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ORIGINAL RESEARCH

Effect of *Bacillus clausii* Capsules in Reducing Adverse Effects Associated with *Helicobacter pylori* Eradication Therapy: A Randomized, Double-Blind, Controlled Trial

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ABSTRACT

Introduction: Antibiotic treatment can alter the gut microbiome and cause short-term gastrointestinal adverse effects (AEs). This study assessed the efficacy of lyophilized capsules containing 2×10^9 spores of *Bacillus clausii* (Enterogermina®; Sanofi Synthelabo) in reducing AEs associated with *Helicobacter pylori* eradication therapy in Italy.

Methods: In this randomized, double-blind, single-center, phase IIIB study, 130 adult outpatients with *H. pylori* infection were assigned to receive one Enterogermina® capsule or placebo three times daily for 2 weeks (1:1). During week 1, all patients received clarithromycin 500 mg, amoxicillin 1 g, and rabeprazole 20 mg twice daily. The primary efficacy outcome was the presence of diarrhea in week 1.

Results: A total of 130 patients were randomized. The incidence of diarrhea in week 1 was 29% in the *B. clausii* group and 48% in the placebo group [relative risk (RR) 0.61; 95% confidence interval (CI) 0.39–0.97; $p = 0.03$]. The incidence of diarrhea remained lower with *B. clausii* than with placebo in week 2 (RR 0.38; 95% CI 0.14–1.02; $p = 0.0422$). In week 1, the number of days without diarrhea was significantly higher in the *B. clausii* group than in the placebo group (6.25 vs. 5.86; $p = 0.0304$). In both groups, the number of days without diarrhea increased significantly ($p < 0.0001$) from week 1 to week 2. A total of three AEs occurred in two patients in the placebo group, but none were serious.

Conclusions: Compared with placebo, Enterogermina® reduced the incidence of, and the number of days with, diarrhea in patients receiving *H. pylori* eradication therapy. Enterogermina® was well tolerated.

Keywords: *Bacillus clausii*; Clinical trial; Diarrhea; Enterogermina; Eradication therapy; *Helicobacter pylori*; Probiotics

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Key Summary Points

Why carry out this study?

Recommended antibiotic treatments for *Heliobacter pylori* eradication cause short-term gastrointestinal adverse effects (AEs), such as diarrhea, nausea, vomiting, bloating, and abdominal pain. Such AEs can result in treatment discontinuation, and may increase the risk of treatment failure and/or developing antibiotic resistance.

This study aimed to assess and confirm the efficacy of a spore-based probiotic containing spores of four antibiotic-resistant *B. clausii* strains (Enterogermina®) in reducing and preventing the AEs associated with *H. pylori* eradication therapy using the galenic form of capsules.

What was learned from the study?

Compared with placebo, Enterogermina® reduced the incidence of, and the number of days with, diarrhea in patients receiving *H. pylori* eradication therapy.

Probiotic supplementation with *B. clausii* capsule formulation during 7-day triple-therapy for *H. pylori* eradication was well tolerated and effective in reducing the incidence of, and the number of days with, diarrhea.

DIGITAL FEATURES

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INTRODUCTION

The recommended treatment for *Helicobacter pylori* eradication in regions of the world with clarithromycin resistance < 15% is proton-pump inhibitor (PPI)–clarithromycin-containing triple therapy, while in areas with high (> 15%) clarithromycin resistance, the recommended therapy is bismuth quadruple or non-bismuth quadruple therapies [1, 2]. However, antibiotic treatment can alter the gut microbiome and cause short-term gastrointestinal adverse effects (AEs), such as diarrhea, nausea, vomiting, bloating, and abdominal pain [1, 3]. These AEs can result in treatment discontinuation [2], which, in turn, may increase the risk of treatment failure and/or developing antibiotic resistance [1, 2, 4, 5].

Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [6, 7]. Probiotics mediate their effects via several mechanisms, including colonization and normalization of perturbed intestinal microbiota, competitive exclusion of pathogens, and modulation of the immune system [8–11]. In addition to improving eradication rates and reducing the development of antibiotic-associated diarrhea [12, 13], certain probiotics, such as *Saccharomyces boulardii* and *Bacillus clausii*, may reduce gastrointestinal AEs associated with *H. pylori* eradication therapies [1, 5].

Bacillus species are ubiquitous in the environment, and found in soil, water, plants, mammals, aquatic animals, insects, and other invertebrates [14]. They are Gram-positive, rod-shaped bacteria with the ability to produce a robust spore to survive environmental stress and harsh conditions, such as extreme pH (e.g., bile fluids), temperatures, and dry conditions [14]. *B. clausii* has been used widely in Italy since the 1960s for antibiotic-related AEs [15]. *B. clausii* spores can survive transit through the gut, where they germinate, grow, and multiply as vegetative cells. Enterogermina® (Sanofi Synthelabo) is a spore-based probiotic containing spores of four antibiotic-resistant *B. clausii* strains (*O/C*, *N/R*, *SIN*, *T*) [16]. It is available in 55 countries around the world in several

formulations: liquid vials or lyophilized capsules containing 2 billion colony-forming units (CFU), liquid vials containing 4 billion CFU, and lyophilized powder for suspension and orodispersible granules containing 6 billion CFU [17]. Lyophilized capsules and liquid vials containing 2 billion CFU were bioequivalent, with *B. clausii* found alive in feces for up to 12 days after administration [16]. A previous study demonstrated the efficacy of Enterogermina® 2 billion vial formulation of *B. clausii* versus placebo in reducing gastrointestinal symptoms (nausea, diarrhea, epigastric pain, and taste disturbance) associated with *H. pylori* eradication using 7-day PPI and antibiotic-based therapy [15].

This study aimed to assess the efficacy of Enterogermina® 2 billion capsule formulation of *B. clausii* in reducing and preventing the AEs associated with *H. pylori* eradication therapy.

METHODS

Study Design and Patients

This randomized, double-blind, single-center, placebo-controlled, parallel-group, phase 3b study was conducted at the Gastroenterology and Internal Medicine Day Hospital, Gemelli Hospital, Rome, Italy.

Between June 2003 and April 2004, 130 consecutive male or female outpatients who had gastric *H. pylori* infection (as confirmed by a ^{13}C-urea breath test) were enrolled. Patients were aged 18–65 years and did not have gastrointestinal symptoms in the 3 months prior to enrolment. Exclusion criteria included any concomitant therapy within 3 months of the study, use of laxatives, antidiarrheal agents or probiotics within 3 months of the study, and acute or chronic gastrointestinal diseases, diabetes, or chronic debilitating diseases.

All patients were required to sign a written informed consent form before participating in the clinical trial. The institution's Independent Ethics Committee (Comitato Etico, Università Cattolica del Sacro Cuore, Policlinico A. Gemelli, Largo Gemelli, 8, 00,168 Roma) approved the study protocol in April 2003, and the study was performed according to the Helsinki declaration and in compliance with the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice guidelines adopted by the European Medicines Agency. The trial was conducted between June 2003 and May 2004, before the European database for clinical trials (EudraCT) was established, and is therefore not registered in a clinical study database.

Treatments

Patients were randomly assigned (1:1) to receive one Enterogermina® capsule (containing 2×10^9 spores of polyantibiotic-resistant *B. clausii*) three times daily (for a total of 6×10^9 CFU per day) or matching placebo capsule three times daily, for 2 weeks. Randomization was conducted using SAS software. During week 1 of treatment, all patients received triple therapy with clarithromycin 500 mg twice daily, amoxicillin 1 g twice daily, and rabeprazole 20 mg twice daily for *H. pylori* eradication. After 2 weeks of treatment, patients were followed-up for another 2 weeks.

