

# Triple therapies plus different probiotics for *Helicobacter pylori* eradication

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**Abstract.** – The *Helicobacter pylori* (*H. pylori*) cure rate following standard triple therapies is decreasing worldwide. Therefore, further approaches aimed to improve standard triple therapy efficacy should be attempted. This prospective, pilot study aimed to evaluate the therapeutic role of either *Lactobacillus reuteri* (*L. reuteri*) or a high concentration of probiotics in addition to standard triple therapies for *H. pylori* eradication.

The study enrolled 65 consecutive dyspeptic patients with *H. pylori* infection. All patients underwent upper endoscopy with gastric biopsies. Patients were assigned to receive one of the following therapies: (a) standard 7-day triple; (b) the same 7-day triple therapy plus *L. reuteri* supplementation; (c) the same 7-day triple therapy plus a probiotic mixture; and d) a 14-day standard triple therapy plus a probiotic mixture. *H. pylori* eradication was checked by using a <sup>13</sup>C-urea breath test performed 4-6 weeks after treatment.

No therapy regimen achieved > 80% eradication rate at both intention-to-treat (ITT) and per protocol (PP) analyses. Although the 14-day therapy plus a probiotic mixture tended to achieve higher eradication rate (71%), no statistically significant difference emerged among the different therapy regimens tested (range: 53-71%). The lowest incidence of side-effects was observed following the 7-day therapy plus *L. reuteri* (6%) and highest with the 14-day triple therapy plus probiotic mixture (33%), although the difference failed to reach the statistical significance.

In conclusion, our data found that 7-14 days triple therapy with or without probiotic supplementation failed to achieved acceptable *H. pylori* eradication rates.

*Key Words:*

*Helicobacter pylori*, Therapy, Probiotics, Triple therapy, Eradication.

## Introduction

*Helicobacter pylori* (*H. pylori*) infection is a widespread disease causing a significant morbidity and mortality, thus requiring an appropriate therapeutic approach<sup>1-4</sup>. However, the cure rate following standard triple therapies suggested in the previous and updated European guidelines is decreasing worldwide<sup>5-7</sup>. Therefore, additional therapies – generally more complex and less effective – are required in more than 20-30% of patients, with a relevant economic impact<sup>8</sup>. Since the best first-line treatment is still regarded as the best 'rescue' therapy<sup>8</sup>, further approaches aimed to improve standard triple therapy efficacy should be attempted.

The possible role of probiotics in the prophylaxis and treatment of *H. pylori* infection is of increasing interest in the literature. Some *in vitro* studies showed that probiotics are able to inhibit *H. pylori* by producing different compounds, such as short-chain fatty acids (formic, acetic, propionic, butyric, lactic) and bacteriocins, including nisin A, pediocin PO<sub>2</sub>, leucocin K, and reuterin (3-hydroxy propionaldehyde)<sup>9,10</sup>. In the last few years, different probiotics have been tested for *H. pylori* eradication. Interestingly, monotherapy with *Lactobacillus* strains showed a reduction in the *H. pylori* bacterial load in some studies<sup>11-14</sup>. Probiotics have been also administered in addition to standard antibiotic therapies, with conflicting results<sup>9,10</sup>. However, it should be hypothesized that different probiotic types and concentrations could exert a different role. Moreover, some data indicate that probiotic administration improves antibiotic therapy tolerability by reducing its side effects, in particular diarrhoea<sup>15,16</sup>.

Some studies found that *Lactobacillus reuteri* (*L. reuteri*) is able to prevent *H. pylori* adhesion to cell receptor molecules *in vitro*<sup>17</sup>. Moreover, it was observed that monotherapy with *L. reuteri* significantly reduced *H. pylori* bacterial load<sup>18</sup>, and that administration of a 30-day dual therapy with *L. reuteri* and omeprazole achieved *H. pylori* eradication in 60% of 15 patients<sup>19</sup>. Furthermore, the addition of *L. reuteri* to antibiotic therapy significantly reduced side effects in *H. pylori* infected children<sup>20</sup>. Another recent study found that a preparation containing a highly concentrated mixture of different probiotics significantly increased *H. pylori* cure rate when administered in addition to standard 7-day triple therapy and lactoferrin<sup>16</sup>.

