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ORIGINAL ARTICLE

Effect of *Saccharomyces boulardii* CNCM I-745 as complementary treatment of *Helicobacter pylori* infection on gut microbiome

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Abstract

Conventional therapy for *H. pylori* infection includes the combination of antibiotics and a proton-pump inhibitor. Addition of probiotics as adjuvants for *H. pylori* antibiotic treatment can increase eradication rate and decrease treatment side effects. Although many studies show the benefits of *S. boulardii* CNCM I-745 in the treatment of *H. pylori* infection, the mechanism by which those benefits are achieved is unknown. Here, we report clinical characteristics and fecal microbiota changes comparing conventional anti-*H. pylori* therapy versus conventional therapy supplemented with *S. boulardii* CNCM I-745. A total of 74 patients were included in the current study; patients positive for *H. pylori* ($n = 63$) were randomly assigned to 2 groups: 34 patients received conventional therapy and 29 antibiotic therapy plus 750 mg of *S. boulardii* CNCM I-745 daily, for 2 weeks. Eleven patients negative for *H. pylori* infection were also studied. Patients provided 3 fecal samples: before initiating the antibiotic treatment, upon its completion, and 1 month after treatment. Patients were contacted every 72 h to inquire about side effects and compliance. DNA was extracted, and 16S rRNA was amplified and sequenced on Illumina MiSeq. Bioinformatic analysis was performed using QIIME2. Patients who received the probiotic had a significantly lower frequency of associated gastrointestinal symptoms ($P = 0.028$); higher number of bacterial diversity evenness ($P = 0.0156$); higher abundance of Enterobacteria; and lower abundance of Bacteroides and Clostridia upon treatment completion. Addition of *S. boulardii* CNCM I-745 induced a lower frequency of gastrointestinal symptoms that could be related to changes in gut microbiota.

Keywords *Saccharomyces boulardii* CNCM I-745 · *Helicobacter pylori* infection · Probiotics · Microbiome · Ecuador

Introduction

Gastric cancer is a public health problem worldwide due to its high incidence and mortality [1, 2]. *Helicobacter pylori* infection is by far the most important risk factor for gastric cancer; 78 % of gastric cancer cases are attributed to this pathogen. The International Agency for Research on Cancer has classified *H. pylori* as a group 1 carcinogen [3]. More than 70% of gastric cancer occurs in developing countries, and Ecuador ranks 15th in the world for incidence of stomach cancer [1, 4]. Among adults in South America, prevalence of *H. pylori* infection ranges from 50 to 95% [5]. There are not studies that show a current overall prevalence of *H. pylori* infection in Ecuador. However, a couple of local studies carried on in children from few cities showed a prevalence of *H. pylori* infection between 63 and 77% [6, 7]. Consequently, *H. pylori* infection is a common health problem in developing countries including Ecuador. Recently, our group reported on factors that increased the risk for and protected against the development of gastric cancer [8]. We found that risk factors associated with the presence of gastric cancer or metaplasia were frequent consumption of reheated food and high consumption of salt while protective factors include frequent use of non-steroidal anti-inflammatory drugs, age less than 58 years old and prior treatment of *H. pylori* infection [8].

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Novel measures to prevent and treat the infection are necessary to decrease the development of gastric cancer [9].

Helicobacter pylori is a major human pathogen, the causative agent of most chronic gastritis, peptic ulcer, gastric adenocarcinoma, and atrophy of gastric mucosa [10]. In addition, *H. pylori* has been associated with other pathologies including idiopathic thrombocytopenic purpura, mucosa-associated lymphoid tissue lymphoma (MALT), dyspepsia, and gastric polyps [11]. *H. pylori* acquisition is thought to be acquired through transmission from person to person, with the mother-to-child transmission early in life being of critical importance [12].

According to the Kyoto global consensus report, all *H. pylori*–infected patients should receive eradication therapy [13]. Elimination of *H. pylori* could prevent further gastric mucosal damage and, consequently, decrease local inflammation, promote mucosal repair including restoration of normal acid secretion, cure peptic ulcers, and decrease risk for developing gastric cancer [11, 14, 15]. On the other hand, recommendations for population-wide screening for and eradication of *H. pylori* infection must carefully consider the potential consequences of such widespread (and, in many developing countries, virtually universal) use of antibiotics, including antibiotic resistance and disruption of the normal gut microbiome [16–18].

