Lactobacillus reuteri in management of *Helicobacter pylori* infection in dyspeptic patients: a double-blind placebo-controlled randomized clinical trial

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Abstract

Introduction: The eradication rate of *Helicobacter pylori* following the standard triple therapy is declining. This study was conducted to test whether the addition of *Lactobacillus reuteri* to the standard triple therapy improves the eradication rates as well as the clinical and pathological aspects in *H. pylori* infection.

Methods: A total of 70 treatment-naïve patients were randomly assigned into group A (the *L. reuteri* treated group) and group B (the placebo control group). Patients were treated by the standard triple therapy for 2 weeks and either *L. reuteri* or placebo for 4 weeks. They were examined by symptom questionnaire, *H. pylori* antigen in stool, upper endoscopy with biopsies for rapid urease test and histopathological examination before treatment and 4 weeks after treatment.

Results: The eradication rate of *H. pylori* infection was 74.3% and 65.7% for both *L. reuteri* and placebo treated groups, respectively. There was a significant difference regarding the reported side effects, where patients treated with *L. reuteri* reported less diarrhea and taste disorders than placebo group. A significant difference within each group was observed after treatment regarding Gastrointestinal Symptom Rating Scale (GSRS) scores; patients treated with *L. reuteri* showed more improvement of gastrointestinal symptoms than the placebo treated group. The severity and activity of *H. pylori* associated gastritis were reduced after 4 weeks of therapy in both groups. The *L. reuteri* treated group showed significant improvement compared with the placebo treated group.

Conclusion: Triple therapy of *H. pylori* supplemented with *L. reuteri* increased eradication rate by 8.6%, improved the GSRS score, reduced the reported side effects and improved the histological features of *H. pylori* infection when compared with placebo-supplemented triple therapy.

Keywords: eradication rate, gastritis, Gastrointestinal Symptom Rating Scale, *Helicobacter pylori*, *Lactobacillus reuteri*, triple therapy

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Introduction

Helicobacter pylori infection is a widespread disease and is endemic in many countries, including Egypt [Hunt *et al.* 2011], with a wide range of morbidity that requires appropriate antimicrobial therapy [Vaira *et al.* 2001]. However, the eradication rate following the standard triple therapy is declining worldwide and this necessitates the introduction of new antimicrobial agents [De Francesco *et al.* 2004]. In view of the *in vivo* and *in vitro* activities against *H. pylori*, the use of different strains of probiotics in the treatment of *H. pylori* may be justifiable. *Lactobacillus reuteri*, through different mechanisms including production of reuterin, has

anti-*H. pylori* activity and has been evaluated for improving the eradication rates of *H. pylori* [Hamilton-Miller, 2003] with contradictory results [Francavilla *et al.* 2008; Scaccianoce *et al.* 2008]. This study was conducted to assess whether the addition of *L. reuteri* to the standard triple therapy improves the eradication rate as well as the clinical and pathological aspects of *H. pylori* infection.

Patients and methods

Study design
We performed a double-blind placebo-controlled randomized prospective study.

Patients
All adult dyspeptic patients presented to the out-patient gastroenterology clinics of the Tropical Medicine and Internal Medicine Departments, Faculty of Medicine, Zagazig University Hospitals, Egypt, from June 2012 to February 2013 were offered to participate in the study. Patients who agreed gave written informed consent to participate in the study and to undergo all interventions needed. A total of 70 treatment naïve patients were enrolled and randomly assigned into two groups, group A (the *L. reuteri* treated group) and group B (the placebo control group).

Inclusion criteria
Patients with the following criteria were included:

1 age 18–60 years;
2 any sex;
3 confirmed *H. pylori* infection;
4 good mentality to understand aim, benefits and steps of the study;
5 assumed availability during the study period.

Exclusion criteria
Patients with the following conditions were excluded from the study:

1 chronic diseases, e.g. diabetes, renal failure and cirrhosis;
2 malignancies;
3 gall bladder disorders;
4 peptic ulcer;
5 prior upper digestive tract surgery;
6 prior probiotic therapy in the last month;

7 antibiotics, proton-pump inhibitors and H2 receptor blockers therapy within 1 month prior to commencement of study;
8 known allergy to the used medications.