The present pilot study aimed to evaluate the therapeutic role of either *L. reuteri* or a mixture of probiotics in addition to standard triple therapies for *H. pylori* eradication.

## Patients and Methods

### Study Design

This was a prospective, open-label, two-center, pilot study. Since it has been suggested that 30-40 patients are sufficient to identify a potentially effective regimen for *H. pylori* eradication in pilot studies<sup>21</sup>, we planned to enroll 30 patients in each therapeutic arm. However, based on the study design (pilot and open), we decided to perform an 'ad interim' analysis when at least 15 patients were enrolled in each therapy groups, 'a priori' establishing to continue the enrollment only whether the eradication rate was > 80% at intention-to-treat (ITT) analysis.

### Patients

The study population consisted of consecutive dyspeptic patients aged > 18 years who were referred by primary care physicians for diagnostic upper endoscopy. Patients were enrolled if *H. pylori* infection was detected. Patients previously treated for *H. pylori* infection were not enrolled in the present study. Moreover, patients were excluded if they were taking proton pump inhibitors, H<sub>2</sub>-receptor antagonists, or antibiotics in the four weeks preceding the study. Those patients with known allergy to antibiotic used in the present study were not enrolled. All patients underwent endoscopy with biopsies for histology (2 samples from the antrum and 2 samples from the corpus). Patients were considered *H. pylori* posi-

tive when bacteria were detected at histology (Giemsa staining), jointly with an active chronic gastritis (haematoxylin/eosin). For the purpose of the study only patients without macroscopic lesions at endoscopic examination were enrolled. All participants gave written informed consent.

### Treatments and Follow-up

Consecutive patients were assigned to receive one of the following regimens: a) standard 7-day triple therapy including lansoprazole 30 mg b.i.d., clarithromycin 500 mg b.i.d., and amoxicillin 1 g b.i.d.; b) the same triple therapy plus Reuflor<sup>®</sup> containing *L. reuteri* (ATCC 55730; 10<sup>8</sup> CFU), 1 tablet b.i.d.; c) the same triple therapy plus Probinul<sup>®</sup> containing a probiotic mixture with *Lactobacillus plantarum* (5 × 10<sup>9</sup>), *L. reuteri* (2 × 10<sup>9</sup>), *Lactobacillus casei* subsp. *rhamnosus* (2 × 10<sup>9</sup>), *Bifidobacterium infantis* (2 × 10<sup>9</sup>), *Bifidobacterium longum* (2 × 10<sup>9</sup>), *Lactobacillus salivarius* (1 × 10<sup>9</sup>), *Lactobacillus acidophilus* (1 × 10<sup>9</sup>), *Streptococcus thermophilus* (5 × 10<sup>9</sup>), and *Lactobacillus sporogenes* (*Lactobacillaceae*) (1 × 10<sup>9</sup>); 5 g/dose b.i.d.; d) a 14-day standard triple therapy plus Probinul<sup>®</sup> 5 g/dose b.i.d..

For each therapy regimen, the proton pump inhibitor and probiotics were prescribed ½ hour before breakfast and dinner, whereas all antibiotics were immediately given after such meals. Patients were asked to return at the end of the treatment to assess the compliance with therapy, and to determine possible side effects. Compliance was defined as consumption of > 90% of the prescribed drugs. Side effects were evaluated using a structured questionnaire by personal interview. Bacterial eradication was checked in all patients 4-6 weeks after treatment by using a <sup>13</sup>C-urea breath test (UBT). Citric acid (1.5 g) as test meal and 75 mg of <sup>13</sup>C-urea as water solution was given to the patients after collection of a baseline sample, obtained by blowing through a disposable plastic straw into a 20 ml container, and a further breath sample was collected 30 min later. The breath samples were considered positive if there was a greater than 5 per 1000 of <sup>13</sup>CO<sub>2</sub> difference over baseline, according to the manufacturer's recommendations.

