Inhibition of *Helicobacter pylori* Infection in Humans by *Lactobacillus reuteri* ATCC 55730 and Effect on Eradication Therapy: A Pilot Study

Ruggiero Francavilla,[*¶] Elena Lionetti,[*] Stefania Paola Castellaneta,[†] Anna Maria Magistà,[*] Giovanni Maurogiovanni,[‡] Nunzia Bucci,[*] Angela De Canio,[*] Flavia Indrio,[*] Luciano Cavallo,[*¶] Enzo Ierardi[§] and Vito Leonardo Miniello[*]

[*]Department of Biomedicina dell'Età Evolutiva, Università degli Studi di Bari, [†]Unità di Pediatria, Ospedale San Paolo, Bari, Italy, [‡]Gastroenterologia Ospedaliera, Policlinico di Bari, Bari, Italy, [§]Dipartimento Scienze Mediche e del Lavoro, Università degli Studi di Foggia, Puglia, Italy, [¶]Centro Interdipartimentale Gastroenterologia ed Epatologia Età Evolutiva, Bari, Italy

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Reprint requests to: Dr Ruggiero Francavilla, Clinica Pediatrica "B. Trambusti", Piazza Giulio Cesare, 11 – Policlinico, Bari, Italy.
Tel.: +39 0805592847; Fax: +39 080 5478911;
E-mail: rfrancavilla@libero.it

Authors Francavilla Ruggiero and Lionetti Elena have equally contributed to the work

Abstract

Background: Several studies report an inhibitory effect of probiotics on *Helicobacter pylori*.
Aim: To test whether *Lactobacillus reuteri* ATCC 55730 reduces *H. pylori* intragastric load in vivo, decreases dyspeptic symptoms, and affects eradication rates after conventional treatment.
Materials and Methods: In a double-blind placebo-controlled study, 40 *H. pylori*-positive subjects were given *L. reuteri* once a day for 4 weeks or placebo. All underwent upper endoscopy, ^{13}C-urea breath test, and *H. pylori* stool antigen determination at entry and ^{13}C-urea breath test and *H. pylori* stool antigen (used as both qualitative and semiquantitative markers) after 4 weeks of treatment. Sequential treatment was administered subsequently to all.
Results: In vivo, *L. reuteri* reduces *H. pylori* load as semiquantitatively assessed by both ^{13}C-urea breath test δ-value and *H. pylori* stool antigen quantification after 4 weeks of treatment ($p < .05$). No change was shown in patients receiving placebo. *L. reuteri* administration was followed by a significant decrease in the Gastrointestinal Symptom Rating Scale as compared to pretreatment value ($p < .05$) that was not present in those receiving placebo (p = not significant). No difference in eradication rates was observed.
Conclusions: *L. reuteri* effectively suppresses *H. pylori* infection in humans and decreases the occurrence of dyspeptic symptoms. Nevertheless, it does not seem to affect antibiotic therapy outcome.

The need to treat *Helicobacter pylori* infection is unequivocally related to the therapy of peptic ulcer disease and gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and further, the possibility of preventing gastric carcinoma has also been emphasized [1–3]. At present, there are numerous treatment options for curing *H. pylori* infection and many are still under investigation. One-week triple therapy, combining acid suppression with clarithromycin, amoxicillin, or nitroimidazolic compounds, represents first-line regimens administered for either 7 days or 14 days as recommended by European and American guidelines, respectively [4]. However, eradication rates in the community setting vary from approximately 65% to 80%, and poor patient compliance, primary and secondary bacterial resistance, and the occurrence of antibiotic side-effects are among the factors that may contribute to treatment failure [5,6].

^{13}C-urea breath test (^{13}C-UBT) is the most accurate noninvasive test for the diagnosis of *H. pylori* infection [7] and is widely used in clinical practice, clinical trials, and epidemiologic studies. According to recent studies, ^{13}C-UBT, by measuring the intragastric urease activity, is able to estimate the *H. pylori* bacterial load [7] and the severity of gastritis activity [8,9].

Strategies targeted to improve treatment tolerability and to raise eradication rates are needed [7], and the report from the Maastricht III Consensus Report on *H. pylori* includes probiotics as "possible" tools for management of the infection [4].

