Aliment Pharmacol Ther 2004; 20: 1181–1188. doi: 10.1111/j.1365-2036.2004.02274.x

Bacillus clausii *therapy to reduce side-effects of anti-*Helicobacter pylori *treatment: randomized, double-blind, placebo controlled trial*

E. C. NISTA*, M. CANDELLI*, F. CREMONINI*, I. A. CAZZATO*, M. A. ZOCCO*, F. FRANCESCHI*, G. CAMMAROTA*, G. GASBARRINI* & A GASBARRINI†
*Departments of *Internal Medicine and †Medical Pathology, Catholic University, Rome, Italy*

Accepted for publication 27 September 2004

SUMMARY

Background: *Helicobacter pylori* eradication fails in about 10% of patients, particularly because of the occurrence of resistance to antibiotics and side-effects. During anti-*H. pylori* therapy, probiotics have been successfully used to reduce the incidence of side-effects.

Aim: To evaluate the effect of *Bacillus clausii*, a probiotic, on incidence (primary variable) and severity of antibiotic-associated side-effects during anti-*H. pylori* therapy.

Methods: One hundred and twenty *H. pylori*-positive patients were randomly screened to receive: (i) a standard 7 days triple therapy with rabeprazole 20 mg b.d., clarithromycin 500 mg b.d. and amoxicillin 1 g b.d. and *B. clausii* t.d.s. (each preparation containing 2×10^9 spores) for 14 days starting from the first day of treatment. (ii) The same 7 days triple therapy and placebo t.d.s. for 14 days starting from the first day of treatment. Side-effects were assessed using a validated questionnaire and were recorded for 4 weeks from the start of therapy.

Results: The incidences of nausea, diarrhoea and epigastric pain in patients treated with *B. clausii* were significantly lower than in placebo group, in both PP and ITT analysis. Equally, intensity of nausea and diarrhoea in patients treated with *B. clausii* was significantly lower than in placebo group. There were no differences in adherence to treatment and *H. pylori* eradication rates between groups.

Conclusion: In symptom-free, *H. pylori*-positive subjects *B. clausii* bacteriotherapy reduces the incidence of the most common side-effects related to anti-*H. pylori* antibiotic therapy compared with placebo.

INTRODUCTION

Therapeutic schemes to eradicate *Helicobacter pylori* infection are based on the association of antibiotics and a proton pump inhibitor.[1–8] Treatment outcome depends on the class of antibiotic administered, dosages used, therapy duration, bacterial resistance and patients' compliance. Gastrointestinal side-effects during antibiotic therapy include diarrhoea, nausea, taste distortion, stomatitis and bloating. These side-effects reduce treatment tolerability and may cause treatment discontinuation and failure to eradicate *H. pylori*. Poor compliance to treatment is one of the determinants of failure to eradicate *H. pylori*. Despite the number of dropouts is relatively small in clinical trials, this may not be the case in clinical practice.

Probiotics are defined as microbial cell preparations or components of microbial cells that can beneficially impact human health.[9] The use of probiotics has recently been proposed to increase patients' tolerability by limiting side-effects of anti-*H. pylori* eradicating therapies.[10–12]

The probiotic strains previously used in *H. pylori* eradication trials include *Lactobacillus GG*, *Saccharomyces boulardii*, or combinations of different strains. In these studies, the addition of probiotics improved the

Correspondence to: Prof. A. Gasbarrini, Department of Internal Medicine, Catholic University, Rome, Italy.
E-mail: angiologia@rm.unicatt.it

© 2004 Blackwell Publishing Ltd

tolerability of eradication regimens, with limited effect on treatment compliance.[9–11]

Bacillus clausii is a probiotic widely used in Italy since the 1960s for viral diarrhoea in children and for antibiotic related side-effects.[13] *B. clausii* spores can survive the gastric pH, activate and reach the intestinal tract where they germinate to vegetative forms.[14, 15] Experimental data suggest that both *B. clausii* spores and cells can adhere to the bowel wall and colonize the mucosa.[16]

Our aim was to assess, in a double-blind, randomized, placebo-controlled trial the effect of oral bacteriotherapy with *B. clausii* on gastrointestinal side-effects occurring during anti-*H. pylori* therapy.

