**Lactobacillus reuteri** therapy to reduce side-effects during anti-*Helicobacter pylori* treatment in children: a randomized placebo controlled trial

E. LIONETTI*, V. L. MINIELLO*, S. P. CASTELLANETA†, A. M. MAGISTÁ*, A. DE CANIO*, G. MAUROGIOVANNI†, E. IERARDI§, L. CAVALLO** & R. FRANCAVILLA*

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

Correspondence to:
Dr R. Francavilla, Clinica Pediatrica ‘B. Trambusti’, Piazza Giulio Cesare, 11 – Policlinico, Bari, Italy.
E-mail: rfrancavilla@libero.it

**SUMMARY**

**Background**

*Helicobacter pylori* eradication fails in about 25–30% of children, particularly because of the occurrence of resistance to antibiotics and side-effects.

**Aim**

To determine whether adding the *Lactobacillus reuteri* to an anti-*H. pylori* regimen could help to prevent or minimize the gastrointestinal side-effects burden in children.

**Methods**

Forty *H. pylori*-positive children (21 males; median age: 12.3 years) were consecutively treated with 10-day sequential therapy [omeprazole + amoxycillin for 5 days, and omeprazole + clarithromycin + tinidazole for other 5 days] and blindly randomized to receive either *L. reuteri* ATCC 55730 (10⁸ CFU) or placebo. All children completed the Gastrointestinal Symptom Rating Scale (GSRS) at entry, during and after treatment. *H. pylori* status was assessed after 8 weeks by ¹³C-urea breath test.

**Results**

Overall, in all probiotic supplemented children when compared with those receiving placebo there was a significant reduction of GSRS score during eradication therapy (4.1 ± 2 vs. 6.2 ± 3; \( P < 0.01 \)) and at the end of follow-up (3.2 ± 2 vs. 5.8 ± 3.4; \( P < 0.009 \)). Overall, children receiving *L. reuteri* report less symptoms than those receiving placebo.

**Conclusion**

*L. reuteri* is capable of reducing frequency and intensity of antibiotic-associated side-effects during eradication therapy for *H. pylori*.

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INTRODUCTION

According to the North American Society for Paediatric Gastroenterology, Hepatology and Nutrition guidelines, one-week triple therapy represents the current most widely used first-line regimen for Helicobacter pylori infection, but the eradication failure rate is more than 30%. Today, bacterial resistance and side-effect occurrence represent the second most frequent cause for anti-\(H. \text{ pylori}\) treatment failure in clinical practise. Indeed, the high prevalence of antibiotic side-effects might induce even motivated dyspeptic patients to discontinue therapy, with a consequent treatment failure as well as a possible development of antibiotic-resistant strains. These manifestations have been related to the quantitative and qualitative changes in the intestinal microflora because of unabsorbed or secreted antibiotics in the intestinal content which results in a reduction of normal saprophytic flora, bacterial overgrowth and the persistence of potentially pathogenic antibiotic resistant indigenous strains.

A strategy targeted to improve the treatment tolerability might increase compliance and eventually raise eradication rate. The use of probiotics has recently been proposed in adults to increase patient tolerability by limiting side-effects of eradicating therapies; nonetheless, the report from the Maastricht 2000 consensus conference on \(H. \text{ pylori}\) include probiotics as possible useful ‘side tools’ for management of the infection.

\textit{Lactobacillus reuteri} is a heterofermentative bacterium that resides in the gastrointestinal tract of humans and animals and is considered to be one of the few true autochthonous \textit{Lactobacillus} species in humans. \textit{L. reuteri} has been shown to exert a beneficial effect in the prevention and treatment of several intestinal conditions.

The demonstration that \textit{L. reuteri} colonizes the stomach and duodenum combined with recent data that \textit{L. reuteri} ATCC 55730 is a potent inhibitor of \(H. \text{ pylori}\) growth prompted our study in children.

We investigated, for the first time in a paediatric population, in a double-blind, randomized, placebo-controlled trial the effect of \textit{L. reuteri} on antibiotic-associated side-effects during and after \(H. \text{ pylori}\) eradication therapy.