Definitions

- *H. pylori* infection is defined in this study as positivity to all these tests: *H. pylori* antigen in stool; histopathological confirmation of *H. pylori* bacilli; and rapid urease test.
- *H. pylori* eradication is defined in this study as concomitant negativity to all previously positive tests 4 weeks after the end of therapy (6 weeks after the end of the standard triple therapy).
- Endoscopic features *for H. pylori: H. pylori* induced gastritis was classified as antral or corpus predominant [Price and Misiewicz, 1991].
- Histology: Histopathological analysis was performed by the pathologist author, who was blinded to the treatment arm and the time points of tissue sampling. All biopsies were stained with hematoxylin and eosin and giemsa stains. All biopsy specimens were evaluated for the presence of *H. pylori* bacilli, mononuclear cells (lymphocytes, plasma cells and monocytes) as markers of inflammation and polymorphonuclear leucocytes (neutrophils) as markers of activity. Inflammation and activity were graded using a semi-quantitative score ranging from 0 (none), 1 (minimal), 2 (mild), 3 (moderate) to 4 (severe) [Price and Misiewicz, 1991; Stolte *et al.* 1995; Pantoflickova *et al.* 2003].
- Gastrointestinal Symptom Rating Scale (GSRS) [Svedlund *et al.* 1988]: All patients attended an interview for the recall of gastrointestinal symptoms. The 14-item GSRS to assess the severity and frequency of symptoms was reported. The following symptoms were specifically investigated: abdominal pain, acid regurgitation, heartburn, sucking sensation in the epigastrium, nausea and vomiting, borborygmus, abdominal distension, eructation, increased flatus, disorders of defecation [decreased/increased passage of stools, consistency of stools (loose/hard), urgency and feeling of incomplete evacuation]. Other symptoms investigated included

inappetence, halitosis, taste disturbance, and urticaria.

- Triple therapy associated diarrhea was defined according to the World Health Organization as three or more abnormally loose bowel movements per 24 hours and was graded as mild, moderate and severe by the patient himself in the symptom questionnaire [Johnston *et al.* 2008].

Patient assessment

All patients were subjected to the following assessments.

1. Full history-taking with symptom questionnaire.
2. Full clinical examination.
3. Abdominal ultrasound examination.
4. *H. pylori* antigen in stool (On Site H. pylori Ag Rapid Test, CTK BIOTECH, USA). This is a qualitative assessment of *H. pylori* antigens depending on the use of monoclonal antibodies against *H. pylori* conjugated with colloid gold. Positive cases were marked with two bands of color changes (one test band and one control band).
5. Upper digestive endoscopy with full comment on pathological lesions, biopsy and rapid urease test. The rapid urease test, a qualitative assessment of urease activity, was performed using fresh antral and corporal biopsies. Biopsies were impeded on the slides without any contaminating blood and results were classified as negative if no color change from the yellow color for 1 hour while sample with color change toward the pink color was considered positive (HelicotecUT-Plus, Strong Biotech Corporation, Taiwan).
6. Histopathological examination of the gastric biopsies (hematoxylin and eosin and giemsa stains) for determining the presence of *H. pylori* bacilli, presence and density of inflammatory cells including lymphocytes, plasma cells and neutrophils.
7. Recording of GSRS at baseline and 4 weeks after completion of therapy. Reporting of the side effects by symptom questionnaire at weeks 1, 2, 3 and 4 of therapy.
8. Four weeks after therapy, re-examination was carried out by *H. pylori* antigen in stool and re-endoscopy for pathological lesions, biopsies and rapid urease test.

Patient management

Group A. Received triple therapy [omeprazole 20 mg twice a day (bid), amoxicillin 1000 mg bid, clarithromycin 500 mg bid] and *L. reuteri* [a mixture of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 6475, delivered in a dose of 1×10^8 colony-forming units (CFU) of each strain in a chewable tablet for a total dose of 2×10^8 CFU/day] for 2 weeks followed by *L. reuteri* alone for another 2 weeks (i.e. *L. reuteri* was given for 4 weeks) [Francavilla *et al.* 2008].

Group B. Received the same triple therapy plus a placebo for 2 weeks followed by placebo alone for another 2 weeks. The appearance and taste of the placebo study product were identical to the active study product.

Randomization and blinding. This was carried out by an independent physician using a computer generated system. He also carried out the task of blinding (patient and investigator) using a computer generated randomization list. The independent physician enrolled participants and assigned participants to the interventions.