Lactobacillus reuteri ATCC 55730, a probiotic of human origin, reportedly exerts a beneficial effect in the prevention and

treatment of several intestinal conditions [10,11] and, further, *L. reuteri* strains have been demonstrated to colonize the human gastric mucosa [12], to inhibit the binding of *H. pylori* to gastric epithelial cell lines [13], to reduce side-effects during anti-*H. pylori* treatment [14], and could therefore play a role in the treatment of *H. pylori* infection in humans.

The primary end-point of the current study was to test the hypothesis that a 28-day supplementation with *L. reuteri* would change the intragastric *H. pylori* bacterial load. The secondary end-points were the improvement of dyspeptic symptoms and the assessment of the effect of the pretreatment with *L. reuteri* on the eradication rate of *H. pylori* after conventional antibiotic therapy.

Methods

Patients

The study was carried out on 40 *H. pylori*-positive dyspeptic patients [23 males; mean age: 55 ± 15 years (range: 35–68)], consecutively referred to the Department of Gastroenterology of the University of Bari, Italy, between January 2006 and September 2006. The study population consisted of subjects who had never been treated for *H. pylori* infection before. Subjects with a positive medical history for upper gastrointestinal surgery, active peptic ulcer disease or neurologic, cardiovascular, metabolic, hematologic or endocrine disorders, and those with previous exposure to drugs that might interfere with *H. pylori* status, such as recent (a month prior to the start of the study) or continuing use of antibiotics, bismuth compounds, proton pump inhibitors, or H_2-receptor antagonists, were excluded from the study.

H. pylori Assessment

At baseline, patients underwent upper endoscopy, ^{13}C-UBT, and *H. pylori* stool antigen determination.

Endoscopy was performed by the same physician after sedation with intravenous midazolam and biopsies for histology (two samples from the antrum and two samples from the corpus), and for rapid urease test (one sample from the antrum) (CP test, Yamanouchi Pharma S.p.A., Carugate, Italy), taken. Histologic examinations were carried out by the same observer using hematoxylin and eosin staining for assessment of gastritis, as stated in the updated Sydney system, and Giemsa stain for detection of *H. pylori*.

In order to achieve the best correlation with ^{13}C-UBT and *H. pylori* bacterial load [8], the test was conducted according to the ^{13}C-UBT European Standard Protocol [14]. ^{13}C-UBT was performed by all patients within 24 hours before the endoscopy. Briefly, after an overnight fasting, a fatty meal (100 mL) and a solution of ^{13}C urea [75 mg ^{13}C urea (AB Analitica srl Padova, Italy)] were given to each patient. Breath samples were collected before and 30 minutes after the dose of urea. The ratio of ^{13}C to ^{12}C in the expired air samples was measured using a dual-inlet-ratio mass spectrometer (Automated Breath ^{13}Carbon Analyzer, Europa Scientific, Ltd, Crawley, West Sussex, UK). Results were expressed as parts per million of excess of $\Delta^{13}CO2$ (by subtraction of the baseline pretest breath sample). The presence of gastric urease activity was revealed by a change of 3.5 per thousand (or more) related to the baseline signal (δ-value) [15]. In the cases where medications such as antibiotics or antiacids were used, the ^{13}C-UBT was differed for at least 4 weeks.

H. pylori in stool specimens (carried out on the day of ^{13}C-UBT) was investigated by a commercial enzymatic immunoassay test (HpSA, Meridian Diagnostic Inc, Cincinnati, OH, USA). This test uses polyclonal anti-*H. pylori* antibodies that are adsorbed into microwells. Diluted stool samples were tested according to the manufacturer's instructions. Plates were read spectrophotometrically at a wavelength of 450 nm. Stool testing was performed blind, without knowledge of the other test results. Our group has previously shown that HpSA, similarly to UBT delta, may represent a semiquantitative determination of bacterial intragastric load [16] after application of the statistical principle of "standard points" [17] to adsorbance units obtained at spectrophtometric reading of stool samples of all patients (Net/Control value).

At entry, patients were considered *H. pylori* positive if three of four tests (histology, rapid urease test, ^{13}C-UBT, HPsA) were positive.