METHODS

Patients

The study was a single centre, double-blind, prospective, randomized, placebo controlled study performed at the Gastroenterology and Internal Medicine Day Hospital of Gemelli Hospital of Rome, Italy. One hundred and twenty consecutive *H. pylori*-positive patients free from gastrointestinal symptoms (spouses or relatives of patients with *H. pylori* associated gastrointestinal diseases who asked to be tested and treated for *H. pylori* infection) were enrolled from November 2001 to June 2002.

Patients were considered eligible to enter the study if they were between 18 and 65 years old, free of gastrointestinal symptoms in the previous 3 months and affected by gastric *H. pylori* infection as confirmed by a ^{13}C-urea breath test. Exclusion criteria were: recent (within the previous 3 months) use of anti-microbial agents, bismuth compounds, proton pump inhibitors and H_2 receptor antagonists, laxatives, anti-diarrhoeals, other probiotic preparations, alcohol or illicit drug abuse. Patients with acute or chronic gastrointestinal diseases, or with major concomitant diseases including psychiatric disorders and pregnant or lactating women were also excluded from the study. Patients under chronic drug treatment were considered suitable if they had been receiving such treatments for >3 months. All patients signed a written informed consent.

Treatment

Using a permuted blocks randomization (1:1), 120 patients were assigned to one of the following parallel groups:

- Sixty patients (male/female 33/27, mean age 46.2 ± 12.83) were randomly assigned to receive a triple therapy based on clarithromycin 500 mg b.d., amoxicillin 1 g b.d., rabeprazole 20 mg b.d. for 7 days plus a probiotic preparation, one vial t.d.s. (each vial containing 2×10^9 spores of *B. clausii*, *Enterogermina*, Sanofi–Synthelabo OTC, Milan, Italy) for 14 days, during eradication therapy and 1 week thereafter.
- Sixty patients (male/female 25/35, mean age 43.1 ± 13.36) were randomly assigned to a placebo preparation, one placebo vial t.d.s., during the 7-day eradication therapy and for 1 week thereafter. The placebo preparation, looked identical in colour, size, shape, weight and taste to the probiotic preparation.

Side-effects

Each patient was required to complete a validated daily diary for 4 weeks, starting from the first day of the eradicating treatment.[11] The diary contains a questionnaire (slightly modified from DeBoer *et al.*)[17] evaluating onset, intensity and frequency of gastrointestinal side-effects: taste distortion, loss of appetite, nausea, vomiting, epigastric pain, bloating, diarrhoea, constipation and skin rash. The intensity of symptoms was rated using a scale, in which 0, 1, 2 and 3, respectively corresponded to absent, mild, moderate and severe symptoms. An overall judgment of tolerability was assessed by the patient at the end of both the first and second weeks of treatment. Treatment tolerability was scored with a scale in which 1, 2, 3, 4 and 5 respectively corresponded to 1, no side-effect observed; 2, mild side-effect(s), non-interfering with daily activities; 3, moderate side-effect(s), slightly interfering with daily activities; 4, severe side-effect(s), interfering with daily activities but not leading to treatment interruption and 5, severe side-effect(s), producing treatment interruption. Adherence to treatment was evaluated by counting the vials returned by the subject [patients who returned <80% of empty vials were not included in the 'per protocol population' (PP) analysis].

Eradication of Helicobacter pylori

Helicobacter pylori eradication was evaluated by means of a ^{13}C-urea breath 6 weeks after the end of the treatment. A delta ^{13}C over baseline value higher than 3.5 was considered positive for active *H. pylori* infection.

Statistical analysis

For statistical analysis the following population were considered:

- 'Safety population', defined as all randomized patients who have taken at least one dose of study treatment.
- 'Intention-to-treat population' (ITT), defined as all randomized patients who have taken at least one dose of study treatment and have returned the first diary, reporting at least one symptom assessment during the 7-day eradication therapy. Missing data because of premature discontinuation of treatment were considered treatment failures for the analysis of symptom incidence and were replaced using the mean of the available daily scores of the patient for the analysis of symptoms intensity and frequency.
- 'Per protocol population', defined as all randomized patients who completed the study without protocol violations.

Descriptive analysis of demographic, anamnestic and clinical data by treatment was performed. Dichotomous variables were analysed by χ^2 test and relative risk (RR) and its 95% confidence interval (CI) were calculated. The differences between the two groups in symptoms intensity, summarized in the overall mean over time, were assessed using analysis of variance, after rank transformation of the summary measure, considering treatment and diary mean factors and treatment by time interaction. For the overall judgements of tolerability, the distribution of patients with side-effects in the two treatment groups was evaluated by Kruskal–Wallis test. Tests were two sided and *P*-value <0.05 was considered statistically significant.

