" containing *L. reuteri*, *Casei*, *Acidophilus*, and *Bifidobacterium* species along with the usual antibiotic treatment in patients with *H. pylori* infection.

## Methods

This was an interventional study. The study population consisted of patients with *H. pylori* infection who were referred to the gastroenterology clinic of Shahid Beheshti Hospital (Qum, Iran).

The study was conducted after obtaining approval from the Vice-Chancellor of Qom University of Medical Sciences, ethics code (IR.MUQ.REC.1400.202) from the ethics committee of the university, and IRCT code (20210719051943N2).

Sampling was performed using the convenience sampling method.

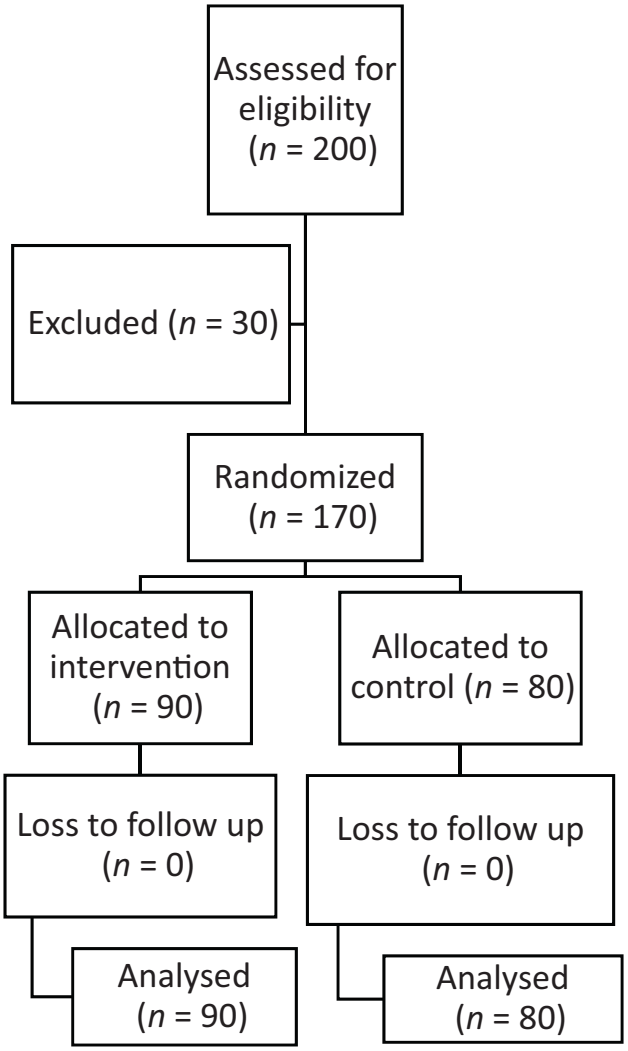


**Figure 1** Consort flow diagram for trial recruitment.

The inclusion criteria were as follows:

1. *H. pylori* infection confirmed by pathology report or urease test on biopsy specimen obtained from endoscopy;
2. age 18 years or older;
3. indication for *H. pylori* eradication treatment;
4. willing to participate in the study and consent to undergo upper gastrointestinal (GI) endoscopy.

The exclusion criteria were as follows:

1. Absence of an appropriate indication for upper GI endoscopy;
2. past medical history of eradication treatment failure;
3. pre-existing allergies and/or contraindications for amoxicillin, clarithromycin, and bismuth;
4. use of any herbal medicine or other probiotics.

Sample size calculations were performed using the MedCal statistical software.