Symptom Assessment

All patients attended an interview to recall history of gastrointestinal symptoms, and the following data were collected: 1, a detailed physical examination, 2, the 15-item Gastrointestinal Symptom Rating Scale (GSRS) to assess severity and frequency of symptoms [18], and 3, questions to assess other variables that may have affected study results (i.e. intercurrent infections, life events). The following symptoms were specifically investigated: epigastric burning and/or pain, abdominal pain, acid regurgitation, heartburn, sucking sensation in the epigastrium, nausea, vomiting, bloating, abdominal distension, eructation, increased flatus, disorders of defecation [decreased/increased passage of stools, consistency of stools (loose/hard), urgency, feeling of incomplete evacuation], inappetence, halitosis, taste disturbance, and urticaria. The patients scored symptoms on a four-point scale: mild (noninterfering with daily activities), moderate (slightly interfering with daily activities), severe (interfering with daily activities), and very severe

(continuous and if on therapy, producing treatment interruption). Stool consistency was graded from hard (0) to watery (4). Data were collected before (1 week before intervention) and after completion of therapy, and patients were invited to return their diaries immediately after the intervention period.

Therapy Regimens

An independent physician prescribed either probiotic or placebo according to a computer-generated randomization list, blindly to researchers. Patients were randomly assigned to receive either *L. reuteri* or placebo both provided by Nóos (BioGaia AB, Sweden) as chewable tablets and included either *L. reuteri* (each tablet containing 10^8 colony-forming units of *L. reuteri* ATCC 55730 (Reuterin, Nóos) or placebo which consisted of tablets identical in taste and appearance to the active study product except for the absence of freeze-dried *L. reuteri* (and cryoprotectants). Boxes containing placebo had the same shape, dimension, indication, and appearance as those containing the viable *L. reuteri* and were provided by the probiotic producer, which ensured that the study was blinded for investigators and patients. Both placebo and probiotics were prescribed as one tablet, once a day (2 hours after meals) for 28 days. Patients were thoroughly instructed and motivated for the therapy. Adherence to treatment was evaluated by counting the tablets returned by the subject; a minimum tablet intake of 95% was considered as acceptable.

On day 29, *H. pylori* status was assessed by repeating a ^{13}C-UBT and HPsA. An informed consent was obtained by all patients.

At the end of the trial, patients received a 10-day sequential therapy comprising of rabeprazole (20 mg twice a day) plus amoxicillin (1 g, twice daily) for 5 days followed by rabeprazole (20 mg twice a day) plus clarithromycin (500 mg, twice daily), and tinidazole (500 mg, twice daily) for the next 5 days [19]. The code was revealed to the researchers once recruitment, data collection, and laboratory analyses and statistical analyses were completed.

The local Ethical Committee approved the study protocol.

Statistical Analysis

All data are expressed as median with a range. All data analysis was carried out according to a pre-established analysis plan. The sample size was calculated assuming a reduction of *H. pylori* bacterial load in at least 65% of treated patients [20], aiming to detect a difference of 35%; based on a 0.80 power to detect significant difference ($p = .05$, two-sided), 20 patients were required for each arm. Proportions were compared by using chi-squared tests with continuity correction or Fisher's exact test when appropriate; comparison of continuous variables was performed using Mann–Whitney. A $p < 0.05$ was considered significant. The statistical analysis was performed using the Software Program Stata System (SPSS) version 13.0 (SPSS Inc., Chicago, IL, USA) program.

Results

Baseline Characteristics

The baseline demographic and clinical characteristics of the enrolled patients are reported in Table 1. As shown, the two groups did not differ for age, sex, and baseline GSRS score, as well as for mean mean ^{13}C-UBT δ and HPsA Net/Co value (Table 1). No difference was found in the endoscopic features detected (Table 2) and in histology, *H. pylori* was observed in the gastric antrum of all patients. The presence of the bacterium was always associated with chronic gastritis (lymphocytes and plasma cells in the lamina propria) with a variable degree of activity. None

Table 1 Baseline demographic and clinical characteristics of trial groups

	Lactobacillus reuteri (n: 20)	Placebo (n: 20)	*p*
Mean age ± SD in years	53.3 ± 13.3	52.4 ± 13.1	1.0
Sex (male/female)	11/9	12/8	0.8
Mean GSRS ± SD	11.4 ± 9.7	11.8 ± 8.5	0.9
Mean ^{13}C-UBT ± SD	33.8 ± 15‰	35.8 ± 15.5‰	0.8
Mean HpSA ± SD	18.1 ± 6.4 Net/Co	17.6 ± 6.5 Net/Co	0.8

^{13}C-UBT, ^{13}C-urea breath test; GSRS, Gastrointestinal Symptom Rating Scale; SD, standard deviation.