Efficacy and Safety Assessments

Patients were assessed at baseline (visit 0) and at weeks 1, 2, and 4 (visits 1, 2, and 3). At visit 0 (baseline), patients were given a diary to record the daily frequency of vomiting and diarrhea and the intensity of the other gastrointestinal symptoms over the first 2 weeks of the study.

The primary efficacy parameter was the presence of diarrhea in week 1 (assessed at visit 1). Secondary efficacy parameters were: the presence/absence of diarrhea in week 2; number of days without diarrhea in week 1 and week 2; presence/absence of other gastrointestinal symptoms (vomiting, taste disturbance, loss of appetite, nausea, epigastric pain, flatulence, constipation, skin rash) in weeks 1 and 2; patient-reported daily frequency of vomiting and diarrhea episodes in weeks 1 and 2; patient-reported daily intensity of taste disturbance, loss of appetite, nausea, epigastric pain,

bloating, constipation, and skin rash in weeks 1 and 2 (assessed on a 4-point scale, in which 0 = no symptoms, 1 = mild, 2 = moderate, and 3 = severe symptoms); and patients' global assessment of tolerability after week 1 and 2 (scored on a 5-point scale, in which 1 = no symptoms, 2 = mild symptoms that did not interfere with daily activities, 3 = moderate symptoms that interfered slightly with daily activities, 4 = severe symptoms that interfered with daily activities but did not result in treatment discontinuation, and 5 = severe symptoms that required treatment discontinuation).

Safety of the study drug was assessed by monitoring the AEs and the vital signs at each study visit.

Sample Size

We estimated that a sample size of 130 would provide 80% power (two-sided $\alpha = 0.05$) to detect the difference in the incidence of diarrhea between the *B. clausii* group and the placebo group, assuming the same incidences of diarrhea as in a previous randomized, double-blind trial (placebo 30.8%, *B. clausii* 9.3%, placebo vs. *B. clausii* odds ratio 4.341) [15].

Statistical Analysis

The primary efficacy analysis was conducted in the intent-to-treat (ITT) population, which comprised all randomized patients who received at least one dose of the study drug and for whom at least one post-baseline efficacy assessment was available. A supportive analysis of the primary endpoint was conducted in the per-protocol (PP) population, comprising all randomized patients who completed the study therapy without any major protocol violation and had valid efficacy data at visit 1. Safety was assessed in all randomized patients who received at least one dose of the study drug (the safety population).

Dichotomous variables were analyzed by χ^2 test and the relative risk (RR) and 95% confidence intervals (CI) were calculated. The daily episodes of diarrhea and vomiting and the daily intensity of other symptoms were summarized as overall means. An analysis of variance for repeated measures was applied to the rank-transformed data. Treatment, time, and the interaction between treatment by time were included in the model. The difference between groups for global assessment of tolerability was evaluated in the ITT population using the Wilcoxon test. All the statistical analyses were performed using the SAS system, v.8.2. All tests were two-sided and a p value of < 0.05 was considered statistically significant.

RESULTS

Patient Disposition and Characteristics

Overall, 130 patients were randomized to receive *B. clausii* ($n = 65$) or placebo ($n = 65$). All patients assigned to the *B. clausii* group completed the study, while one patient in the placebo group discontinued because of an AE (intestinal infection) 8 days after treatment initiation. Patient disposition is shown in Fig. 1. Patient demographic characteristics were well balanced between the treatment groups (Table 1).

Primary Efficacy Outcome

In the ITT population, the incidence of diarrhea at week 1 was significantly lower in patients receiving *B. clausii* than those receiving placebo (29% vs. 48%, $p = 0.03$), corresponding to a 39% reduction in the risk of diarrhea with *B. clausii* (RR 0.61; 95% CI 0.39–0.97; Table 2). Results in the PP population supported the primary efficacy analysis, with a 39% reduction in the risk of diarrhea in patients receiving *B. clausii* than in those receiving placebo ($p = 0.0381$; Table 2).