Current guidelines for *H. pylori* eradication favor a clarithromycin-based triple therapy regimen that includes a proton-pump inhibitor (PPI), clarithromycin, amoxicillin, or nitroimidazole for 10–14 days in areas of low clarithromycin resistance [19]. However, this triple therapy regimen should not be used in areas where clarithromycin resistance is common [20]. Other recommended first-line therapies for *H pylori* infection are available and their use should be based on patient's clinical characteristics including drug allergies and resistance to antibiotics [21].

Antibiotic regimens for *H. pylori* infection result in different rates of eradication and gastrointestinal side effects; the latter include taste alterations, nausea, diarrhea, and abdominal pain [22, 23]. The addition of probiotics as adjuvant agents against *H. pylori* can increase eradication rates and decrease side effects [24, 25]. Yet the role of the healthy mycobiome in *H. pylori* infection is poorly understood, it seems is not affected by the use of antibacterial-antibiotics. Moreover, *Saccharomyces boulardii* CNCM I-745, a non-pathogenic probiotic yeast, is used as a complementary component in *H. pylori* anti-microbial therapy [26]. It has been shown extensively in clinical studies that *Saccharomyces boulardii* provides enhanced eradication rate and decreased side effects [27, 28]. However, the precise mechanism of action of these *S. boulardii*–mediated effects is not fully understood. *Saccharomyces boulardii* CNCM I-745 limits *H. pylori* adherence to epithelial cells, modulates the gastric immune response, modifies the gut microbiota, and restores intestinal barrier function [29–32]. In the current study, we explored potential changes in the gut microbiota in patients infected with *H. pylori* that received conventional antibiotic treatment in combination with *S. boulardii*. We report clinical characteristics and fecal microbiota changes using 16S rRNA gene microbiota profiling in patients treated with conventional anti-*H. pylori* therapy compared with patients treated with conventional therapy supplemented with a daily dose of 750 mg of *S. boulardii* CNCM I-745 (corresponding to approximately 22.5×10^9 CFU).

Subjects, material, and methods

Study design

This was a single-blind randomized trial that assessed the effect of the addition of *S. boulardii* to conventional triple therapy on clinical side effects and the microbiota. We included patients of both sexes from 18 to 55 years of age with typical dyspepsia symptoms from whom an upper gastrointestinal endoscopy with biopsies was done for histopathological examination. A total of 178 patients were initially invited to participate of whom 40 were negative for *H. pylori* infection on histopathology (Fig. 1), and 11 of those were randomly selected for microbiome analysis. Gastric biopsy samples were obtained following the Sydney protocol and placed in paraffin for histological studies [33]. Analysis of samples was carried out by an experienced pathologist. Out of 138 positive patients on histopathology, only 63 decided to receive treatment during the study period; these were stratified by age and gender and then randomly assigned to receive the conventional treatment (amoxicillin 1 g tid, tinidazole 1 g qd, and omeprazole 40 mg bid; $n = 34$) or conventional treatment plus *S. boulardii* CNCM I-745 (approximately 22.5×10^9 CFU; $n = 29$). Once volunteers were stratified by age and gender, they were subsequently allocated by simple randomization to receive one of the two treatments.

Clinical follow-up and fecal sample collection

Research staff contacted by phone the patients during the 2 weeks of antibiotic treatment, at days three, seven, and thirteen. Patients were asked about medication compliance, gastrointestinal symptoms, and any other potential side effect. During the last phone call, patients were scheduled for a follow-up appointment upon completion of antibiotic treatment. We scored the presence of each symptom as 1 point and the absence as 0, and registered the mean number of symptoms for each treatment group. Since patients were assessed three times during the follow-up, each symptom