Table 2 Endoscopic and histologic findings in the two treatment groups

	Lactobacillus reuteri (n: 20)	Placebo (n: 20)	*p*
Endoscopic findings			
Macroscopic nodular antral gastritis with hyperemia	16 (80%)	14 (70%)	0.9
Antral hyperaemia without macroscopic nodularity	1 (5%)	3 (15%)	0.6
Pangastritis	15 (75%)	16 (80%)	1
Gastric ulcer	Nil	Nil	–
Duodenal ulcer	Nil	Nil	–
Erosive bulbitis	5 (25%)	4 (20%)	1
Esophagitis	Nil	Nil	1
Histologic findings			
Pangastritis	13 (65%)	14 (70%)	1
Antral gastritis mild	4 (50%)	5 (50%)	0.7
Antral gastritis moderate	14 (70%)	12 (60%)	0.7
Antral gastritis severe	2 (10%)	3 (15%)	0.7

had gastric atrophy or ulcer or intestinal metaplasia (Table 2). In all patients, all the four tests were positive.

Trial Flow

All patients completed the study and there were no protocol deviations. Compliance to the treatment was good (> 95%) in all enrolled cases, and no patient discontinued therapy. There were no differences in adherence to treatment schedules (100% in both groups); however, seven patients (four in the probiotic and three in the placebo group) did not present at follow-up 8 weeks after the completion of the eradicating regimen.

^{13}C-UBT

After 4 weeks of treatment, the urease activity, as determined by ^{13}C-UBT δ-value, decreased from 33.8 ± 15‰ (95%CI: 26.8–40.0‰) to 27.3 ± 12.1‰ (95%CI: 21.6–33‰) ($p < .05$) in those receiving *L. reuteri* as compared to unchanged results from 35.8 ± 15‰ (95%CI: 25.9–41.5‰) to 37.3 ± 16.2‰ (95%CI: 29.7–44.9‰) in those receiving placebo ($p = .8$) (Fig. 1A). ^{13}C-UBT decreased by at least 10% in 13 of 20 (65%) receiving *L. reuteri* and in six of the 20 (30%) receiving placebo ($p < .03$). Overall, we observed a decrease of ^{13}C-UBT δ-value of about 13% in those receiving *L. reuteri* as compared to a 3% increase in those receiving placebo [–13.7 ± 28‰ (95%CI: –26.8–13.5‰) vs. 3.6 ± 34.8‰ (95%CI: –12.8–41.5‰); $p < .009$] (Fig. 2A).