Secondary Efficacy Outcomes

The risk of diarrhea remained significantly lower with *B. clausii* than with placebo at week 2, both in the ITT (RR 0.38; 95% CI 0.14–1.02; $p = 0.0422$) and PP populations (RR 95% CI 0.25; 0.07–0.84; $p = 0.0130$; Table 2). No

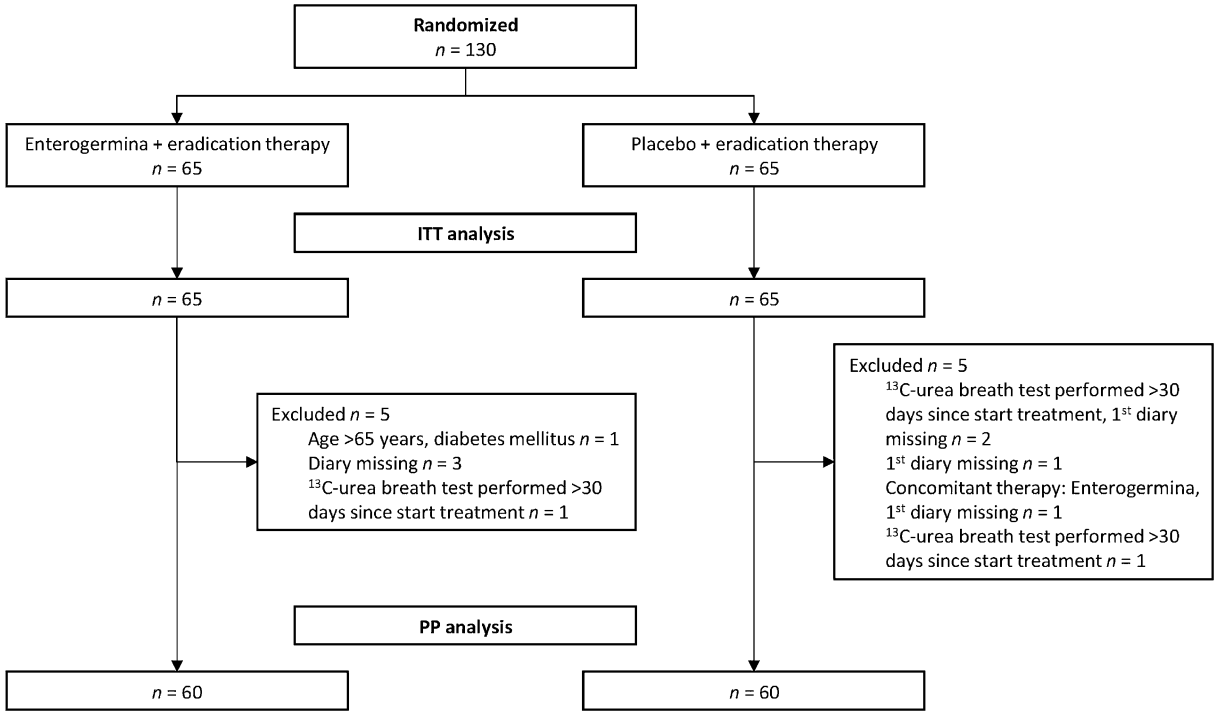

Fig. 1 Patient disposition

Table 1 Demographic characteristics (ITT population)

Demographic data	*Bacillus clausii* *n* = 65	*n* = 65
Male[a], *n* (%)	28 (43.08)	26 (40.00)
Female[a], *n* (%)	37 (56.92)	39 (60.00)
Age[b], years (mean ± SD)	44.23 (13.48)	42.38 (13.02)
Race[c], *n* (%)		
Caucasian	62 (95.38)	62 (95.38)
Black	1 (1.54)	0 (0.00)
Oriental	0 (0.00)	1 (1.54)
Other	2 (3.08)	2 (3.08)

ITT intent-to-treat, *SD* standard deviation
[a] $p = 0.7229$
[b] $p = 0.4286$
[c] $p = 0.5756$

significant difference between treatment groups was observed in the incidence of the other gastrointestinal symptoms at week 1 (Table 2). At week 2, the incidence of epigastric pain was significantly lower with *B. clausii* than with placebo (RR 0.50; 95% CI 0.25–0.98; $p = 0.0374$; Table 2).