Outcomes

Primary end point. The primary end point was eradication of *H. pylori* infection 4 weeks after completion of therapy.

Secondary end point. The secondary end point was the development of severe adverse effects to the used medications and dietary supplements.

Statistical analysis

Sample size

A total of 70 patients were included with 35 in each arm. The sample size was calculated assuming eradication of *H. pylori* in at least 70% of treated patients [Hunt *et al.* 2011], aiming to detect a difference of 30% based on a 0.80 power to detect significant difference ($p = 0.05$, two-sided).

Data were checked, entered and analyzed using SPSS version 16 for data processing and statistics. Data were expressed as number and percentage for qualitative variables and mean ± standard deviation (SD) for quantitative ones. A p value <0.05 indicates significant results. Comparison between the two groups was done using t test and Chi square tests, while the paired t test was used
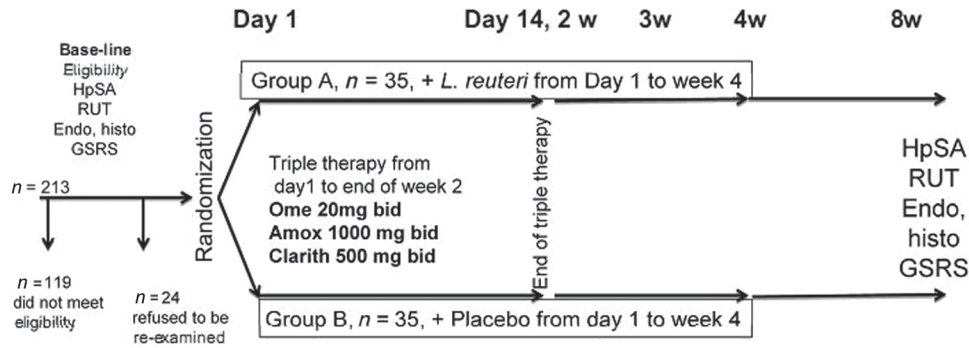
Figure 1. Study flow chart. Out of 213 patients, 94 patients met the inclusion criteria of whom 70 were randomly assigned to the *L. reuteri* and placebo groups.

HpSA, *H. pylori* stool antigen; RUT, rapid urease test; Endo, endoscopic; histo, histopathological confirmation; GSRS, Gastrointestinal Symptom Rating Scale; Ome, omeprazole; Amox, amoxicillin; Clarith, clarithromycin; bid, twice daily; w, week.

to assess variability within the same group before and after therapy.

Ethical considerations

The study protocol was approved by the Institutional Review Board of the faculty of Medicine, Zagazig University, Egypt (ZU-IRB # 395/29-4-2012) [Clinicaltrials.gov identifier: NCT01593592]. All patients gave written informed consent to participate in the study and for performing all relevant interventions.

Results

Trial flow

Out of 213 patients presented to the outpatient gastroenterology clinics during the study period, 70 patients were included. A total of 119 patients were excluded according to our criteria; 94 patients met the inclusion criteria but 24 patients refused to be re-endoscoped and hence were also excluded (Figure 1). There were no dropouts and all subjects remained in the study until the end, and were fully compliant according to the regular interviews. Confirmation of pill intake was recorded by pill count and patients were instructed to bring the vials even when empty. At each visit patients were given a total number of pills sufficient for the period until the next interview. Overall the compliance was good and more than 98% of the chewable tablets were consumed.

Study patients

A total of 70 subjects with confirmed *H. pylori* infection, of which 61.3% were females and 38.7% were males, with a mean age of 35.00 ± 12.65 years (range 18–60 years) participated in the study. They were randomized into two study arms: 35 patients in the *L. reuteri* group (group A) and 35 patients in the placebo group (group B). Most of our patients were of rural residence (68.6%) and nonsmokers (90%). The two treatment groups were similar in their demographic, clinical and endoscopic characteristics at baseline (Table 1).

Endoscopic features

All patients had gastritis at the time of first endoscopy. Antral predominant gastritis and corpus predominant gastritis were encountered in 88.6% and 11.4% of patients in the *L. reuteri* group and in the placebo group respectively. At the end of therapy there was no change in the distribution of gastritis (*p* = 1.0) (Table 1).