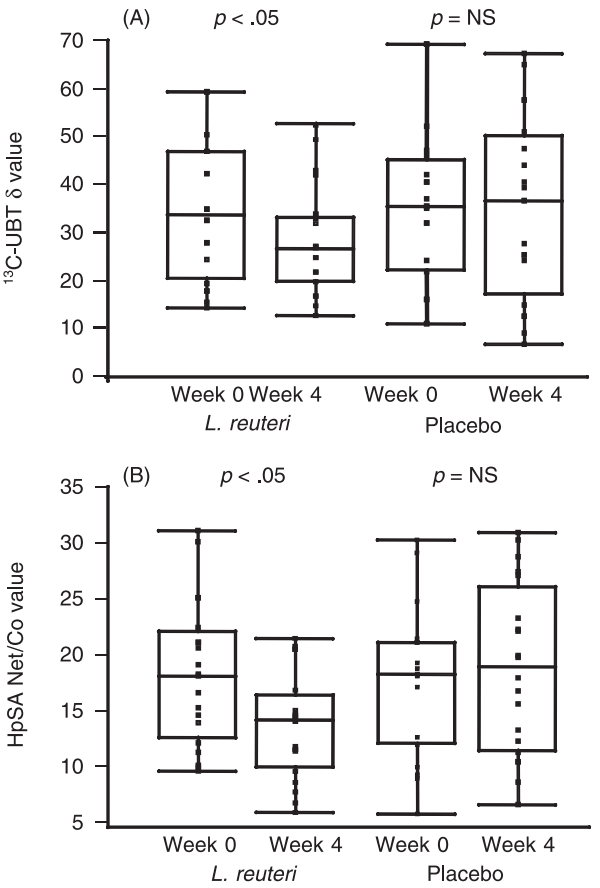

Figure 1 *H. pylori* bacterial load assessed by ^{13}C-urea breath test (A) and HpSA (B) before and after placebo or *Lactobacillus reuteri* supplementation.

HpSA

After 4 weeks of treatment, the HpSA decreased from 18.1 ± 6.4 Net/Co (95%CI: 21.1 ± 15.2 Net/Co) to 14.4 ± 5.2 Net/Co (95%CI: 12–16.8 Net/Co) in those receiving *L. reuteri* as compared to unchanged results from 17.6 ± 6.5 Net/Co (95%CI: 14.6–20.7 Net/Co) to 18.8 ± 7.0 Net/Co (95%CI: 15.6–22.1 Net/Co) in those receiving placebo ($p < .05$) (Fig. 1B). HpSA decreased by at least 10% in 14 of 20 receiving *L. reuteri* and in five of the 20 receiving placebo (65% vs. 25%; $p < .004$). Overall, we observed a decrease of HpSA of about 15% in those receiving *L. reuteri* as compared to a 10% increase in those receiving placebo [–15 ± 26.8 Net/Co (95%CI: –29.6–9.4 Net/Co) vs. 12.4 ± 35.6 Net/Co (95%CI: –8.3–29.1 Net/Co); $p < .006$] (Fig. 2B).

Symptom Assessment

In patients receiving *L. reuteri* for 4 weeks, we observed a significant decrease of GSRS as compared to the pretreatment value [7.9 ± 4.1 (95%CI: 6.3–9.8) vs. 11.8 ± 8.5 (95%CI: 7.8–15.7); $p < .05$] that was not present in those receiving placebo [9.7 ± 8.7 (95%CI: 5.6–13.8) vs. 11.4 ± 9.7 (95%CI: 6.8–15.9); p = not significant], although a reduction was still noted (Fig. 3). No difference was found in the numbers of patients that reported a decrease of the GSRS (14 of 20 in each group; $p = 1.0$). In detail, when the frequencies of the symptoms were evaluated by taking into account only the occurrence of new or worsened symptoms after the weeks of treatment compared to the baseline, we found that patients receiving *L. reuteri* referred less frequently than those receiving placebo, abdominal distension (25% vs. 55%; difference: –30%; $p < .05$), disorders of defecation (10% vs. 35%; difference: –25%; $p < .05$), and flatus (5% vs. 30%; difference: –25%; $p < .04$) than those receiving placebo. No adverse events were reported.

H. pylori Eradication

After the probiotic/placebo supplementation, none of the patients had eradicated the *H. pylori* infection. After the 10-day sequential regimen, there was no difference in the

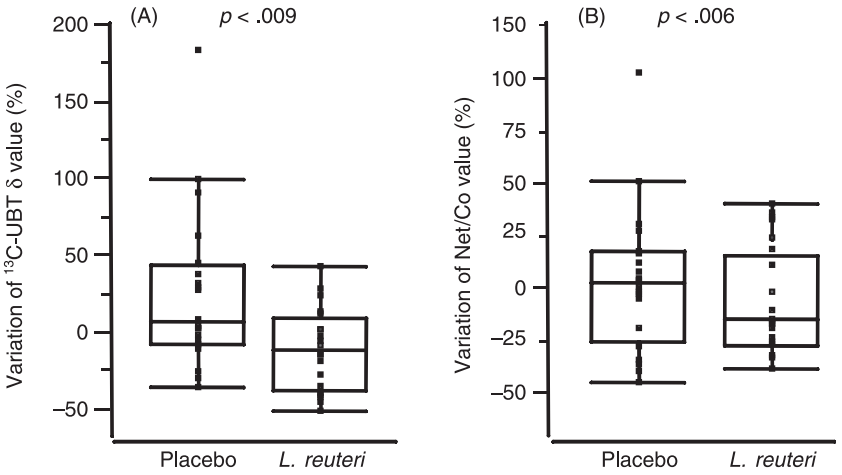

Figure 2 Percentage of variation of ^{13}C-urea breath test (A) and HpSA (B) after placebo or *Lactobacillus* supplementation.