In the ITT population, patients receiving *B. clausii* had significantly ($p = 0.0304$) more days without diarrhea in week 1 than patients receiving placebo (6.25 vs. 5.86; Table 3). In both, the *B. clausii* and placebo groups, the number of days without diarrhea increased significantly ($p < 0.0001$) from week 1 to week 2, according to a repeated measures analysis (Table 3). In a repeated measures analysis, no significant difference between groups was observed in the number of days without symptoms (Table 3) or the patient-reported overall mean frequency/intensity (Table 4) of vomiting, taste disturbance, loss of appetite, nausea, epigastric pain, flatulence, constipation, or skin rash. In both treatment groups, for the majority of symptoms, the number of symptom-free days increased significantly (Table 3), and patient-reported frequency/intensity decreased significantly (Table 4) from week 1 to week 2. A significant interaction between treatment and

Table 2 Incidence of symptoms

Symptoms	Population	Week 1			Week 2		
		Bacillus clausii (%)	Placebo (%)	RR; 95% CI	*Bacillus clausii* (%)	Placebo (%)	RR; 95% CI
Primary outcome							
Diarrhea	ITT	29.23	47.69	0.61; 0.39–0.97*	7.69	20.00	0.38; 0.14–1.02*
	PP	28.33	46.67	0.61; 0.37–0.99*	5.00	20.00	0.25; 0.07–0.84*
Secondary outcomes							
Vomiting	ITT	6.15	3.08	2.00; 0.38–10.54[a]	3.08	3.08	1.00; 0.14–6.89[a]
Taste disturbance	ITT	52.31	56.92	0.92; 0.67–1.26	18.46	23.08	0.80; 0.41–1.57
Loss of appetite	ITT	24.62	20.00	1.23; 0.64–2.35	6.15	10.77	0.57; 0.18–1.86
Nausea	ITT	35.38	27.69	1.28; 0.77–2.13	15.38	16.92	0.91; 0.41–1.99
Epigastric pain	ITT	46.15	44.62	1.03; 0.71–1.51	15.38	30.77	0.50; 0.25–0.98*
Flatulence	ITT	41.54	41.54	1.00; 0.66–1.50	16.92	29.23	0.58; 0.30–1.12
Constipation	ITT	27.69	26.15	1.06; 0.60–1.87	21.54	23.08	0.93; 0.49–1.77
Skin rash	ITT	4.62	9.23	0.50; 0.13–1.91[a]	10.77	4.62	2.33; 0.63–8.63

CI confidence interval, *ITT* intent-to-treat population, *PP* per-protocol population, *RR* risk ratio
X^2 test $^*p < 0.05$ vs. placebo
[a] Fischer exact test; X^2 test was not valid as counts of < 5 were expected in 50% of the cells

time was observed for the intensity of skin rash ($p = 0.0077$; Table 4).

Patients' global assessment of tolerability did not differ significantly between the two treatment groups (Table 5).

Safety Outcomes

In the safety population ($n = 130$), the mean duration of exposure to treatment was 14.14 [± 0.46 standard deviation (SD)] days in the *B. clausii* group and 14.09 (± 1.07 SD) days in the placebo group.