### Statistical Analysis

The differences between the proportions eradicated for different treatments were calculated at both intention-to-treat (ITT) and per protocol (PP) analyses. For all other variables, the Fisher's exact test and t-test were used as appropriate, and *P* values less than 0.05 were considered significant.

**Table I.** Eradication rates at intention-to-treat (ITT) and per protocol (PP) analyses.

Therapy	Patients (N)	Mean age (years)	M/F	ITT eradication	PP eradication
7-day standard triple therapy	16	48	6/10	10/16 (62%)	10/15 (67%)
7-day standard triple therapy plus <i>L. reuteri</i>	17	51	8/9	9/17 (53%)	9/17 (53%)
7-day triple therapy plus probiotic mixture	15	50	7/8	8/15 (53%)	8/15 (53%)
14-day triple therapy plus probiotic mixture	17	52	7/10	12/17 (71%)	12/15 (80%)

No statistically significant difference emerged among sub-groups for each comparison.

## Results

### *H. pylori* Eradication Rates

Overall, 65 consecutive dyspeptic patients (mean age: 51 years, range 19-71; M/F: 28/37) were enrolled in the study. All but 3 patients completed the study. In detail, 1 patient in the standard 7-day triple therapy and 2 patients in the 14-day triple therapy plus probiotic mixture were lost to the follow-up. Therefore, the final PP population consisted of 62 patients. *H. pylori* eradication rate at ITT and PP analyses following each treatment regimen are provided in Table I. As shown, although the 14-day therapy tended to achieve higher eradication rate, no statistically significant difference emerged among different therapy regimens, both at ITT and PP analyses. At the scheduled “*ad interim*” analysis, no therapy regimen achieved > 80% eradication rate at ITT analysis, and consequently the enrolment of patients was censored for each sub-group. Indeed, we calculated that increasing enrolment to the planned 30 cases, the cure rate still remained lower than 80% in 3 of 4 therapeutic arms, even assuming that all the added patients would have achieved *H. pylori* eradication.

### Compliance and Side-effects

The reported compliance to the therapy was excellent in all but 1 patient in the 7-day standard therapy and 1 patient in 14-day triple therapy plus probiotic mixture who early stopped the treatment due to side effects. As shown in Table II, the lowest incidence of side-effects was observed following the 7-day therapy plus *L. reuteri* and the highest with the 14-day triple therapy plus probiotic mixture, although the difference failed to reach the statistical significance. All side effects were self-limiting after therapy ending. The overall number of drop-out patients did not differ among the therapy groups, being 1 in the 7-day standard therapy, 2 in the 14-day regimen and none in the other two remaining regimens.

## Discussion

In the last years, probiotic has received a considerable attention in the literature<sup>22,23</sup>. In different disorders, such as irritable bowel disease<sup>24</sup>, diverticular disease<sup>25</sup>, inflammatory

**Table II.** Side-effects complained with the study therapy regimens.

Therapy	Patients with side-effects	Side effects
7-day standard	4 (26.7%)	Mild diarrhoea = 2 Abdominal pain = 1 Metallic taste = 1
7-day plus <i>L. reuteri</i>	1 (5.9%)*	Abdominal pain = 1
7-day plus probiotic mixture	3 (20%)	Mild diarrhoea = 2 Metallic taste = 1
14-day plus probiotic mixture	5 (33.3%)*	Mild diarrhoea = 3 Metallic taste = 2

\* $P = 0.056$

bowel diseases<sup>26</sup>, non steroid anti-inflammatory drugs (NSAIDs) damage protection<sup>27</sup>, lactose malabsorption<sup>28</sup>, urogenital infections<sup>29</sup>, and atopic dermatitis<sup>30</sup> probiotic supplementation has been attempted with some promising results.