H. pylori eradication

After the probiotic/placebo supplementation, the overall eradication rate of the *H. pylori* infection was 70% (49/70), distributed as 74.3% (26/35) and 65.7% (23/35) for the *L. reuteri* and placebo groups respectively. Although the eradication rate was higher in the *L. reuteri* treated group by 8.6%, this difference was not statistically significant (*p* = 0.603).
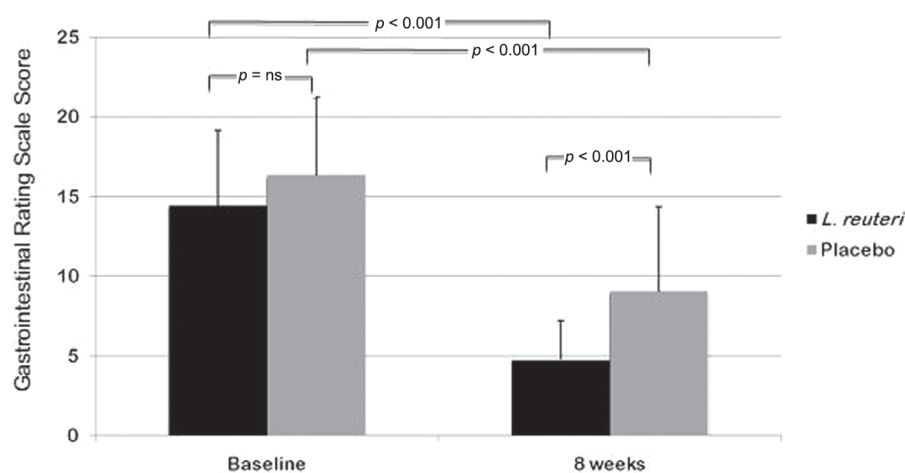
Gastrointestinal symptoms

The baseline GSRS scores for both the *L. reuteri* group and placebo group were 14.42 ± 4.745 and

Table 1. Characteristics of the two groups.

	Group A	Group B	*p*
Age (years)			
Mean ± SD	33.20 ± 13.974	36.80 ± 11.085	0.237
Range	(18–60)	(22–60)	
Sex			
Male	13 (37.1%)	11 (31.4%)	0.615
Female	22 (62.9)	24 (68.6%)	
Residence			
Rural	22	26	0.303
Urban	13	9	
Smoking			
Yes	4	3	0.690
No	31	32	
Endoscopic pattern of gastritis			
Antral predominant	31	31	1.00
Corpus predominant	4	4	

SD, standard deviation.

Figure 2. Gastrointestinal Symptom Rating Scale (GSRS) values between both groups at baseline and 8 weeks later and between each group before and 4 weeks after end of treatment.
ns, not significant.

16.34 ± 4.869 respectively (Figure 2) with no significant difference (p = 0.052).

After treatment (8 weeks from the baseline), GSRS scores for both *L. reuteri* group and placebo group were 4.77 ± 2.446 and 9.06 ± 5.291 respectively (Figure 2); patients treated with *L. reuteri* showed more improvements in gastrointestinal symptoms (p < 0.001).

In patients receiving triple therapy for 2 weeks plus *L. reuteri* for 4 weeks, we observed a significant decrease of GSRS 8 weeks from the baseline compared with the pretreatment value [14.42 ± 4.745 *versus* 4.77 ± 2.446; 95% confidence interval (CI) of the difference: 8.380–10.911; p < 0.001] (Figure 2). The same was also noticed in those receiving triple therapy plus placebo [16.34 ± 4.869 *versus* 9.06 ± 5.291; 95%CI of the difference: 5.409–9.163; p < 0.001] (Figure 2). We found a difference in the number of patients who reported a decrease of the GSRS although it was not statistically significant (35/35 in *L. reuteri* group; 30/35 in placebo group, p = 0.054).

Table 2. Reported side effects in the two groups during the 4 weeks of therapy.

	Group A	Group B	p
Taste disorders	2	8	0.002
Diarrhea	1	10	
Distension	5	4	
Vomiting	2	2	

When the frequencies of the symptoms were evaluated, we found that patients receiving *L. reuteri* showed marked improvement of these symptoms, epigastric pain, sucking sensation in the epigastrium and eructation.

Both study products were well tolerated by all subjects and no serious adverse events were reported during the period of intake. The types of adverse events reported in this study did not differ significantly between the two groups.