PATIENTS AND METHODS

Patients

The study enrolled only patients at the first diagnosis of \textit{H. pylori} infection. A total of 42 consecutive symptomatic children [23 males (54.8%), median age 12.3 years (range: 3.3–18 years)] were candidates for inclusion in the study at the Department of Paediatric Gastroenterology of the University of Bari (Italy) between November 2004 and December 2005. Exclusion criteria were one of the following: (i) consumption of PPI, \(H_2\) receptor antagonist, bismuth compounds, antibiotics, probiotics, in the previous 4 weeks, (ii) previous gastric surgery, (iii) known allergy to antibiotics and (iv) acute or chronic gastrointestinal disease.

\textit{Helicobacter pylori} assessment

At baseline, patients underwent endoscopy with biopsies for histology (two samples from the antrum and two samples from the corpus), and a rapid urease test (one sample from the antrum) (CP test; Yamanouchi Pharma S.p.A., Carugate, Italy). Endoscopy was performed by the same physician (R.F.) (endoscope model GIF XP20; Olympus, Tokyo, Japan) after sedation with intravenous midazolam. Histological examinations were carried out by the same observer using haematoxylin–eosin staining for assessment of gastritis and Gram stain for detection of \textit{H. pylori}. Within 24 h of the endoscopy, patients completed a standard \(^{13}\text{C}\)-urea breath test (\(^{13}\text{C}\)-UBT) after overnight fasting. A fatty meal (100 mL) and a solution of \(^{13}\text{C}\) urea [75 mg \(^{13}\text{C}\) urea (AB Analitica srl Padova, Italy)] were fed to each patient. Breath samples were collected before and 30 min after the dose of urea. In children <6 years of age, breath samples were collected by using a face-mask with a bag (made in house). The ratio of \(^{13}\text{C}\) to \(^{12}\text{C}\) in the expired air samples was measured using a dual-inlet-ratio mass spectrometer (Automated Breath \(^{13}\text{Carbon Analyzer};\) Europa Scientific, Ltd, Crawley, West Sussex, UK). Results were expressed as parts per million of excess of \(\Delta^{13}\text{CO}_2\) (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.\(^{15}\)

\(^{13}\text{C}\)-UBT, as described, had been validated in children.
of our geographic area by our group. In case of antibiotic or antiacid medications, ¹³C-UBT was deferred to at least 4 weeks. At entry, patients were considered H. pylori positive if two of three tests (histology, rapid urease test, ¹³C-UBT) were positive.

**Symptom assessment**

According to current guidelines,¹ only symptomatic children deserve anti-H. pylori treatment; therefore, in infancy studying the emergence of anti-H. pylori-related side-effect as performed in adults, is unfeasible and not ethic.

All children (or family member) attended an interview to recall history of GI symptoms and the following data were collected: (i) a detailed physical examination, (ii) the 15-item Gastrointestinal Symptom Rating Scale (GSRS) to assess severity and frequency of symptoms¹⁷ and (iii) 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 symptoms were scored by the child (or family member) on a four-point scale: mild (non-interfering with daily activities), moderate (slightly interfering with daily activities), severe (interfering with daily activities), very severe (continuous and if on therapy, producing treatment interruption). Stool consistency was graded from hard (0) to watery (4). A similar scale has been used in paediatric populations with a 0.84 inter-rater reliability in children.¹⁸ Data were collected before (1 week before intervention), during (5th and 10th day) and after completion of eradicating therapy (15th and 20th day) and patients were invited to return their diaries immediately after the intervention period.

**Therapy regimens**

In the current study we tested the hypothesis that L. reuteri would reduce the rate of side-effects secondary to the use of antibiotics during H. pylori eradication therapy and increase compliance to therapy.

All children received a 10-day sequential therapy comprising of omeprazole (1 mg/kg/die) plus amoxycillin (50 mg/kg/die) for 5 days followed by omeprazole (1 mg/kg/die) plus clarithromycin (15 mg/kg/die) and tinidazole (20 mg/kg/die) for the next 5 days,¹⁹ omeprazole was prescribed before breakfast and dinner, the antibiotics were administered after meals. Patients were randomly assigned to receive either L. reuteri or placebo both provided by Nöös (BioGaia, Sweden) in pills form and included either L. reuteri (each pill containing 10⁸ CFU of L. reuteri ATCC 55730 (SD2112), Reuterin, Nöös) 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 (cryoprotectants). Boxes containing placebo had the same shape, dimension, trade mark, indication and contained the same amount of sachets of boxes containing the viable L. reuteri and were provided by the probiotic producer. Both placebo and probiotics were prescribed one pill once daily (2 h after lunch) for a period of 20 days.