Several *in vitro* studies, *in vivo* models and humans have assessed the possible role of different probiotics in *H. pylori* infection therapy. Indeed, many live probiotics, such as *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus johnsonii*, *Lactobacillus salivarius*, *Lactobacillus reuteri*, *Lactobacillus gasseri*, and *Saccharomyces boulardii*, or their supernatant filtrates have been tested with a certain benefit against *H. pylori* infection<sup>31</sup>. Although mechanisms by which probiotics interfere with *H. pylori* are largely unclear, a competitive action, an immunomodulator effect, and production of both lactate and bacteriocin-like substances have been advocated to play a role<sup>9,10</sup>. Some clinical trials have tested the effect of administration of probiotics alone, showing a reduction in the *H. pylori* bacterial load<sup>11-13</sup>. Interestingly, in a double-blind, placebo-controlled study<sup>19</sup>, a 4-week, antibiotic-free therapy with omeprazole and *L. reuteri* was able to cure *H. pylori* in 60% of patients. In the present study, the same *L. reuteri* dose supplemented to standard triple therapy failed to increase *H. pylori* eradication, the cure rate being even tendentially lower than that of standard regimen. A similar result was observed in a recent study in which *L. reuteri* supplementation did not significantly increase efficacy of sequential therapy in children<sup>20</sup>.

A recent study found that when a probiotic mixture and lactoferrin were supplemented to standard triple therapy – i.e. a quintuple therapy – a significantly higher cure rate was achieved as compared to the same triple therapy without supplementation (88.6% versus 72.5%)<sup>16</sup>. Since the same triple therapy with only lactoferrin – i.e. a quadruple therapy – failed to improve the eradication rate as compared to standard triple therapy<sup>32,33</sup>, it was suggested that therapeutic gain observed following the quintuple therapy could mainly depend on the probiotic mixture used<sup>34</sup>. Unfortunately, the present study failed to confirm that a quadruple therapy with the same probiotic mixture was able to increase *H. pylori* eradication rate. Our observations add further information on this field. Indeed, a previous systematic review

found that when probiotics were used as an adjunct to antibiotic therapy, the eradication rate was significantly increased in 2 studies, whilst it was unchanged in 4 other trials<sup>13</sup>.

Some data indicate that probiotic administration improves antibiotic therapy tolerability by reducing its side effects, in particular diarrhoea. We observed that triple therapy with *L. reuteri* supplementation tended to reduce the overall incidence of side effects, and such a result is in agreement with data of a previous study in children<sup>20</sup>. However, this observation deserves further confirmations in other studies, and cost considerations should be also taken into account before to suggest its administration in all patients. Probably, *L. reuteri* supplementation could have a role in few, selected patients who experienced diarrhoea during a previous therapeutic attempt. On the contrary, the probiotic mixture seemed to be ineffective in reducing the incidence of antibiotic-related side effects in our study. Therefore, our data failed to confirm data of a previous study in which the same probiotic combination was used<sup>16</sup>.

In the updated Italian guidelines on the *H. pylori* management<sup>35</sup>, it has been advised that a standard 7-14 triple therapy should be used as first line therapy regimen, the longer regimen being indicated in those geographic areas where high primary clarithromycin prevalence is present. However, two recent, large trials failed to demonstrate a significant therapeutic gain of 14-day triple therapy as compared to the 7-day regimen<sup>36,37</sup>. In detail, a multicenter study involving 906 duodenal ulcer patients, *H. pylori* infection was cured in 79.7% and 81.7% of patients receiving a 7-day or a 14-day triple therapy, respectively<sup>35</sup>. In the present study, we assessed whether a probiotic mixture supplementation was able to improve the cure rate of a 2-week triple therapy regimen. Although the eradication rate achieved following the prolonged regimen tended to be higher than standard therapy, the cure rate still remained < 80% at ITT analysis. Moreover, the therapeutic cost of this regimen is obviously double as compared with the 7-day therapy, further questioning on its actual role in the clinical practice.

In conclusion, the role of probiotics supplementation in *H. pylori* therapy is of current interest. Our data failed to demonstrate a significant role of both *L. reuteri* and a probiotics mixture in improving *H. pylori* therapy success.

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