The most common side effects were diarrhea, taste disorders and distension (Table 2). There was a significant difference between the two groups concerning the reported side effects; patients treated with *L. reuteri* reported less diarrhea and taste disorders than placebo group ($p = 0.002$). All reported side effects were rather mild and there was no reported treatment discontinuation because of the side effects.

Pathological lesions
Inflammation. The severity of *H. pylori* associated gastritis was reduced after 8 weeks from the beginning of therapy in the *L. reuteri* treated group compared with pretreatment period (inflammatory cell score 2.63 ± 0.598 *versus* 1.57 ± 0.558; $p < 0.001$). The severity of *H. pylori* associated gastritis was also reduced after 8 weeks from the beginning of therapy in the placebo treated group compared with pretreatment period (inflammatory cell score 2.43 ± 0.502 *versus* $2.20 \pm .531$; $p = 0.019$). The *L. reuteri* treated group showed more improvement in the inflammatory cell score than placebo treated group ($p < 0.001$) (Figure 3).

Activity. The activity of *H. pylori* associated gastritis was reduced after 8 weeks from the beginning of therapy in *L. reuteri* treated group as compared to the pretreatment period (inflammatory cell score 2.54 ± 0.611 *versus* 1.26 ± 0.886; $p < 0.001$). The activity of *H. pylori* associated gastritis was reduced after 8 weeks from the beginning of therapy in the placebo treated group compared with pretreatment period (inflammatory cell score 2.37 ± 0.490 *versus* 1.66 ± 0.691; $p < 0.001$). The *L. reuteri* showed more improvements in the inflammatory cell score than the placebo treated group ($p = 0.034$) (Figure 4).
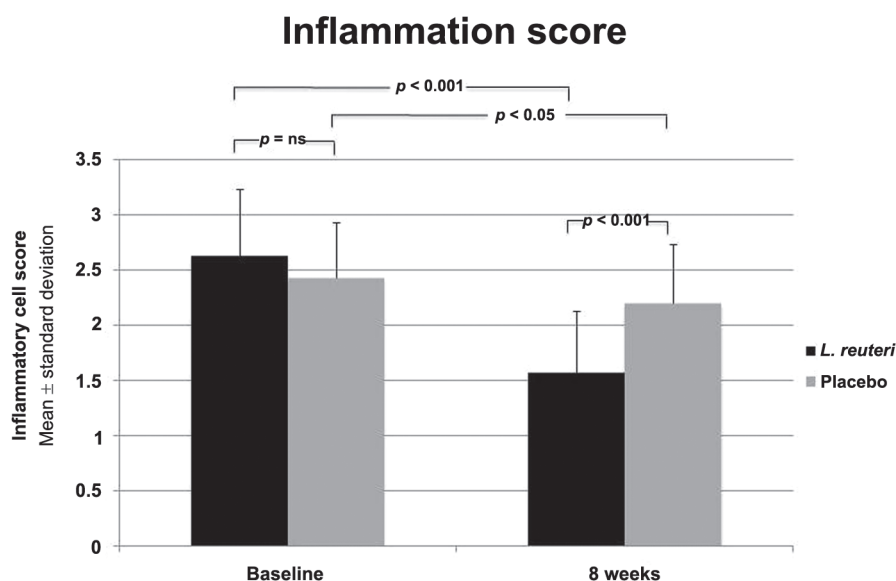
Figure 3. Inflammatory cell score (lymphocytes, plasma cells) between both groups at baseline and 8 weeks later and between each group before and 4 weeks after end of treatment.
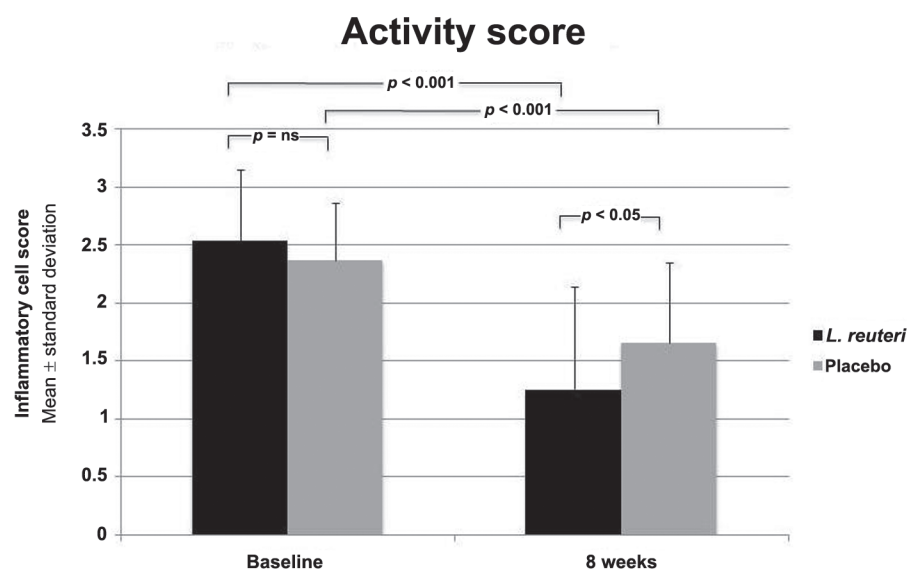
Activity score