Considering the study power of 80%, type 1 error of 5%, and the eradication rate in the two groups equal to 92.1% and

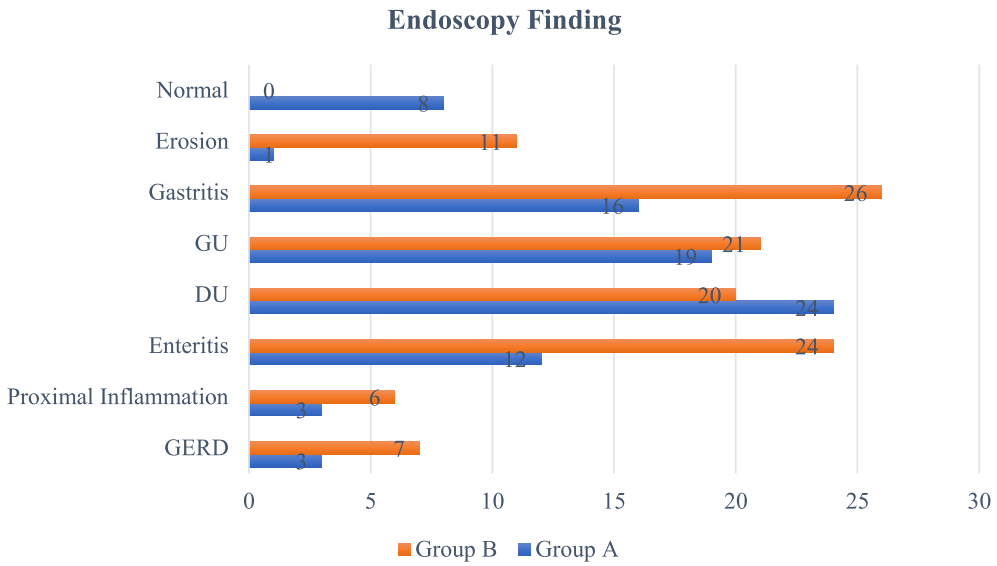


**Figure 2** Frequency of endoscopic findings in two groups.

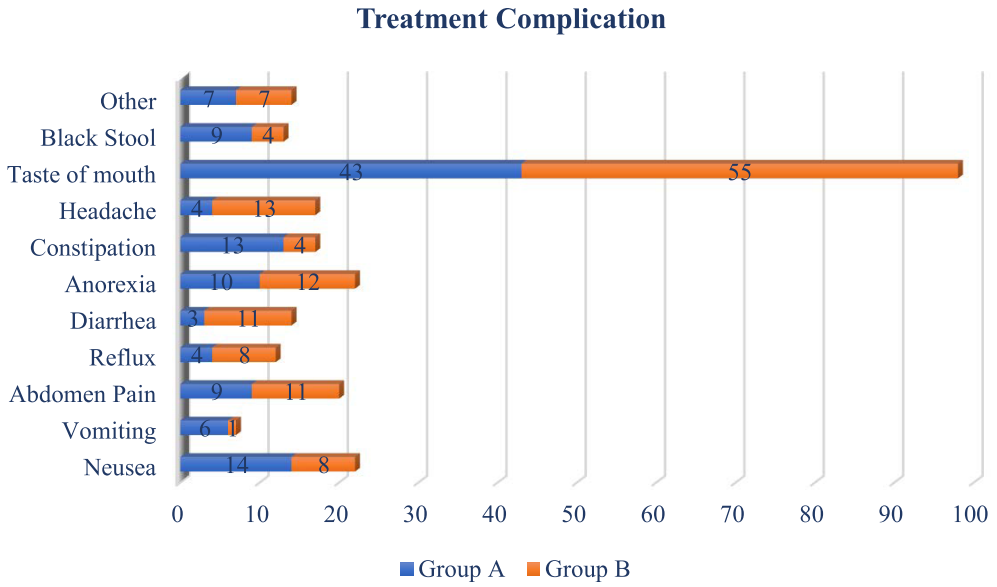


**Figure 3** Frequency of treatment adverse events in two groups.

3.62% (based on the study by Shafaghi *et al.*), the minimum required sample size was determined to be 67 patients in each group.