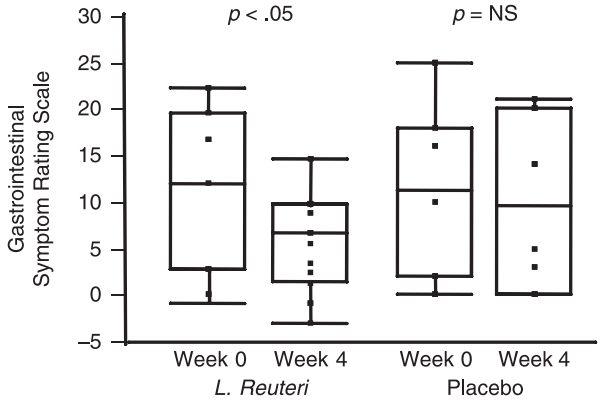

Figure 3 Gastrointestinal Symptom Rating Scale (GSRS) assessed before and after placebo or *Lactobacillus reuteri* supplementation.

rate of *H. pylori* eradication in those who had previously received probiotic or placebo (88% vs. 82%; $p = .8$).

Discussion

In this randomized, placebo-controlled pilot study, we have shown that *L. reuteri* may exert an inhibitory effect on *H. pylori* growth as demonstrated by the simultaneous and significant decrease of both ^{13}C-UBT δ and HpSA Net/Co. Moreover, *L. reuteri* appears to be able to decrease dyspeptic symptoms in infected individuals.

The ability of a probiotic to decrease the ^{13}C-UBT δ and therefore the intragastric urease activity has some important implications. Indeed, recent studies have clearly reported a significant correlation between the ^{13}C-UBT, *H. pylori* bacterial load [8,21] (assessed on the whole gastric mucosa and not in the antrum alone), grade of gastritis activity (neutrophils infiltration of gastric mucosa) [8,9], and gastric mucosal myeloperoxidase activity that is a quantitative marker of gastrointestinal inflammation [22,23]. Despite most reports agree about the role of ^{13}C-UBT as a marker of intragastic bacterial load, this statement has been recently reviewed [9]. Nevertheless, δ-value undoubtfully reflects urease activity, which often is directly correlated to the number of viable organisms in the stomach. In truth, it is possible that this value may be affected by variables other than bacterial load (i.e. environmental conditions favoring *H. pylori* growth and CagA status [16]) even if no semiquantitative determination may express a perfect verdict since both culture (technical problems even in expert hands) and histology (patchy distribution of bacteria on mucosal surface) cannot be still considered as a reliable gold standard. Therefore, it is possible to speculate that *L. reuteri* may exert a beneficial effect during *H. pylori* infection determining a reduction of the bacterial load and consequently of gastric inflammation. Finally, a reduction of bacterial load seems to be associated with an reduced risk of gastroduodenal endoscopic lesions, such as peptic ulcer disease and its complications [24,25]. We do believe that our finding is genuine since: 1, both tests (^{13}C-UBT and HpSA) showed a similar and proportional reduction in patients receiving *L. reuteri* and 2, the effect was observed in the absolute value of the tests, the percentage of its variation as well as at the number of patients achieving a decrease of at least 10% vs. baseline. Nevertheless, this result is not unexpected and agrees with earlier studies where the administration of *L. gasseri* [26], *L. casei* [20], or a strain of *L. acidophilus* La1 [27] decreased *H. pylori* density (measured by ^{13}C-UBT δ) in adults and in children [28]. Interestingly, in a study by Myllyluoma [29] only patients receiving the probiotic (but not the placebo), who were not eradicated, had borderline results from ^{13}C-UBT after completion of eradicating therapy. Finally, as reported in a recent review [30], the effect on *H. pylori* is strain specific with some strains having no effect on modulation of intragastric bacterial load and/or gastric inflammation [20,31].