An independent physician prescribed either probiotic or placebo according to a computer generated randomization list, blindly to researchers. The code was revealed to the researchers once recruitment, data collection and laboratory analyses and statistical analyses were completed.

Patients were thoroughly instructed and motivated for the therapy. Adherence to treatment was evaluated by counting the pills returned by the subject; a minimum pill intake of 95% was considered as acceptable. Eight weeks after completion of therapy H. pylori eradication was assessed by using a ¹³C-UBT. An informed consent was obtained by all parents (or guardian). 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 starting from the assumption of having at least one antibiotic-associated side-effects in at least 80% of the treated children aiming to detect a difference in amelioration of symptom rate of 35%. Based on a 0.80 power to detect significant difference (P = 0.05, 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 test. The median regression analysis was used to estimate difference between adjusted medians with the baseline value as covariate. A probability (P)-value <0.05 was considered significant. The statistical analysis was performed using the Software Program Stata System (spss) v13.0 (Chicago, IL, USA) program.

RESULTS
Forty children [21 males (52.5%), median age 12.3 years (range: 3.3–18 years)] were recruited while two were excluded (Figure 1). The baseline demographic and clinical characteristics of the enrolled patients are reported in Table 1. As shown, patients in the two groups did not differ for age, sex, clinical and duration of symptoms and baseline GSRS score, as well as for the endoscopic features detected (Table 2). At 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 had gastric atrophy or intestinal metaplasia (Table 2). In all children, all the three tests resulted positive.

Trial flow
Forty patients completed the study. No children underwent protocol deviation. Compliance to the therapy was good (>95%) in all enrolled cases, and no patient discontinued therapy because of side-effects. There were no differences in adherence to treatment schedules (97% in both groups).

_Helicobacter pylori_ eradication
No significant differences were observed between the groups in the success of _H. pylori_ eradication. Treatment was successful in 17 of 20 [85% (95%CI: 68–100)] patients in probiotic supplemented when compared with 16 of 20 patients in placebo group [80% (95%CI: 61–99)] (P = NS).

Symptom assessment
Overall, in all probiotic supplemented children when compared with those receiving placebo there was a significant reduction of GSRS score during eradication therapy [4.1 ± 2 (95%CI: 2.9–5.9) vs. 6.2 ± 3 (95%CI: 5.2–8.3 P < 0.01] which became markedly evident at the end of follow-up [3.2 ± 2 (95%CI: 2.4–4) vs. 5.8 ± 3.4 (95%CI: 4.8–6.9); P < 0.009] (Figure 2). In detail, when the frequencies of the symptoms were evaluated by taking into account only the occurrence of new or aggravated symptoms during and after the eradication week relative to the baseline, we found that children receiving _L. reuteri_ refer less frequently epigastric pain during eradicating treatment (15% vs. 45%; difference: –30%; P < 0.04) and abdominal distension (0% vs. 25%; difference: –25%; P < 0.02),

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42 eligible participants

Two excluded
Reasons:
- One consumption of antibiotics in the previous 4 weeks
- One known allergy to antibiotics

40 randomized

20 allocated to _L. reuteri_

Lost to follow-up n: 0

20 analysed

20 allocated to placebo

Lost to follow-up n: 0

20 analysed

Figure 1. Flow diagram of participants through each stage.
eructation (5% vs. 35%; difference: −30%; P < 0.04), disorders of defecation (15% vs. 45%; difference: −30%; P < 0.04) and halitosis (5% vs. 35%; difference: −30%; P < 0.04) thereafter (Table 3). No adverse events were reported.

DISCUSSION

In this randomized, placebo-controlled study, children on _H. pylori_ eradication therapy receiving _L. reuteri_ when compared with peers receiving placebo, reported a significant reduction of the total symptom score, which takes into account both the frequency and the severity of the symptoms. Symptoms which were most positively influenced by _L. reuteri_ were abdominal distension, disorders of defecation, epigastric pain, eructation and halitosis.