Considering the possibility of dropout, 100 patients in each group (total number: 200 patients) were included in the study, of which 30 patients were excluded. Thus, 80 patients in the control group and 90 patients in the intervention group were included in the study.

Based on the study objective, subjects were randomly divided into two groups: case (Pyloshot) and control (Pyloshot placebo). All patients were treated for 14 days with a standard four-drug regimen: amoxicillin in the form of 500 mg capsules twice a day (every 12 h), clarithromycin in the form of 500 mg capsules twice a day (every 12 h), bismuth in the form of tablets twice a day (every 12 h), and pantoprazole in the form of 40 mg capsules twice a day (every 12 h). In addition to the standard four-drug regimen, patients in the case group received Pyloshot-R capsules (twice a day), while patients in the control group received Pyloshot-R placebo capsules (twice a day).

Pyloshot capsules containing *Lactobacillus* bacteria (including *L. reuteri*, *Casei*, *Acidophilus*, and *Bifidobacterium*) are produced by "Bonian Salamat Kasra" Pharmaceutical Company (BSK) in Iran, under the brand name: Pyloshot-R.

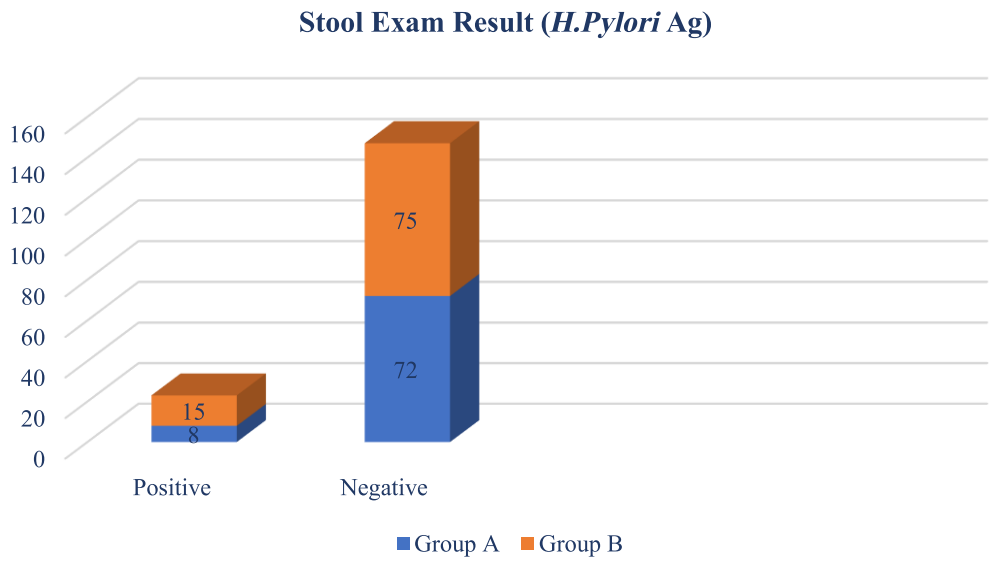


**Figure 4** Comparison of stool antigen test results in two groups.

Patients were not allowed to take any other medicine for 1 month after completion of the medication period. After this interval, the clinical symptoms of all patients were evaluated and *H. pylori* eradication was assessed using a stool antigen test.

Data were statistically analyzed using SSPS (v.26) statistical software.

Data analysis was done using descriptive statistics including mean, standard deviation, and percentage. To compare the two study groups in terms of success or failure in bacterial eradication, independent *t*-tests were used for quantitative variables, and the chi-square test was used for qualitative variables.

To comply with ethical considerations, informed consent was obtained from all patients before participating in the study. Personal information was not disclosed and the checklists were coded. Patients were allowed to withdraw from the study at any stage of the treatment, and they were assured that there would be no change in their normal treatment process after withdrawing from the study.

## Results

In this study, 170 patients were included, 80 in the control group and 90 in the intervention group.