Sample size calculation

Based on a previous study,[9] a total sample size of 120 was calculated as adequate to detect, with an 80% power and a two sided 5% significance level, a difference between the two groups ≥20% in occurrence of nausea, diarrhoea and taste distortion.

RESULTS

Baseline characteristics. Participant characteristics are showed in Table 1. There were no significant differences in age and baseline symptom scores (all patients were symptom free at enrolments) between the placebo and the *B. clausii* groups. On the contrary, a higher prevalence of male gender was observed in *B. clausii* group and female gender in placebo group.

Table 1. Demographic data of the patients included in the safety population ($n = 114$)

Demographic data	*Bacillus clausii*	Placebo
Males (*n*, %)	33 (58)	22 (38)
Females (*n*, %)	24 (42)	35 (61)
Age: years (mean ± s.d.)	46 ± 13	43 ± 13

Trial flow. Six patients (three in the *B. clausii* group, three in the placebo group) did not start the assigned treatment. Eight patients (three in the *B. clausii* group, five in the placebo group) did not return the first diary. Further six patients (four in the *B. clausii* group, two in the placebo group) were not included in the per-protocol analysis because they did not return the second diary or because of withdrawal or poor compliance (i.e. <80% of the vials were recovered) (Figure 1).

The *H. pylori* eradication rate was similar between *B. clausii* and placebo groups. In particular, ITT analysis has shown *H. pylori* was eradicated in 39 of 54 patients (72.2%) in the *B. clausii* group and in 37 of 52 patients (71.15%) in the placebo group. In PP population, *H. pylori* was eradicated in 39 of 50 patients (78%) in the *B. clausii* group and in 37 of 50 patients (74%) in the placebo group.

Effect of Bacillus clausii *on the incidence and intensity of side-effects.* Both ITT and PP analysis showed a significant difference between the two treatments in the incidence of nausea, diarrhoea and epigastric pain. In the ITT analysis, RR of occurrence of nausea were halved in patients treated with *B. clausii* compared with placebo after one (RR = 0.5; 95% CI 0.31–0.88) and 2 weeks (RR = 0.45; 95% CI 0.21–0.96) of bacteriotherapy. A greater reduction in the risk of diarrhoea was observed in the *B. clausii* group compared with the placebo group after one (RR = 0.30; 95% CI 0.12–0.76) and 2 weeks (RR = 0.38; 95% CI 0.08–1.9). For epigastric pain, after 1 week, the relative risk was 0.68, 95% CI 0.48–0.97 (Table 2). The incidence of vomiting, constipation and skin rash were also lower in the *B. clausii* group