Figure 4. Inflammatory cell score (neutrophils) between both groups at baseline and 8 weeks later and between each group before and 4 weeks after end of treatment.

Discussion

Treatment of *H. pylori* infection is becoming a very relevant problem, especially in the developing countries. Although different therapeutic regimens are currently available, treatment failure remains a growing problem in daily medical practice. Several factors could play a role in the eradication failure, but the most relevant are antibiotic resistance and patient's compliance [Ibrahim *et al.* 2012].

The overall eradication rate reported in this study is 70%, which lies between different eradication rates ranging from 60% [Yaşar *et al.* 2010] to 94.4% [Manfredi *et al.* 2012] either with the standard triple therapy or when the sequential therapy is used. The eradication rate increased when probiotics were added to the triple therapy; in our study the eradication rate increased to 74.3%. Different rates were reported from different studies and this may point to a strain specific anti-*H. pylori* activity. For *L. reuteri*, different eradication rates were reported ranging from 53% to 88% [Lionetti *et al.* 2006; Francavilla *et al.* 2008; Scaccianoce *et al.* 2008; Ojetti *et al.* 2012].

Resistant strains of *H. pylori* are now widely prevalent in the west, and the eradication rate with current regimens fails in 10% to 20% of patients [Vakil *et al.* 1998; Mégraud *et al.* 2013].

The 30% failure rate in this study could be attributed to the growing antimicrobial resistance, although it was not examined in the current study. Several investigators have documented a high response rate of *H. pylori* isolates to amoxicillin [Mégraud and Lehours, 2007; Seck *et al.* 2009], others reported variable resistance rates ranging from 18.5% to 100% [Godoy *et al.* 2003; Thyagarajan *et al.* 2003; Kim *et al.* 2006; Abordin *et al.* 2007]. In our community resistance to amoxicillin have been recently reported to be 3.7% [Ibrahim *et al.* 2012].

In our rural communities, the prevalent populations in this study, we expect higher rates of amoxicillin resistance because this antibiotic is widely available in primary care units and is used for different sorts of infection in particular respiratory tract infections. In addition, this antibiotic has been available for a long time and widely abused in self medication, a common custom in the Egyptian rural community.

Resistance to clarithromycin is becoming more prevalent in some European countries, where the prevalence may be as high as 17% [Romano and Cuomo, 2004; Mégraud *et al.* 2013]. Clarithromycin resistance in *H. pylori* infection has sharply decreased the success of eradication by 40% to 50% [Njume *et al.* 2009]. Also a growing clarithromycin resistance has been reported in the Egyptian population; a recent *H. pylori* study reported the rate of resistance to be 11.1% in gastric isolates and 27% in dental isolates [Ibrahim *et al.* 2012].

An important issue in the eradication of *H. pylori* is patient compliance, which is largely affected by side effects related to the triple therapy used [Njume *et al.* 2009]. Addition of *L. reuteri* to the triple therapy reduced the frequency of side effects, particularly diarrhea; this should improve patient compliance and adherence to therapy. Similar results were reported from different studies that used *L. reuteri* as adjuvant to the triple therapy in *H. pylori* eradication [Francavilla *et al.* 2008; Scaccianoce *et al.* 2008; Efrati *et al.* 2012; Ojetti *et al.* 2012]. Although all patients in the present study were compliant with the regimen prescribed, in daily clinical practice reduced side effects would increase the patients' compliance especially with retreat patients who experienced side effects with previous triple therapies and therefore were not motivated to be retreated due to intolerable side effects.