Forty-three cases in the control group (53.8%) and 53 cases in the intervention group (58.9%) were male. The mean ages of the subjects in the control and intervention groups were 44.16 ± 14.42 years and 45.80 ± 12.55 years, respectively.

As shown in Table 1, there was no statistically significant difference in the mean body mass index (BMI) gastrointestinal of the subjects. The mean BMI of the control group was 26.01 kg/m$^2$ and that of the intervention group was 27.38 kg/m$^2$.

The level of education was a bachelor's degree in 52.2% of the control group and a high school diploma in 33.3% of the intervention group (Table 2).

In the present study, most subjects reported a negative history of underlying diseases, including gastrointestinal, pulmonary, cardiac, and renal diseases, as well as a negative history of corticosteroid, non steroid anti inflammatory drugs (NSAID), or antiplatelet medicine consumption, both in themselves and in their families.

Based on the investigation of the results obtained from this study, and unlike the level of education ($P = 0.011$), there was no statistically significant relationship between any of the demographic variables of this study, such as gender, age, BMI, past medical history, family history, and medicine consumption ($P > 0.05$).

**Table 1** Demographic variables and their frequencies in two groups

| Variable | Groups | | *P*-value |
|---|---|---|---|
| | A | B | |
| Gender (male), *n* (%) | 43 (53.8) | 53 (58.9) | 0.50 |
| BMI (mean ± SD) | 26.01 ± 6.23 | 27.38 ± 6.29 | 0.157 |
| Education, *n* (%) | | | |
| <Diploma | 4 (5.0) | 11 (12.2) | 0.011 |
| Diploma | 18 (22.5) | 30 (33.3) | |
| Bachelor | 42 (52.5) | 23 (25.6) | |
| >Bachelor | 11 (13.8) | 17 (18.9) | |
| PhD and more | 5 (6.3) | 9 (10.0) | |

**Table 2** Underlying diseases and past medical history in two groups

| | Group | | *P*-value |
|---|---|---|---|
| | A | B | |
| NSAID, *n* (%) | 1 (1.3) | 5 (5.6) | 0.129 |
| Antiplatelet, *n* (%) | 3 (3.8) | 1 (1.1) | 0.257 |
| GI cancer, *n* (%) | 0 (0.0) | 1 (1.1) | 0.34 |
| Heart disease, *n* (%) | 3 (3.8) | 8 (8.9) | 0.17 |
| Kidney disease, *n* (%) | 3 (3.8) | 1 (1.1) | 0.25 |
| Lung disease, *n* (%) | 3 (3.8) | 7 (7.8) | 0.26 |
| Liver disease, *n* (%) | 3 (3.8) | 10 (11.1) | 0.07 |

Based on the predefined goals of this study, patients were examined in terms of clinical signs and symptoms (including nausea and vomiting, abdominal pain, flatulence, heartburn, early satiety, weight loss, and reflux) before study initiation and after completion of the treatment period. The results indicate abdominal pain as the most reported clinical symptom, followed by flatulence and reflux, although no statistically significant difference was found. However, there was a significant relationship between nausea and vomiting ($P = 0.02$) and heartburn ($P = 0.03$). The frequency of nausea and vomiting in the control and intervention groups was 11.1% *versus* 23.8%, and the frequency of heartburn in the intervention and control groups was 51.1% *versus* 35%. Regarding clinical symptoms, there was a statistically significant relationship between heartburn and reflux, both of which were more frequently reported in the control group than in the intervention group.

In this study, all subjects underwent upper GI endoscopy, and the endoscopic findings of all patients were examined. Based on the obtained results, the most common endoscopic findings were GERD, proximal inflammation, duodenal ulcer (DU), gastric ulcer (GU), gastritis, erosion, and enteritis. The endoscopic result in eight subjects from the control group was normal, although in none of the subjects in the intervention group, normal endoscopy results were reported.