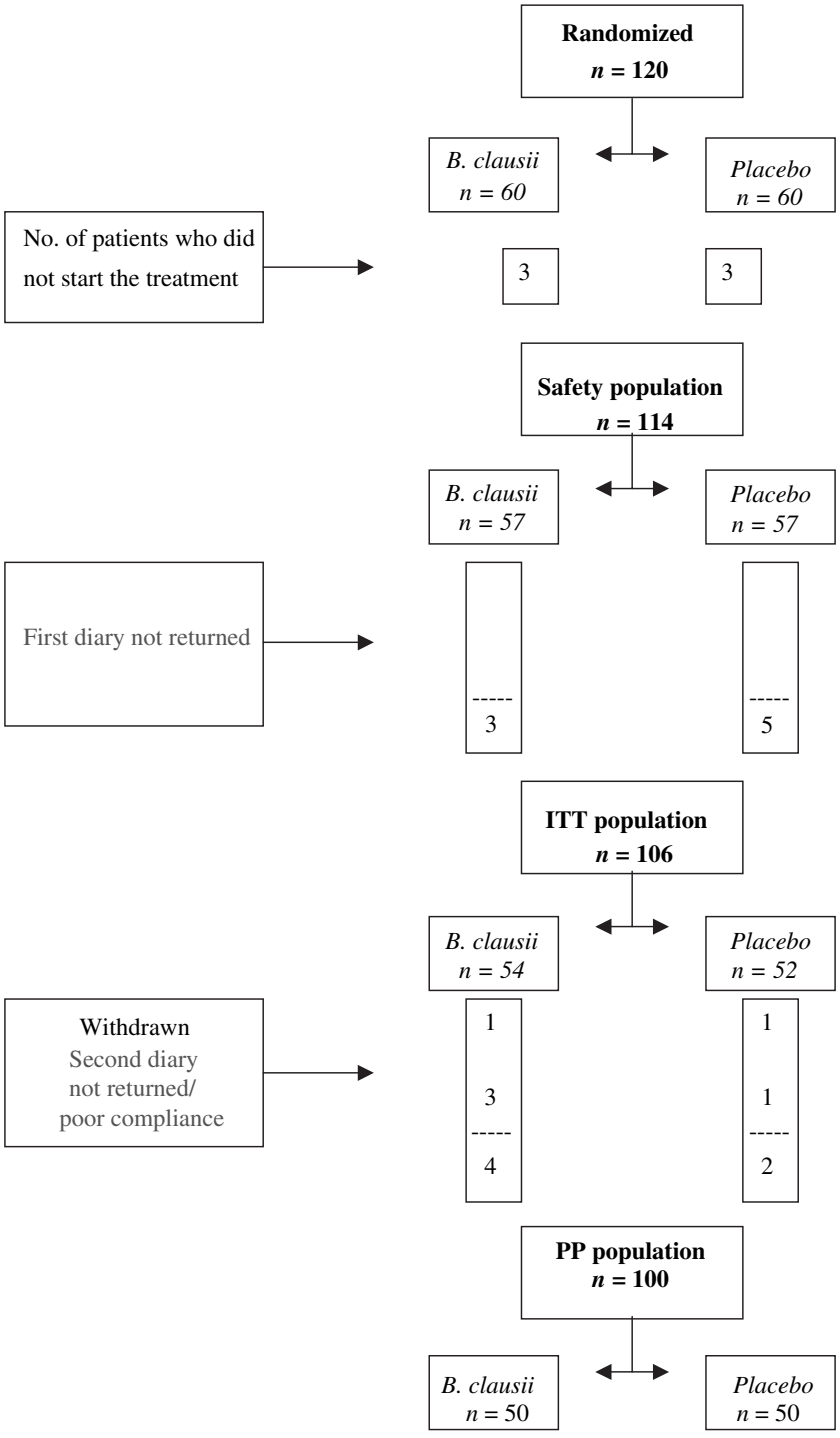

Figure 1. Trial flow.

compared with the placebo group, although the differences were not statistically significant.

The mean intensities and frequencies of nausea and diarrhoea were also significantly lower in the *B. clausii* group (Table 3).

The individual patients' overall assessment of tolerability in ITT population was better in the group treated with *B. clausii* than in the placebo group. The difference was statistically significant after 2 weeks of treatment ($P < 0.05$) (Table 4).

Table 2. Incidence of symptoms during treatment. Data are presented as percentages and relative risks with 95% confidence intervals

	PP			ITT		
Symptoms	*Bacillus clausii* (%)	Placebo (%)	RR; 95% CI	*Bacillus clausii* (%)	Placebo (%)	RR; 95% CI
Nausea						
First week	24	48	0.5; 0.28–0.89*	25.9	50	0.519; 0.31–0.88*
Second week	12	30	0.4; 0.17–0.95*	14.8	32.7	0.453; 0.21–0.96*
Diarrhoea						
First week	10	30	0.333; 0.13–0.85*	9.3	30.8	0.301; 0.12–0.76**
Second week	4	8	0.5; 0.10–2.61	3.7	9.6	0.385; 0.08–1.90
Epigastric pain						
First week	44	64	0.688; 0.47–1.0*	44.4	65.4	0.68; 0.48–0.97*
Second week	30	34	0.882; 0.49–1.57	31.5	36.5	0.862; 0.51–1.47
Vomiting						
First week	6	6	1.0; 0.21–4.72	7.4	9.6	0.777; 0.22–2.71
Second week	2	6	0.333; 0.04–3.10	3.7	7.7	0.481; 0.09–2.52
Taste distortion						
First week	52	60	0.867; 0.61–1.23	53.7	59.6	0.901; 0.65–1.26
Second week	12	26	0.462; 0.19–1.12	16.7	26.9	0.619; 0.29–1.31
Loss of appetite						
First week	14	20	0.7; 0.29–1.69	16.7	19.2	0.867; 0.38–1.96
Second week	6	10	0.6; 0.15–2.38	9.3	9.6	0.963; 0.29–3.13
Bloating						
First week	36.7	46	0.799; 0.49–1.28	37.7	48.1	0.785; 0.50–1.23
Second week	20	32	0.625; 0.32–1.24	22.2	34.6	0.642; 0.34–1.20
Constipation						
First week	12	22	0.545; 0.22–1.36	11.1	21.2	0.525; 0.21–1.32
Second week	14	26	0.538; 0.24–1.24	12.9	25	0.519; 0.23–1.20
Skin rash						
First week	8	16	0.5; 0.16–1.55	7.4	15.4	0.481; 0.15–1.50
Second week	4	14	0.286; 0.06–1.31	3.7	13.5	0.275; 0.06–1.26