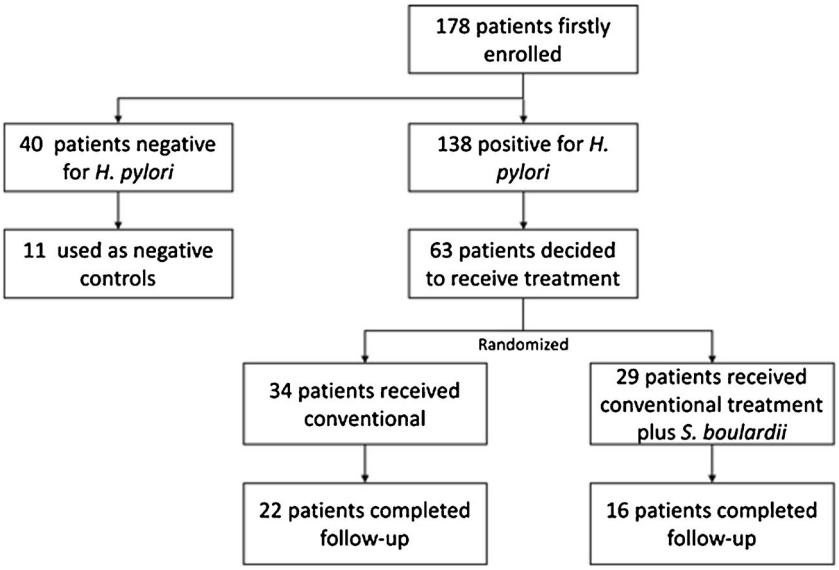

Fig. 1 Flowchart of the number of patients that were initially invited to participate in the trial

could have had a score range from 0 to 3; and since we evaluated the presence of 7 symptoms, the total possible maximal symptomatology score was 21 points. *H. pylori* eradication rates were not determined directly by laboratory test but only clinically.

Participating patients were also asked to provide three fecal samples during the study, the first before initiation of antibiotics (S1), the second immediately after completion of antibiotics (S2), and the last sample 1 month after finishing the treatment (S3). Patients were instructed to collect stool samples at home in sterile plastic containers and store them at 4 °C until delivery to the laboratory; in general, samples were stored less than 8 h before reaching the laboratory. Immediately upon arrival, stool samples were stored at − 80 °C until DNA extraction.

DNA extraction, 16S rRNA library preparation, and sequencing

DNA was extracted from 500-mg fecal material using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, USA), following the manufacturer's protocol. At the end of extraction protocol, 50 μl of DNA was stored at − 80 °C until further PCR amplifications. The DNA quality and quantity were evaluated by measuring absorbance at 260 and 280 nm with a BioTek Synergy HT Multi-Mode reader spectrophotometer (BioTek® Insttruments, Inc., USA). DNA was transported on dry ice to the Carolina Center for Genome Sciences at the University of North Carolina at Chapel Hill, NC, USA. Amplification of the V3-V4 regions of the 16S rRNA gene was performed using barcoded primers; multiplexing was performed with the Nextera kit and sequencing was performed using Illumina's MiSeq technology.

Sequence data analysis

The raw data set reads (fastq files) were imported into QIIME version 2.0 [2]. Paired-end reads were demultiplexed and featured tables were constructed by DADA2 by clustering using uclust method [34] into operational taxonomic units (OTUs) against the Greengenes Ribosomal Data Project (RDP) database for taxonomy assignation [35]. The alignment of OTU representative sequences was accomplished against a template of the Greengenes core reference alignment file [36] using the PyNAST alignment method [36]. The alignment was filtered to reduce OTUs in low number, and subsequently a maximum likelihood phylogenetic tree (rooted and unrooted) was created using FastTree building method [36]. Community diversity analysis was also done in QIIME2 [2]. For alpha diversity calculations (number of bacterial species per sample), we used the Shannon index (quantitative community richness) and observed OTUs (qualitative community richness), Faith's Phylogenetic Diversity (qualitative community richness with phylogenetic relationships) [37], and Pielou's evenness (to evaluate equitability). Beta diversity analysis (differences of the complete microbiome patterns) was performed to evaluate dissimilarities in microbial communities between groups using Jaccard distance (qualitative community dissimilarity) [38], Bray-Curtis distance (quantitative community dissimilarity), unweighted UniFrac distance (qualitative community with phylogenetic relationships), and weighted UniFrac distance (quantitative community dissimilarity with phylogenetic relationships) [39]. The raw sequences obtained were demultiplexed and uploaded to the European Nucleotide Archive (ENA) with the accession numbers ERX3120558 to ERX3120665.

Statistical analysis

Descriptive statistics were used for categorical variables as frequencies and percentages. Central tendency and their respective dispersion measures were applied for continuous variables. Chi-square was calculated for hypothesis tests between categorical variables. ANOVA was applied to test hypothesis between categorical and continuous variables. Sample composition was compared in the context of categorical metadata using PERMANOVA. Additionally, analysis of microbiome composition (ANCOM) [40] used to compare between microbial communities and differential abundance of taxa was performed using Gneiss [39]. To adjust for multiple comparisons for clinical parameters, we used the method of Benjamini Hochberg [41]. Adjusted P values ≤ 0.05 were considered statistically significant.