The most reported endoscopic findings in the control and intervention groups were DU (24 cases, 30.0%) and gastritis (26 cases; 28.9%), respectively.

The least reported endoscopic findings in the control and intervention groups were erosion (1 case; 3.1%) and proximal inflammation (6 cases; 6.7%), respectively.

In this study, the adverse effects of treatment were also evaluated. As shown in the diagram, the most reported adverse effect was taste perversion (bitter taste on the tongue), which was reported in 43 cases (53.8%) in the control group and 55 cases (61.1%) in the intervention group.

The least reported adverse effects were vomiting, which was reported in just one case in the control group, and diarrhea, which was reported in three cases from the intervention group.

Other adverse events such as weakness and lethargy were also reported by the patients, the frequency of which was equal in both control and intervention groups.

There was a statistically significant relationship between vomiting ($P = 0.03$), diarrhea ($P = 0.04$), constipation ($P = 0.01$), and headache ($P = 0.04$); vomiting and constipation were most frequent in the control group, whereas diarrhea and headache were most frequent in the intervention group.

Finally, the eradication status of *H. pylori* was also investigated using stool antigen tests. The stool antigen test was negative in 72 cases of the control group and 75 cases of the intervention group, indicating a failure in *H. pylori* eradication in some cases (8 cases from the control group and 15 cases from the intervention group). There was no statistically significant relationship between stool antigen test results and the two groups of patients in this study ($P = 0.20$).

## Discussion

This study was conducted to investigate the efficacy of the adjunct Pyloshot probiotic and standard four-drug therapy in patients with *H. pylori* infection.

Based on the results obtained from the current study, despite the greater eradication of *H. pylori* infection in the Pyloshot group, no significant statistical relationship was observed between the intervention and control groups. In line with the present study, in the study of Wang *et al.*[21] and Naqibzadeh *et al.*,[22] despite the greater eradication rate of *H. pylori* infection in the intervention group, no statistically significant difference was observed between the two groups in terms of stool antigen test results. In the study by Zhang *et al.*, who reviewed the results of 45 trials, the eradication rate of *H. pylori* infection was reported to be higher in the probiotic group than in the control group; and unlike the present study, there has been a significant statistical relationship in the studied groups.[23] Also, in the study by Viazis *et al.*, the eradication rate of *H. pylori* in the placebo group was lower than that of the Pyloshot group, and a statistically significant relationship was observed between the two groups.[24] This contradiction between the results of the current study and those of previous studies can be attributed to the small statistical population as well as the lack of tolerance and completion of the treatment period. Despite the physical distribution of the studied medicine for all participants, approximately 15% of the subjects did not complete the treatment period due to intolerance of the prescribed drugs and their side effects, and thus were excluded from the study. In addition, the contradiction can be regarded as the type and dosage of probiotics examined in the current study, which is different from that in previous studies.

In the present study, drug side effects in both groups were also investigated. The frequency of side effects was higher in patients receiving standard treatment than those who received probiotic treatment. This agrees with the study by Zhang *et al*. in which fewer side effects were reported in the probiotics group than in the standard treatment group.[23] In two studies performed by Wang *et al.*[21] and Fallone *et al.*,[25] the addition of probiotics resulted in a reduction in treatment-related side effects. Based on these results, it can be assumed that in patients with *H. pylori* infection, adding Probiotics to the treatment regimen is a good option due to its fewer side effects and will result in better patient compliance and subsequently improve the effectiveness of the treatment regimen.

## Conclusion

According to the results of this study, no statistically significant relationship was found between Pyloshot and the eradication of *H. pylori* infection. However, the incidence of side effects was lower in the group treated with Pyloshot. Thus, Pyloshot can be considered an appropriate medicinal supplement for patients with *H. pylori* infection, leading to better drug tolerance and increasing the probability of completing the treatment period.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Acknowledgments

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## Patient consent statement

Informed consent was provided by all patients enrolled in the survey.

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