The onset of side-effects during anti- _H. pylori_ therapy is mainly due to the use of antibiotics in moderate to high-dose or in combination. Antibiotic-related side-effects are common and usually affect the gastrointestinal system. The intestinal microbiota is characterized by a high bacterial concentration, up to $10^{14}$ CFU/mL in the colon; these bacteria and colonic mucosal cells coexist in a delicate equilibrium that can be easily altered by antibacterial drugs causing the emergence of potentially pathogen species over the saprophytic flora. Unfortunately, the most frequently used antibiotics for _H. pylori_ eradication (amoxycillin, clarithromycin, metronidazole) are frequently accompanied by gastrointestinal side-effects and clarithromycin in particular, determines an increased contractility of gastrointestinal smooth muscle, leading to accelerated transit with diarrhoea.

Probiotics are ‘living micro-organisms which upon ingestion in certain numbers favourably influence the health of the host by improving the indigenous microbiota’. Many studies have documented the effectiveness of prophylactic probiotics taken with antibiotics in preventing or lowering the antibiotic-related gastrointestinal side-effect burden. It is up to the experts in microbiology, nutritional sciences

| Table 1. Baseline demographic and clinical characteristics of trial groups (n = 20) |
|---------------------------------|-----------------------|-------------------|
|                                | _Lactobacillus reuteri_ | Placebo           |
| Median age (range) years        | 11.0 (3.3–18)          | 9.9 (4.3–17.6)    |
| Sex (M/F)                       | 12/8                  | 9/11              |
| Duration of symptoms, months (range) | 18 (8.5–36)        | 20 (9.3–33)       |
| Ulcer-like dyspepsia (%)        | 9 (45)                | 8 (40)            |
| Dysmotility like dyspepsia (%)  | 11 (55)               | 12 (60)           |
| GSRS score at entry             | 6.9 ± 3.5             | 7.1 ± 3.2         |
|                                 | (95%CI: 4.9–9)        | (95%CI: 5.3–9.2)  |

| Table 2. Endoscopic and histological findings in the two treatment groups (n = 20) |
|---------------------------------|---------------------|----------------|
|                                | _Lactobacillus reuteri_ [n(%)] | Placebo [n(%)] | P    |
| Endoscopic findings            |                     |                 |
| Macroscopic nodular antral gastritis with hyperaemia | 13 (65)         | 14 (70)        | 1   |
| Antral hyperaemia without macroscopic nodularity | 1 (5)            | 3 (15)         | 0.6 |
| Pangastritis                    | 13 (65)             | 14 (70)        | 1   |
| Gastric ulcer                   | Nil                 | nil             | –   |
| Duodenal ulcer                  | Nil                 | nil             | –   |
| Erosive bulbitis                | 5 (25)              | 4 (20)         | 1   |
| Oesophagitis                    | 1 (5)               | nil             | 1   |
| Histological findings           |                     |                 |
| Pangastritis                    | 13 (65)             | 14 (70)        | 1   |
| Antral gastritis mild           | 10 (50)             | 10 (50)        | 0.7 |
| Antral gastritis moderate       | 6 (30)              | 8 (40)         | 0.7 |
| Antral gastritis severe         | 5 (25)              | 5 (25)         | 0.7 |

Figure 2. Gastrointestinal Symptom Rating Scale (GSRS) in children receiving the _Lactobacillus reuteri_ or placebo before, during and after _Helicobacter pylori_ eradication therapy. Comparison of continuous variables was performed using Mann–Whitney.

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and food technology to formulate unequivocal requirements for probiotic bacterial strains: bile and acid resistance, adhesion to the mucosa and/or to enterocytes, at least temporary colonization of the human gut, ability to inhibit known gut pathogens and antimicrobial substances, stability and activity during manufacture and storage, safety in human use and proven health-promoting effects. 25

*L. reuteri* shares most of these general characteristics. 12, 26–29

Our choice to use *L. reuteri* ATCC 55730 derives from different observations. *L. reuteri* has been extensively studied and is widely used as a food additive to improve human gastrointestinal health.26 Oral administration delivers *L. reuteri* ATCC 55730 to the gastrointestinal tract, leading to the shedding of live bacteria in the faeces. 27, 28 Clinical trials have shown that after its administration to healthy volunteers, it was possible to detect this strain adhering to epithelial cells from corpus and antral gastric biopsies providing the first clear and direct evidence of colonization of the human stomach by *L. reuteri*.14