Overall, three AEs were reported in the study, occurring in two placebo recipients (gastrointestinal mycosis and gastroenteritis due to enteropathogenic *E. coli*; and mild aphthous stomatitis). The AEs were not serious and were not considered related to treatment. The patient who had gastrointestinal mycosis and gastroenteritis required treatment interruption and specific treatment for these AEs. There was no significant difference between the two groups in the incidence of AEs ($p = 0.4961$).

No serious AEs, deaths or other significant AEs were reported in the study.

DISCUSSION

In this randomized, double-blind, placebo-controlled study, patients receiving 7-day triple-therapy for *H. pylori* eradication had a significantly lower incidence of diarrhea if they also received probiotic supplementation with *B. clausii* than if they received placebo. This benefit was observed in week 1 and sustained in week 2. Patients receiving *B. clausii* also had significantly more days without diarrhea than

Table 3 Number of days without symptoms (ITT population)

Symptoms	Overall mean ± SD		*p* value[a] (treatment; time; treatment × time interaction)
	Bacillus clausii n = 65	*n* = 65	
Diarrhea			
Week 1	6.25 ± 1.67	5.86 ± 1.88	0.0304; < 0.0001; 0.9028
Week 2	6.77 ± 1.26	6.31 ± 1.85	
Vomiting			
Week 1	6.97 ± 0.82	7.03 ± 0.39	0.8369; 0.8070; 0.7993
Week 2	7.03 ± 0.35	7.02 ± 0.28	
Taste disturbance			
Week 1	4.71 ± 2.93	4.34 ± 2.89	0.1373; < 0.0001; 0.9748
Week 2	6.80 ± 0.67	6.16 ± 2.03	
Loss of appetite			
Week 1	6.06 ± 2.23	6.23 ± 1.90	0.7756; 0.0004; 0.3541
Week 2	6.88 ± 0.65	6.55 ± 1.75	
Nausea (intensity)			
Week 1	5.70 ± 2.39	6.26 ± 1.75	0.6678; < 0.0001; 0.1719
Week 2	6.72 ± 1.00	6.73 ± 0.88	
Epigastric pain			
Week 1	5.67 ± 2.22	5.55 ± 2.28	0.3505; < 0.0001; 0.1828
Week 2	6.38 ± 1.73	6.09 ± 1.81	
Flatulence			
Week 1	5.50 ± 2.39	5.41 ± 2.44	0.4939; < 0.0001; 0.1788
Week 2	6.28 ± 1.89	5.77 ± 2.48	
Constipation			
Week 1	6.31 ± 1.76	6.28 ± 1.83	0.8618; 0.3638; 0.6237
Week 2	6.30 ± 1.84	6.16 ± 2.05	
Skin rash			
Week 1	7.03 ± 0.53	6.82 ± 1.09	0.9508; 0.6420; 0.0627
Week 2	6.66 ± 1.37	6.86 ± 1.04	

ITT intent-to-treat, *SD* standard deviation

[a] Repeated Measures ANOVA (*p* value of *F* test)

those receiving placebo. Except for a lower incidence of epigastric pain with *B. clausii* at week 2, the incidence of other gastrointestinal symptoms did not differ significantly between the two treatment groups. The *B. clausii* capsule formulation was well tolerated with no

Table 4 Overall mean of patient-assessed frequency/intensity of gastrointestinal symptoms (ITT population)