Currently, there are at least 10 clinical trials on the use of single or mixtures of probiotic strains administered alone (or with dairy products) for the treatment of *H. pylori*. The results vary with some showing no effect [20,31], while others being able to eradicate the infection in some patients [30], to decrease the value of ^{13}C-UBT or HpSA [28,32,33,34], or to decrease gastric inflammation [35]. These trials have used *L. johnsonii* La1 given for a minimum of two [32] and up to 16 weeks [35], or different strains of *L. acidophilus* [31,33,36], *L. casei* [21,31], or *L. gasseri* [26]. Unfortunately, these studies are difficult to compare since only four were randomized and placebo controlled [20,26,27,32], and the probiotics were delivered in different forms, for different periods of time with different doses, and in some cases the bacteria used were killed organisms. Our choice to use *L. reuteri* ATCC 55730 is based on several observations. *L. reuteri* has been extensively studied and is widely used as a food additive to improve human gastrointestinal health. Oral administration delivers *L. reuteri* ATCC 55730 to the gastrointestinal tract, leading to shedding of live bacteria in the feces [37]. Clinical trials have shown that *L. reuteri* ATCC 55730 administration is safe [38] and significantly reduces gastrointestinal infections [11]. Being acid resistant, it persists in the stomach longer than other bacteria surviving in high proportions (> 80%) in the gastric environment for periods of 2 hours. *L. reuteri* adheres to gastric epithelial cells in vitro [39], and recently Valeur et al. have shown the adhesion of *L. reuteri* to epithelial cells from corpus and antral gastric biopsies providing the first clear and direct evidence of colonization of the human stomach [12]. Finally, in vitro studies have demonstrated that *L. reuteri* exerts a significant inhibitory effect on *H. pylori* growth on the plate and bacterial diffusion disk method showed an annular radius of inhibition on the plate by this probiotic strain [40]. The mechanisms by which *L. reuteri* could be responsible for the inhibition of *H. pylori* growth [39,41–43] may be: 1, direct and nonspecific bacteriostatic activity, 2, production of inhibitory compounds such as lactate, hydrogen peroxide, and bacteriocidal substances (reuterin), 3, competition for nutrients, 4, nonspecific immunostimulation of mucosal IgA production, and 4, adherent capacity of *L. reuteri* to gastric epithelial cells.

The second aim of our study was to assess whether *L. reuteri* could be of help in ameliorating the symptoms perceived by *H. pylori*-infected individuals. We have shown that only those receiving the probiotic experienced a significant improvement of the GSRS, while no difference was observed in those receiving the placebo. However, we cannot be sure that the effect we observed reflects the improvement of *H. pylori* status since the symptoms of dyspepsia have a multifactorial origin and an overlap of manifestations of different conditions, such as irritable bowel syndrome (IBS), may strongly be involved. IBS is a symptomatic motility and sensory disorder of the lower gastrointestinal tract and it is a wide spread condition in the adult population with a prevalence in Europe and North America of about 15–30% [44]. The main symptoms of IBS such as abdominal discomfort, bloating, and altered bowel activity are those that were significantly decreased in the *L. reuteri* group, and it is known that such symptoms can be controlled by the administration of probiotics [45], and therefore our finding may reflect a control of IBS rather than of *H. pylori*-related dyspepsia. More studies on larger samples are needed before definite conclusion can be drawn on this issue.

We did not find any difference in rates of eradication between those who did or did not receive the probiotic prior to treatment. This result is in agreement with previous experience with probiotics where eradication has occurred sporadically [30] and mainly in children [36] in whom *H. pylori* infection can be transient [46]. Moreover, due to the high eradication rates that we have achieved with the sequential treatment, to detect a 10% increase in eradication secondary to the use of a probiotic strain and maintaining the statistical power of 80%, we would have needed 150 patients in each arm.

We are aware of the limit of our study, namely the absence of a follow-up gastric biopsy and the small sample size; however, a second endoscopy would not have been accepted by patients who are not affected by major gastric lesions such as ulcer or preneoplastic disease.

In summary, we report that a 4-week supplementation with *L. reuteri* is effective in reducing *H. pylori* bacterial load in humans and theoretically may help to control gastric inflammation; although this pilot study open new areas of investigation, further studies on larger number of patients are needed to define its real clinical application.

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