RR, relative risks; CI, confidence intervals; PP, per protocol population; ITT, intention-to-treat population.
* $P < 0.05$, ** $P < 0.01$.

DISCUSSION

In the present study, we report that patients using *B. clausii* during *H. pylori* eradication therapy experienced a lower incidence of side-effects than subjects whose regimen was supplemented with placebo. The symptoms with a lower incidence in the group of patients treated with *B. clausii* compared with those supplemented with placebo were diarrhoea, nausea and epigastric pain.

The differences were more marked in the first week of treatment when antibiotics were given and when the incidence of side-effects was higher in both groups. The incidence of nausea, diarrhoea and epigastric pain was affected by *B. clausii* treatment, in particular with regard to the intensity and frequency of diarrhoea as it was significantly lower in the probiotic treated group than the placebo group. In previous studies,[9, 11] oral probiotic treatments during anti-*H. pylori* regimens (PPI, clarithromycin and tinidazole) were able to reduce the incidence diarrhoea, nausea and taste distortion. In the current study, amoxicillin was used instead of tinidazole for the first time based on the Maastricht 2–2000 Consensus guidelines.[6] The different antibiotic administered could explain the low incidence of taste distortion experienced by patients in our study as taste distortion is one of the commonest side-effects related to use of tinidazole and other nitroimidazoles.

Antibiotic related side-effects are common and usually affect the gastrointestinal system. The bowel milieu is characterized by a high bacterial concentration, up to 10^{12}–10^{14} CFU/mL in the colon; such bacteria coexist in equilibrium with colonic mucosal cells. Antibacterial drugs can alter this equilibrium causing potentially pathogen species to prevail over the normal resident

Table 3. Summary measure of symptoms intensity and frequency

	PP			ITT		
Symptoms	*Bacillus clausii* (overall mean ± s.d.)	Placebo (overall mean ± s.d.)	*P*-value (treatment; time × treatment interaction)	*Bacillus clausii* (overall mean ± s.d.)	Placebo (overall mean ± s.d.)	*P*-value (treatment; time × treatment interaction)
Nausea (intensity)						
First week	0.15 ± 0.4	0.39 ± 0.6	0.006; 0.188	0.25 ± 0.7	0.40 ± 0.6	0.018; 0.094
Second week	0.08 ± 0.3	0.18 ± 0.4		0.08 ± 0.3	0.21 ± 0.5	
Fourth week	0.05 ± 0.3	0.10 ± 0.3		0.05 ± 0.3	0.10 ± 0.3	
Nausea (frequency)						
First week	0.14 ± 0.3	0.55 ± 1.1	0.019; 0.263	0.61 ± 2.8	0.56 ± 1.1	0.048; 0.146
Second week	0.08 ± 0.3	0.17 ± 0.5		0.08 ± 0.3	0.48 ± 2.2	
Fourth week	0.03 ± 0.2	0.13 ± 0.4		0.03 ± 0.2	0.12 ± 0.4	
Diarrhoea (intensity)						
First week	0.01 ± 0.04	0.16 ± 0.4	0.024; 0.041	0.01 ± 0.04	0.16 ± 0.4	0.010; 0.026
Second week	0.01 ± 0.03	0.03 ± 0.1		0.01 ± 0.03	0.03 ± 0.1	
Fourth week	0.01 ± 0.04	0.02 ± 0.1		0.01 ± 0.04	0.02 ± 0.1	
Diarrhoea (frequency)						
First week	0.02 ± 0.1	0.21 ± 0.5	0.018; 0.047	1.24 ± 3.5	0.20 ± 0.5	0.008; 0.029
Second week	0.01 ± 0.1	0.04 ± 0.2		0.06 ± 0.2	0.05 ± 0.2	
Fourth week	0.01 ± 0.1	0.01 ± 0.1		0.02 ± 0.2	0.01 ± 0.1	
Taste distortion (intensity)						
First week	0.45 ± 0.6	0.70 ± 0.8	0.131; 0.469	0.54 ± 0.7	0.68 ± 0.8	0.553; 0.389
Second week	0.07 ± 0.2	0.14 ± 0.3		0.07 ± 0.2	0.13 ± 0.3	
Fourth week	0.04 ± 0.2	0.02 ± 0.1		0.04 ± 0.2	0.02 ± 0.1	
Taste distortion (frequency)						
First week	0.82 ± 2.4	1.13 ± 2.1	0.044; 0.552	1.24 ± 3.5	1.10 ± 2.0	0.304; 0.461
Second week	0.06 ± 0.2	0.15 ± 0.4		0.06 ± 0.2	0.14 ± 0.3	
Fourth week	0.02 ± 0.2	0.02 ± 0.1		0.02 ± 0.2	0.02 ± 0.1	