Results

Sixteen patients from the group that received the probiotic and 22 patients that received the conventional triple therapy completed the 45-day follow-up period and provided all three fecal samples for analysis (35.3% dropout in the conventional treatment group and 44.8% dropout in the plus *S. boulardii* group) (Fig. 1).

Patient characteristics

There were no statistically significant differences between treatment groups with regard to demographic characteristics at the beginning of the study (Table 1). Patients without *H. pylori* infection also had similar demographic characteristics (not shown).

Additionally, at baseline, microbiome analysis on alpha diversity (Shannon, evenness, and Faith indexes) and beta diversity (Bray-Curtis, Canberra, weighted, and unweighted UNIFRAC) was not significantly different ($P > 0.05$ on the six indexes) between the two treatment groups.

Symptomatology of *H. pylori*–infected patients treated with triple therapy with or without S. boulardii

Data on patients' count of gastrointestinal symptoms collected by phone on days 3, 7, 13, and at end of study visit (day 30) showed fewer gastrointestinal symptoms in those individuals that received *S. boulardii* compared with the conventional treatment group (5.3 ± 3.0 vs 9.0 ± 3.1, $P = 0.028$). Moreover, there was a statistically significant lower frequency of abdominal pain in the *S. boulardii* treatment group compared with the standard treatment. At the end of treatment, patients in both groups showed an overall decrease of the initial dyspepsia; however, no laboratory tests were made to assess *H. pylori* eradication.

Comparison of fecal microbiome composition of *H. pylori*–infected patients versus uninfected individuals at baseline

Initially, the microbiome composition of individuals with and without *H. pylori* infection was compared (data not showed) and displayed a non-significant difference in alpha diversity ($P > 0.05$ on the Shannon, evenness, and Faith indexes). Furthermore, beta diversity (Bray-Curtis, Canberra, weighted, and unweighted UNIFRAC) and the relative abundance of specific OTUs were not different in infected and non-infected individuals.

Comparison of fecal microbiome composition in *H. pylori*–infected patients within treatment groups

In the group that received the probiotic, there were statistically significant differences in relative abundance (verified by compositionally awareness test ANCOM) between fecal samples collected at baseline S1 versus S2 or S3. Several OTUs of Clostridia (principally Clostridiales, Lachnospiracea), Bacteroidia (*Bacteroides* and *Prevotella*), and Bacilli (*Lactobacillus*) in S2 and S3 were higher compared with

Table 1 Demographic characteristics of patients

Demographic characteristics	Triple therapy alone ($n = 22$)	Triple therapy + *S. boulardii* ($n = 16$)	P value between treatment groups	*H. pylori*–negative controls ($n = 11$)
Age (years)	39.5 ± 10.7	37.9 ± 7.2	0.664	34.7 ± 7.7
Male	36.4%	43.8%	0.508	45.4%
Female	63.6%	56.3%		54.6%
BMI (kg/m^2)	27.1 ± 2.0	26.4 ± 2.9	0.540	26.4 ± 24.1
Level of education				
Lower than complete high school	90%	80%	0.468	64%
Complete high school or higher	10%	20%		36%

baseline samples, while other Clostridia OTUs (Ruminococcaceae) and Bacteroidia (*Bacteroides* and other undefined genera OTUs) decreased compared with S1 (Fig. 2). On the other hand, in the group that received the conventional treatment, there were not statistically significant differences from baseline to the other two time points examined.

Comparison of fecal microbiome composition in *H. pylori*–infected patients between treatment groups

At the end of the 2-week treatment period, alpha diversity evenness was higher in the group of individuals that received the probiotic ($P = 0.0156$). In contrast, beta diversity comparison was similar in the two treatment groups. Strikingly, on relative abundance analysis, patients that received *S. boulardii* showed a lower abundance of Bacteroides (*Prevotella*) and Clostridia (*Lachnospira* and *Ruminococcus*) after the 2-week treatment, while they presented a higher abundance of Gammaproteobacteria (*Escherichia* spp. and another Enterobacteriaceae OTUs) (Fig. 3). Finally, 1 month after completion of treatments, these differences in relative abundance between the two groups were maintained.

Discussion

These results indicate that patients who received *S. boulardii* as a complement to triple therapy for *H. pylori* infection had a significantly lower prevalence of occurrence of abdominal pain. Results also show a trend toward a lower occurrence of other gastrointestinal side effects accompanied by a greater bacterial diversity and lower abundance of Bacteroides and Clostridia, and a higher abundance of Enterobacteria immediately at the end of antibiotic treatment and 1 month later.