Our experience in children on eradication therapy showed that *L. reuteri* is effective in reducing the total symptom score and the onset of bloating, disorders of defeation and halitosis similarly to adult data after the administration of a single strain of *Lactobacillus GG* (LGG)4 or *Bacillus clausii*6 or *Saccharomyces boulardii* or a mixture of *Lactobacillus acidophilus* and *Bifidobacterium lactis*.7 However, what we have found more difficult to explain is the effect of *L. reuteri* on symptoms such as eructation and epigastric pain which are more likely related to *H. pylori* infection. This is not a novel finding since other authors have described a significant reduction of epigastric pain in the probiotic treated arm during *H. pylori* eradication7, 33 although no explanation was advocated. It is of interest a recent evidence that some *L. reuteri* strains were able to compete with *H. pylori* for the binding to its receptor on gastric epithelial cells (asialo-GM1 and sulfatide) suggesting that a binding inhibitor(s) is associated with the bacterial cell

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Before treatment</th>
<th>During therapy</th>
<th>Follow-up</th>
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<tbody>
<tr>
<td></td>
<td><em>Lactobacillus reuteri</em> [n (%)]</td>
<td>Placebo [n (%)]</td>
<td><em>Lactobacillus reuteri</em> [n (%)]</td>
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<tr>
<td>Epigastric pain</td>
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<tr>
<td>Abdominal pain</td>
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<td>8 (40)</td>
<td>0.7</td>
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<td>4 (20)</td>
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<tr>
<td>Heartburn</td>
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<tr>
<td>Sucking sensation in epigastrum</td>
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<td>8 (40)</td>
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<td>Nausea and/or vomiting</td>
<td>3 (15)</td>
<td>5 (25)</td>
<td>0.7</td>
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<tr>
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<td>1</td>
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<td>Abdominal distension</td>
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<td>1</td>
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<tr>
<td>Ercution</td>
<td>2 (10)</td>
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<tr>
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<td>0.7</td>
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<td>1 (5)</td>
<td>1</td>
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<tr>
<td>Urticaria*</td>
<td>2 (10)</td>
<td>1 (5)</td>
<td>0.6</td>
</tr>
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* Assessed before (history of urticaria acute or chronic), during (acute urticaria) and after therapy (in the following 20 days).
surface. Therefore, it is possible that the concomitant use of a probiotic along with eradication therapy may decrease more rapidly the bacterial load thus ameliorating symptoms such as epigastric pain. Indeed, we have demonstrated that L. reuteri ATCC 55730 is capable of decreasing the bacterial load when administered to untreated patients (R.F., personal unpublished data) as shown for other probiotic strains. Moreover, it is documented that probiotics have an anti-inflammatory effect that might contribute to reduce gastric inflammation when given to H. pylori colonized animals. Despite the suitability of this hypothesis, caution should be used in attributing symptomatic benefits to probiotic oral therapy until the mechanistic bases of action of probiotics are fully understood.

A possible confounding factor in our study may be that all enrolled children were symptomatic, reason for which these children were tested and treated since in childhood there is no indication for either in the absence of symptoms. However, taking into account only the occurrence of new or aggravated symptoms during and after the eradication therapy when compared with baseline we have demonstrated the superiority of L. reuteri when compared with placebo at least for some symptoms.

Dealing with children, we always have a double aim when we decide to eradicate the H. pylori, being the elimination of the bacterium and the resolution of symptoms. Our data demonstrate that symptoms relief, although in the short term, is better achieved by the concomitant administration of L. reuteri together with antibiotics; in our experience, the use of L. reuteri does not improve the eradication rate, however, the small sample size of our series does not allow to draw firm conclusion, although in line with previous studies in adults. Finally, similar to previous experiences, the overall compliance of patients was not increased by L. reuteri, however, the treatment tolerability had significantly improved.

In Italy a box of L. reuteri costs 11.00 euros for 10 pills that makes 1.1 euro for each treatment day, therefore, its use would increase the overall costs of approximately 20 euros. Moreover, we have to consider that often, after an antibiotic treatment, symptomatic children receive a probiotic that it is usually what the family have at home and rarely one with proved efficacy for a particular indication.

In conclusion, our study shows, for the first time in the paediatric population, that probiotic supplementation, during and after a sequential treatment for H. pylori eradication, may positively affect therapy-related symptoms and overall treatment tolerance.

ACKNOWLEDGEMENT

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