PP, per protocol population; ITT, intention-to-treat population.

Table 4. Overall judgment on tolerability by time and treatment (intention-to-treat population population)

Overall judgement	*Bacillus clausii* [*n* (%)]	Placebo [*n* (%)]	Total [*n* (%)]
First week			
No side-effects	13 (24.5)	8 (15.7)	21 (20.2)
Mild	35 (66.0)	32 (62.7)	67 (64.4)
Moderate/severe	5 (9.4)	11 (21.6)	16 (15.4)
Overall	53	51	104*
Second week			
No side-effects	33 (62.3)	22 (43.1)	55 (52.9)
Mild	19 (35.8)	27 (52.9)	46 (44.2)
Moderate/severe	2 (3.9)	3 (2.9)	
1 (1.9)			
Overall	53	51	104*

Kruskal–Wallis test: $P = 0.07$ during the first week, $P < 0.05$ during the second week.

* For two patients data are not available.

microflora. Modifications in the composition of the intestinal bacteria may be induced by any antibiotic, but the broad spectrum antibiotics (such as tetracyclines, aminoglycosides, macrolides and penicillins) are considered the most frequent drugs responsible for these gastrointestinal side-effects. Moreover, macrolide antibiotics, such as clarithromycin, have been related to increased contractility of gastrointestinal smooth muscle, which may lead to increased motility and accelerated transit with diarrhoea.[18]

Probiotics can also modify gut microflora. The probiotic species most commonly used include spore formers, lactic acid bacteria and yeasts. Probiotics have been shown to be useful and effective in a number of gastrointestinal diseases such as antibiotic-associated diarrhoea[19] and inflammatory bowel diseases.[20] The explanations for the effects of probiotics in human

disease include the synthesis of anti-microbial substances, the competition with pathogenic microorganisms for nutrients and microbial adhesion sites, the modification of toxins or toxin receptors, the incomplete lactose digestion and immune system modulation.[21]

The use of *B. clausii* as a probiotic species has been based on more over than 40 years of clinical usage in Italy with excellent tolerability and no report of side-effects. Moreover it has several unique properties such as the resistance to commonly used antibiotics, [22] the absence in normal resident intestinal flora and the sporogenic activity.[13, 23–25] *Bacillus clausii* spores can survive in the acid gastric environment, activate and reach the intestinal tract where they germinate to vegetative forms.[14, 15] Yet, the actual mechanism of action of *B. clausii* spores in the restoration of intestinal flora has not been fully clarified. Experimental data suggest that both *B. clausii* spores and cells can adhere to the bowel wall, allowing mucosal colonization.[16] A previous study showed a positive action of the probiotic in case of rotavirus diarrhoea and in children with antibiotic-related gastrointestinal side-effects.[13]