Symptoms	Overall mean ± SD		*p* value (treatment; time; treatment × time interaction)
	Bacillus clausii n = 65	*n* = 65	
Diarrhea (frequency)			
Week 1	0.22 ± 0.46	0.26 ± 0.51	0.0585; < 0.0001; 0.7085
Week 2	0.10 ± 0.41	0.14 ± 0.37	
Vomiting (frequency)			
Week 1	0.03 ± 0.17	0.01 ± 0.07	0.4605; 0.4697; 0.9799
Week 2	0.01 ± 0.04	0.00 ± 0.02	
Taste disturbance (intensity)			
Week 1	0.56 ± 0.78	0.60 ± 0.75	0.3325; < 0.0001; 0.8767
Week 2	0.04 ± 0.11	0.21 ± 0.57	
Loss of appetite (intensity)			
Week 1	0.17 ± 0.38	0.17 ± 0.40	0.9818; < 0.0001; 0.2476
Week 2	0.02 ± 0.10	0.10 ± 0.36	
Nausea (intensity)			
Week 1	0.28 ± 0.55	0.16 ± 0.37	0.4693; < 0.0001; 0.2055
Week 2	0.05 ± 0.16	0.04 ± 0.12	
Epigastric pain (intensity)			
Week 1	0.30 ± 0.52	0.30 ± 0.53	0.4410; < 0.0001; 0.1008
Week 2	0.12 ± 0.33	0.19 ± 0.39	
Flatulence (intensity)			
Week 1	0.32 ± 0.57	0.35 ± 0.60	0.4311; < 0.0001; 0.1128
Week 2	0.13 ± 0.39	0.26 ± 0.58	
Constipation (intensity)			
Week 1	0.17 ± 0.46	0.13 ± 0.31	0.8995; 0.1549; 0.6481
Week 2	0.16 ± 0.48	0.17 ± 0.45	
Skin rash (intensity)			
Week 1	0.01 ± 0.04	0.05 ± 0.24	0.7858; 0.7822; 0.0077
Week 2	0.06 ± 0.23	0.04 ± 0.26	

Frequency is the mean number of daily episodes
ITT intent-to-treat, *SD* standard deviation
[a] Repeated Measures ANOVA (*p* value of *F* test)

treatment-associated AEs reported in patients receiving the formulation.

A similar study by Nista and colleagues with a *B. clausii* vial formulation (containing a watery

Table 5 Patients' global assessment of tolerability (ITT population)

Global assessment	*Bacillus clausii* *n* = 65	*n* = 65
Week 1[a], *n* (%)		
No symptoms	14 (21.54)	6 (9.23)
Mild symptoms not interfering with daily activities	32 (49.23)	49 (75.38)
Moderate symptoms interfering slightly in daily activities	15 (23.08)	7 (10.77)
Severe symptoms, seriously interfering with daily activities but not requiring discontinuation	4 (6.15)	2 (3.08)
Severe symptoms requiring discontinuation	0 (0.00)	1 (1.54)
Week 2[b], *n* (%)		
No symptoms	35 (53.85)	24 (36.92)
Mild symptoms not interfering with daily activities	26 (40.00)	38 (58.46)
Moderate symptoms interfering slightly in daily activities	3 (4.62)	2 (3.08)
Severe symptoms, seriously interfering with daily activities but not requiring discontinuation	1 (1.54)	0 (0.00)
Severe symptoms requiring discontinuation	0 (0.00)	0 (0.00)

ITT intent-to-treat
[a] Wilcoxon test $p = 0.8531$ vs. placebo
[b] $p = 0.1269$ for week 2

suspension of 2×10^9 spores) also showed a significant reduction in the incidence of diarrhea compared with placebo in patients receiving 7-day triple therapy for *H. pylori* eradication therapy [15]. In addition, the incidence of nausea at week 1 and week 2, as well as the incidence of epigastric pain at week 1, were significantly reduced with *B. clausii* vial formulation than with placebo [15]. Of note, although the studies were very similar in their design, treatment, and duration, the primary objective of the study by Nista and colleagues was to determine the effect of treatment on the incidence of symptoms (such as diarrhea, epigastric pain, etc.), as well as *H. pylori* eradication rates. The incidence of diarrhea in the control group in this study was greater than that in the study by Nista [15]. Our results might be considered high by the standards of clinical trials, but within the range of what is seen in clinical settings in this indication. Recent meta-analyses have demonstrated that probiotic supplementation during *H. pylori* eradication therapy reduces the incidence of gastrointestinal symptoms, and that diarrhea incidence during *H. pylori* eradication therapy varies significantly between trials and might be as high as 58% [2, 18–21].