Previous work in children and adults showed that the addition of *S. boulardii* to proton-pump inhibitor triple therapy significantly reduced antibiotic side effects. Zhao et al. show that children infected with *H. pylori* that received amoxicillin 40 mg/(kg day), clarithromycin 15 mg/(kg day), and omeprazole 0.7–0.8 mg/(kg day), plus *S. boulardii* (250 mg) had a significantly lower incidence of diarrhea, constipation, and stomatitis than children that received triple therapy without *S. boulardii* [42]. In a similar study in adults, the addition of *S. boulardii* (250 mg) to standard triple therapy resulted in a lower frequency of side effects including nausea, diarrhea, abdominal discomfort, and bloating compared with patients that received antibiotic therapy alone [43, 44]. In agreement with these studies, we also observed a lower occurrence of abdominal pain in patients who received S. *boulardii*. Together, current evidence indicates that the addition of S. *boulardii* to antibiotic treatment for *H. pylori* infection significantly attenuates gastrointestinal side effects [27, 45].

Though a number of mechanisms have been postulated to explain the positive effects of *S. boulardii* in the treatment of *H. pylori* infection [29–31], there is very little information on its impact on the gut microbiota in this context. Wang et al. using culture-based techniques described quantitative and qualitative changes in gut microbiota in subjects that receive conventional anti-*H. pylori* therapy with or without S. *boulardii* supplementation [30]. They reported an increase in the numbers of colony-forming units of total aerobes per gram of wet feces after both antibiotic treatment alone and the combination of antibiotics with *S. boulardii* supplementation; however, they did not

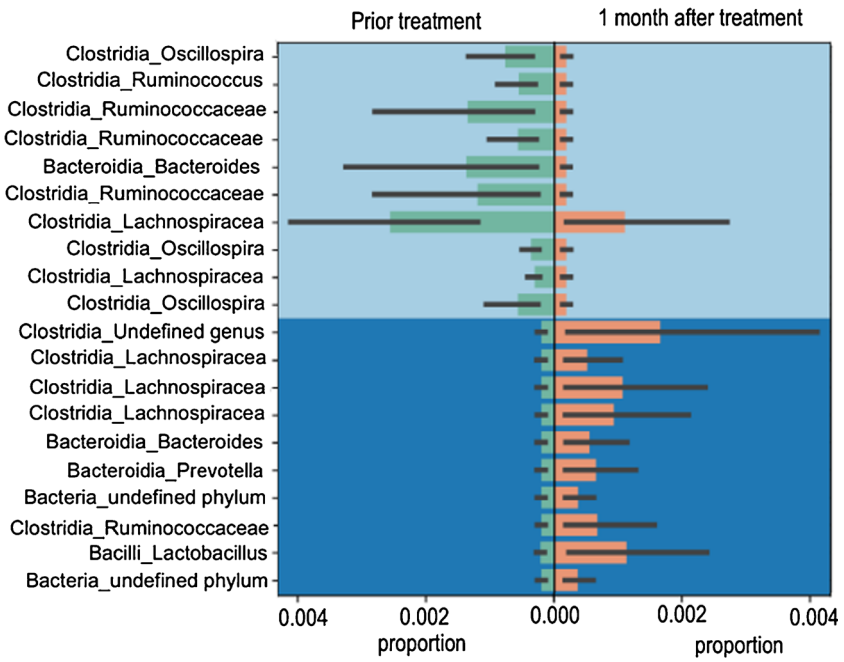

Fig. 2 Statistically significant differences in relative abundance between fecal samples collected at baseline-S1 vs S3