The reported side effects in this study are not different from side effects reported in other studies [Francavilla *et al.* 2008; Scaccianoce *et al.* 2008] and all were self limited after therapy. No serious adverse events were reported; furthermore no manifestations related to systemic invasion of *L. reuteri* were reported, confirming safety issues in the use of these strains in *H. pylori* treatment.

Improvement of clinical manifestations is a marker of successful therapy; both groups showed improvements in the GSRS after therapy, but the *L. reuteri* group showed more. These improvements of symptoms should advocate patients for more compliance and adhesion to therapy. Improvement was greater in symptoms related to the fore gut including epigastric pain, sucking sensation in the epigastrium, and eructation which support the conclusion that *H. pylori* eradication is the direct cause for symptom amelioration.

Another important issue in *H. pylori* infection is gastritis in terms of pathological features. In this study an improvement of gastritis inflammatory score as well as activity was noted. Improvement of the histological features in *H. pylori* infection is not a novel finding of this study as many investigators have noticed improvement of gastric antral activity with the use of different probiotic preparations and strains [Felley *et al.* 2001; Pantoflickova *et al.* 2003; Wang *et al.* 2004]. The novel finding in our study is that *L. reuteri* also has an impact on the *H. pylori* histopathological features, a valuable effect added to its clinical aspects and

improvement in eradication rate confirmed in this study and other studies [Lionetti *et al.* 2006; Francavilla *et al.* 2008; Scaccianoce *et al.* 2008]. This would certainly reduce the risk of atrophic gastritis and gastric malignancies related to *H. pylori* in the long term.

Several mechanisms could contribute to an improvement of *H. pylori* associated gastric inflammation and activity by the use of probiotics. First, probiotic therapy may reinforce gut innate defenses. In the gastric mucosa, *H. pylori* may interact with epithelial cells through secretory components or as a result of adherence [Chauviere *et al.* 1992]. Second, some strains may prevent *H. pylori* adherence by increasing mucin expression [Mack *et al.* 1999] that is usually downregulated in *H. pylori* infection [Byrd *et al.* 2000]. Third, probiotics could secrete antibacterial substances. It has been suggested that the antibacterial activity of the *L. reuteri* is related to an antimicrobial substance called reuterin which has bactericidal and bacteriostatic activities against a number of gram positive and negative bacteria and even some fungi [Hamilton-Miller, 2003; Spinler *et al.* 2008]. Fourth, some strains of probiotics are known to have anti-inflammatory activity (e.g. the *L. reuteri* ATCC PTA 6475 that was used in this study was proved to suppress local pro-inflammatory cytokines production) [Thomas *et al.* 2012]. Finally, Lactobacilli may exert its favorable effect on the gastric mucosa through local or systemic immunomodulation [Cunningham-Rundles *et al.* 2000; Gill *et al.* 2000; Vitini *et al.* 2000]. Lactic acid bacteria may strengthen the gastric immunologic barrier through stimulation of local immunoglobulin A responses and alleviation of inflammatory responses, thus leading to a mucosa-stabilizing effect [Cunningham-Rundles *et al.* 2000].

Although improvements in *H. pylori* induced gastritis were noticed with different antibiotics used in *H. pylori* eradication in this study and also in previous studies [Marshall *et al.* 1987; Graham *et al.* 1993], probiotics associated improvements in the histopathological changes have particular importance due to various aspects. The effect is long lasting compared with the relatively short effect of antibiotics [Pantoflickova *et al.* 2003] and this may be due to its impact on the gut barriers and immunity [Vitini *et al.* 2000]. In addition, it is associated with higher eradication rate [Manfredi *et al.* 2012] and probably low recurrence rates. Finally, it is not associated with more

side effects; in contrast, marked symptom improvements were noticed [Francavilla *et al.* 2008].

In conclusion, eradication triple therapy of *H. pylori* supplemented by *L. reuteri* increased the eradication rate by 8.6% (nonsignificant), improved the GSRS score (significant), reduced the side effects (significant) and improved the histological features (significant) in *H. pylori* infection compared with triple therapy supplemented with placebo.

Conflict of interest statement
The authors declare no conflicts of interest in preparing this article.

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