This study however has some limitations. First, the occurrence of side-effects in anti-*H. pylori* therapy is mostly attributed to the use of antibiotics in moderate to high doses and in combination. Although some specific side-effects such as diarrhoea could be related to the disruption of gut microflora by antibiotics, the link with bowel microecology for other common side-effects such as nausea and epigastric pain is unproven. Until the mechanistic bases of the actions of probiotics are fully understood, caution should be used in attributing symptomatic benefits to probiotic oral therapy. Secondly, we did not perform stool assessment for bacterial recovery. However, faecal recovery studies have previously confirmed presence of *B. clausii* in the stools after oral administration of commercially available preparations and *B. clausii* has been shown to be resistant to the antibiotics used in the present study. Thirdly, the results from our study can be generalized only to subjects who undergo antibiotic therapy without having gastrointestinal symptoms. In fact, we enrolled symptom-free subjects who wished to eradicate their *H. pylori* infection, in order to assess newly occurring therapy-related side-effects. The inclusion of symptom-free subjects allowed for clearer interpretation of the effect of supplementation with the probiotic on side-effects occurrence, but limited the effect size on symptom intensity because the baseline scores were close to zero. We anticipate that in dyspeptic patients the supplementation with *B. clausii* will reduce the burden of side-effects to an extent that is more clinically relevant, however, it must be acknowledged that these data were not collected to prove this hypothesis. Thus an additional clinical study would be needed to prove this point.

It should also be noted that we did not observe any difference between two groups in the *H. pylori* eradication rate. This result is in agreement with previous studies with living probiotic species, [9, 11] and suggest that *B. clausii* in the form and concentration we administered does not possess antibacterial activity against *H. pylori*. Of relevance, in both arms we attained eradication rates lower than in previous studies using the same antibiotic regimen.[26] These results were unexpected and might be partly attributable to the rising antibiotic resistance in our geographical area. Resistance to clarithromycinin in Italy has been reported to be around 10%.[27] Similar to previous experience [9, 11] overall compliance of patients was not affected by *B. clausii*. However, the sample size was chosen to evaluate difference in incidence of side-effects and not in eradication rate and overall compliance. Moreover, patients enrolled in this study asked to be eradicated. Thus, they might have been particularly motivated to complete the treatment independently on the incidence of side-effects. Although eradication rate and overall compliance are not affected by *B. clausii* supplementation, it is still be an advantage to reduce side-effects. On a case-by-case basis, the clinician should determine whether it is worth supplementing the patient with *B. clausii* to prevent mild to moderate side-effects, without affecting compliance. Cost consideration and the preference of the individual patient should also be taken into account.

In conclusion, this study shows that *B. clausii* treatment during and after a standard seven day anti-*H. pylori* regimen is associated with lower incidence of self-reported side-effects and with a better tolerability to multiple antibiotic treatment when compared with placebo.

REFERENCES

1 Basset C, Holton J, Ricci C, *et al.* Review article: diagnosis and treatment of *Helicobacter*: a 2002 updated review. Alimen Pharmacol Ther 2003; 17: 89–97.

2 Di Caro S, Assunta Zocco M, Cremonini F, *et al.* Levofloxacin based regimens for the eradication of *Helicobacter pylori*. Eur J Gastroenterol Hepatol 2002; 14: 1309–12.