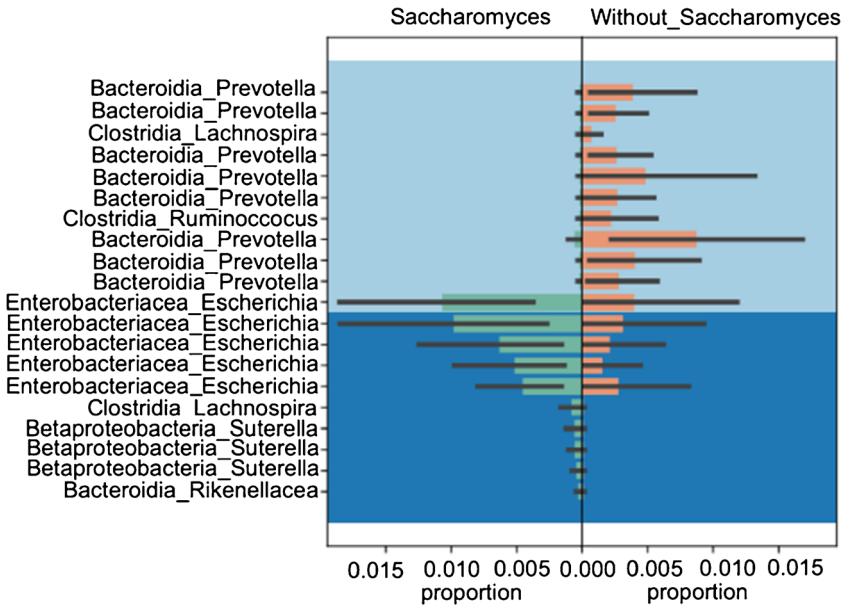

Fig. 3 Statistically significant differences in relative abundance between the two treatmetn groups at S2

observe changes in the number of anaerobes. These changes were not evident when reassessed 71 days after completing concomitant therapy. In that study, there were no significant differences between treatment groups. Several limitations of Wang's study including those inherent to microbiota culture (e.g., failure to grow, difficulties in microbe identification, low selectivity of culture media used); low number of patients in each treatment group ($n = 10$) and that not all of them completed the study preclude definitive conclusions regarding changes in the microbiome [30].

Alpha diversity analysis in the current study showed an increased diversity in the group that received S. *boulardii*. To our knowledge, this is the first report on alpha diversity in the context of *H. pylori* infection or eradication therapies. The current study also reports a lower abundance of Clostridia and Bacteroides in the group that received the probiotic. These strains have been previously implicated as antibiotic multiresistant and pro-inflammatory [46]; however, Clostridia and Bacteroides are considered key regulators on the gut microbiome homeostasis [47]. We also observed higher abundance of Enterobacteriaceae in patients that received *S. boulardii*. In this regard, a study with mice showed that treatment with vancomycin increased Enterobacteriaceae, and this was associated with higher intestinal loads of *C. albicans* and *S. boulardii* [48]; these changes were not observed in mice treated with colistin or untreated animals [48]. The authors concluded that in a non-colitis context, Enterobacteriaceae interact with fungi, including *S. boulardii*, in a manner that favors their colonization [48]. It is possible that supplementation of *S. boulardii* increases Enterobacteriaceae levels in the intestinal microbiota. It will be important to study interactions between fungi and specific bacteria to unravel dynamics between probiotics, microbiota, and the host.

We acknowledge as a limitation of the present work that a significant number of patients that received the diagnosis of *H. pylori* infection decided not to receive antibiotic treatment during the study period and, also, not all the patients that were randomly assigned to treatment groups completed the study; both factors could have introduced selection bias. Additionally, because there was lack of laboratory methods to assess eradication rates in Ecuador at the time the trial was performed, we could not measure this directly. However, present findings agree with previous observations and add new information on microbiota changes associated with *S. boulardii* CNCM I-745 consumption. Further studies are necessary to expand on these findings.

Conclusions

The addition of *S. boulardii* CNCM I-745 as a complement to conventional triple therapy for *Helicobacter pylori* infection decreased the frequency of gastrointestinal side effects that could be related to changes in gut microbiota. The addition of *S. boulardii* CNCM I-745 increased bacterial diversity, lowered the abundance of *Bacteroides* and *Clostridia*, and increased the abundance of Enterobacteria.

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Formal analysis: PC, MF, MEB.
Funding acquisition: HC, MEB, PC.
Investigation: IS, OC, MEB, PC, MF, DG, BP, NF.
Methodology: MEB, PC, MF, HC, IS, OC.

Project administration: MEB, PC, HC, MF.
Resources: MEB, PC, MF, IS, OC.
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Visualization: PC, DG, BP, NF, MF, HC, IS, OC, MEB.
Writing – original draft: MEB, PC, MF.
Writing – review and editing: PC, DG, BP, NF, MF, HC, IS, OC, MEB.

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Compliance with ethical standards

The human subjects Protection Committee at Universidad de Las Américas approved the study. Patients signed an informed consent form after receiving a full explanation of the research protocol to be included in the study.

Disclosure Biocodex was not involved in study design, patient recruitment, and data analysis.

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