Healthy gut microbiota has been recognized as key to several aspects of overall health, with one of its major properties being competitive exclusion of pathogens [22, 23]. Antibiotic therapy can disrupt this competitive exclusion machinery by various mechanisms, such as

reducing the diversity and abundance of gut microbiota, resulting in overgrowth of pathogenic bacteria (e.g., diarrhea-causing bacteria) [23, 24]. Probiotics help to restore the normal flora of the gut, thereby reducing AEs associated with the use of antibiotics [25]. Additionally, probiotics may have a beneficial effect on *H. pylori* eradication [1, 18, 25]. However, our study did not assess the eradication of *H. pylori*, as this was not one of its objectives. In the previous study with *B. clausii* vial formulation, the *H. pylori* eradication rate was similar between *B. clausii* and placebo groups. The *H. pylori* eradication rate in this study is expected to be similar as the capsule and vial formulations are bioequivalent [16]; however, this remains to be confirmed. The fact that this study was performed in a single country (Italy) could be considered a limitation. Since the intestinal microbiota of a patient varies geographically [26], and our study was performed in a single country, the effects observed with treatment are likely to vary depending on the geographic location and diet of other patients.

It should be noted that this study was conducted in 2003–2004 and used PPI–clarithromycin-containing triple therapy for *H. pylori* eradication. The effects of PPIs on gastrointestinal microbiota have been the subject of recent research interest [27]. By increasing gastric pH, PPIs appear to promote the colonization of the gastrointestinal tract by orally-derived bacteria, particularly *Streptococcus* species [27]. An increase in the relative abundance of *Streptococcus* was observed in patients receiving PPIs regardless of their *H. pylori* infection status [28]. PPIs are some of the most widely used medications, and whether the use of *B. clausii* probiotics can ameliorate their effects on gastrointestinal microbiota is a promising future research area. Currently, however, triple therapy is not recommended for use in regions of the world where clarithromycin resistance is > 15%, and additional studies are needed to assess the efficacy of the *B. clausii* capsule and vial formulations with bismuth quadruple or non-bismuth quadruple therapies [1].

CONCLUSION

In conclusion, this study shows that probiotic supplementation with *B. clausii* capsule formulation during 7-day triple-therapy for *H. pylori* eradication was well tolerated and effective in reducing the incidence of, and the number of days with, diarrhea relative to supplementation with placebo.

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Authorship Contributions. MP participated in the analysis of the data and contributed to drafting of the manuscript and revising each draft for important intellectual content. MP III and DG contributed to the analysis of the data and critically revised the drafts for important intellectual content.

Disclosures. Manuel Plomer, Marcos Perez III and Dorothea Maren Greifenberg are employees of Sanofi-Aventis.

Compliance with Ethics Guidelines. All patients were required to sign a written informed consent form before participating in the clinical trial. The protocol, patient information sheet, and all the other study-related documents were submitted for approval to the institution's Independent Ethics Committee (IEC, Comitato Etico, Università Cattolica del Sacro Cuore, Policlinico A. Gemelli, Largo Gemelli, 8, 00168 Roma). Following local regulations, the IEC of the Centre released the "Giudizio di Notorietà" on April 23rd 2003, i.e. the permission to study Enterogermina in capsules formulation. On the same date the IEC approved the study protocol and the related study documentation. The study was performed according to the Helsinki declaration and in compliance with the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice guidelines adopted by the European Medicines Agency. This trial was conducted between June 2003 and May 2004, before the European database for clinical trials (EudraCT) was established, and is therefore not registered in a clinical study database.

Data Availability. Qualified researchers may request access to patient level data and related study documents including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan and dataset specifications. Patient level data will be anonymized, and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi's data sharing criteria, eligible studies and process for requesting access can be found at https://www.clinicalstudydatarequest.com.

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