3 Gene E, Calvet X, Azagra R, Gisbert JP. Triple vs. quadruple therapy for treating *Helicobacter pylori* infection: a meta-analysis. Alimen Pharmacol Ther 2003; 17: 1137–43.
4 Katelaris PH, Forbes GM, Talley NJ, Crotty B. A randomized comparison of quadruple and triple therapies for *Helicobacter pylori* eradication: the quadrate study. Gastroenterology 2002; 123: 1763–9.
5 Laine L, Hunt R, El-Zimaity H, Nguyen B, Osato M, Spenard J. Bismuth-based quadruple therapy using a single capsule of bismuth biskalcitrate, metronidazole, and tetracycline given with omeprazole versus omeprazole, amoxicyllin, and clarithromycin for eradication of *Helicobacter pylori* in duodenal ulcer patients: a prospective, randomized, multicenter, North American trial. Am J Gastroenterol 2003; 98: 562–7.
6 Malfertheiner P, Megraud F, O'Morain C, *et al.* Current concepts in the management of *Helicobacter pylori* infection – the Maastricht 2–2000 Consensus Report. Alimen Pharmacol Ther 2002; 16: 167–80.
7 Mascitelli L, Pezzetta F. Quadruple treatments for *Helicobacter pylori* (comment). Lancet 2003; 361: 86.
8 Zullo A, Vaira D, Vakil N, *et al.* High eradication rates of *Helicobacter pylori* with a new sequential treatment (erratum appears in Aliment Pharmacol Ther 2003 May 1;17:1205). Alimen Pharmacol Ther 2003; 17: 719–26.
9 Armuzzi A, Cremonini F, Bartolozzi F, *et al.* The effect of oral administration of *Lactobacillus GG* on antibiotic-associated gastrointestinal side-effects during *Helicobacter pylori* eradication therapy. Alimen Pharmacol Ther 2001; 15: 163–9.
10 Cremonini F, Canducci F, Di Caro S, *et al. Helicobacter pylori* treatment: a role for probiotics? Dig Dis 2001; 19: 144–7.
11 Cremonini F, Di Caro S, Covino M, *et al.* Effect of different probiotic preparations on anti-*Helicobacter pylori* therapy-related side effects: a parallel group, triple blind, placebo-controlled study. Am J Gastroenterol 2002; 97: 2744–9.
12 Fuller R. Probiotics in human medicine. Gut 1991; 32: 439–42.
13 Benoni G, Marcer V, Cuzzolin L, Raimo F. Antibiotic administration and oral bacterial therapy in infants. Chemioterapia 1984; 3: 291–4.
14 Ciffo F, Da Carro C, Giovannetti M. Gastric resistance of *Bacillus subtilis* spores used in oral bacteriotherapy: in vitro studies. Farmaci e terapia 1987; 3: 163–169.
15 Urdaci MC, Bressollier P, Pinchuk I, Bacillus clausii probiotic strains: antimicrobial and immunomodulatory activities. J Clin Gastroenterol 2004; 38: S86–90.
16 Angioi A, Zanetti S, Sanna A. Adhesiveness of *Bacillus subtilis* strains to epithelial cells cultured in vitro. Microbial Ecology in Health and Disease 1995; 8: 71–77.
17 de Boer WA, Thys JC, Borody TJ, Graham DY, O'Morain C, Tytgat GN. Proposal for use of a standard side effect scoring system in studies exploring *Helicobacter pylori* treatment regimens. Eur J Gastroenterol Hepatol 1996; 8: 641–3.
18 Peters DH, Clissold SP. Clarithromycin. A review of its antimicrobial activity, pharmacokinetic properties and therapeutic potential. Drugs 1992; 44: 117–64.
19 Cremonini F, DiCaro S, Nista EC, *et al.* Meta-analysis: the effect of probiotic administration on antibiotic-associated diarrhoea. Alimen Pharmacol Ther 2002; 16: 1461–7.
20 Gionchetti P, Amadini C, Rizzello F, *et al.* Probiotics–role in inflammatory bowel disease. Digestive & Liver Disease 2002; 34: S58–62.
21 Isolauri E. Probiotics in human disease. Am J Clin Nutr 2001; 73: 1142S–6S.
22 Bozdogan B, Galopin S, Gerbaud G, Courvalin P, Leclercq R. Chromosomal aadD2 encodes an aminoglycoside nucleotidyltransferase in *Bacillus clausii*. Antimicrob Agents Chemother 2003; 47: 1343–6.
23 Senesi S, Celandroni F, Tavanti A, Ghelardi E. Molecular characterization and identification of *Bacillus clausii* Strains marketed for use in oral bacteriotherapy. Applied & Environmental Microbiology 2001; 67: 834–9.
24 Mazza P, Zani F, Martelli P. Studies on the antibiotic resistance of *Bacillus subtilis* strains used in oral bacteriotherapy. Boll Chim Farm 1992; 131: 401–8.
25 Ciffo F. Determination of the spectrum of antibiotic resistance of the '*Bacillus subtilis*' strains of Enterogermina. Chemioterapia 1984; 3: 45–52.
26 Danese S, Armuzzi A, Romano A, *et al.* Efficacy and tolerability of antibiotics in patients undergoing *H. pylori* eradication. Hepatogastroenterology 2001; 48: 465–7.
27 Franzin L, Pennazio L, Cabodi D, Rossigni F, Gioannini P. Clarithromycin and amoxicillin susceptibility of *Helicobacter pylori* strains isolated from adults with gastric or duodenal ulcer in Italy. Curr Microbiol 2000; 40: 96–100.