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ORIGINAL RESEARCH ARTICLE

Efficacy and Safety of the Adjuvant Use of Probiotic *Bacillus clausii* Strains in Pediatric Irritable Bowel Syndrome: A Randomized, Double-Blind, Placebo-Controlled Study

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Abstract

Objectives Current irritable bowel syndrome (IBS) treatments have limited efficacy and probiotics like *Bacillus clausii* (*B. clausii*) were found to be effective in the management of several gastrointestinal disorders. This phase III trial assessed the efficacy and safety of adding *B. clausii* (four strains: O/C, N/R, SIN, T), versus placebo, to conventional treatment of pediatric IBS in Mexico.

Methods Patients aged 6–17 years 11 months with IBS (Rome IV) for at least 2 months were randomized to receive either *B. clausii* (oral suspension, total dose 4 billion spores/day) or placebo once daily for 8 weeks. All patients also received conventional treatment. The primary endpoint was the difference in the proportion of patients with clinical improvements at Week 8 (Global Assessment Questions [GAQ]). Secondary endpoints included responders by Subject's Global Assessment of Relief for Children with IBS (SGARC); number/consistency of stools; abdominal distention/bloating; abdominal pain/intensity; and IBS behavior.

Results 73.6% (95% confidence interval [CI] 67.3–80.0; *B. clausii* $n = 129$) and 78.5% (95% CI 72.5–84.4; placebo $n = 130$) of patients had symptom improvement ($p = 0.8182$). For Week 8 SGARC, 19.2% (*B. clausii*) and 20.9% (placebo) reported complete symptom relief. Stool evaluations, bloating, abdominal pain/intensity, and IBS behavior were similar between groups. Both treatments were well tolerated.

Conclusion No significant differences in efficacy between *B. clausii* and placebo were demonstrated in addition to conventional treatment. The sample size calculation was based on an expected placebo/conventional treatment response of 30–40%. However, the actual treatment response observed was 80% and, thus, a study with larger population would be warranted. In addition, this study was conducted during the COVID-19 pandemic, when such controlled social conditions may have resulted in better diet, greater family stability, less psychological stress, and lower risk of infections exacerbating IBS, thereby improving symptoms in both groups.

EudraCT number 2018-004519-31.

1 Introduction

The estimated prevalence of irritable bowel syndrome (IBS) in children ranges from 2.8 to 22.6% (Rome III or IV criteria), reflecting geography and study types [1]. IBS is linked to reduction in quality of life [1], stress, anxiety, depression, and emotional problems [2], and results in absenteeism [3]. Thus, IBS is considered both a health and a socioeconomic burden.

Curative treatment for IBS is currently not available and current management strategies vary. Conventional treatment includes diet adjustment and confirmation [4], explanation of pain experience and reassurance, symptomatic treatments such as laxatives for constipation and possibly analgesics [5] or antispasmodics [6], with limited efficacy. Pharmacological therapy may reduce discomfort caused by diarrhea [7] or

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Key Points

Probiotics targeting the gut microbiota have beneficial effects in functional gastrointestinal disorders in children and adults.

This phase III trial in children with IBS demonstrated that treatment with *Bacillus clausii* strains as add-on to conventional treatment was well tolerated; although no additional benefit was seen in relieving symptoms over 8 weeks compared with placebo with conventional treatment, significant improvements in abdominal pain episodes (Week 4) and significant decreases in abdominal bloating in patients with IBS with constipation occurred with *B. clausii* versus placebo.

Placebo responses were higher than expected; as the study was conducted under controlled social conditions during the COVID-19 pandemic, improved conditions (e.g., better diet, greater family stability, reduced school-induced stress, lower risk of infections) may have improved IBS symptoms in both groups.

constipation [8]. Antidepressants and psychological therapies are also used in IBS [9]; although there are concerns with using antidepressants in children.

Gut microbiota dysbiosis is increasingly considered as a vital factor in the etiopathogenesis of IBS; thus, gut microbiota are a potential therapeutic target [10]. A commercial probiotic (Enterogermina®) comprises four strains of *Bacillus clausii* (*B. clausii*; O/C, N/R, SIN, T) [11]. *B. clausii* has demonstrated beneficial effects in adults, such as in ameliorating bacterial overgrowth [12], acute diarrhea [13, 14], diarrhea associated with *Clostridium difficile* [15], and as an adjuvant in treating *Helicobacter pylori* [16, 17]. Importantly, benefits of *B. clausii* have also been demonstrated in children, such as in antibiotic-associated diarrhea [18, 19], diarrhea [13, 14, 20–25], and rotavirus-associated diarrhea [26, 27].

Data from a small pilot study in a third-level hospital in Mexico demonstrated that *B. clausii* treatment significantly improved several symptoms in children with IBS [28]. Consequently, the phase III trial reported herein investigated the efficacy and safety of *B. clausii* plus conventional treatment, compared with placebo plus conventional treatment, in children with IBS in Mexico.

2 Methods

2.1 Study Design and Patients

BaclauSII (EudraCT number: 2018-004519-31) was a phase III, multicenter, randomized, placebo-controlled, double-blind, parallel clinical trial conducted at 15 study sites in Mexico. There were three study phases, a 2-week run-in (in which ongoing IBS treatment was continued), an 8-week treatment period, and an 8-week follow-up period (no treatment) (Supplementary Fig. 1, see electronic supplementary material [ESM]), which are in keeping with published recommendations [29, 30].

Male or female patients aged 6–17 years and 11 months with IBS (Rome IV criteria [31]) were eligible. IBS diagnostic criteria had to be met for at least 2 months before diagnosis, and had to include all of the following: abdominal pain for ≥ 4 days per month (associated with > 1 of the following: related to a bowel movement; a change in stool frequency; a change in stool appearance); in children with constipation, the pain did not resolve with resolution of the constipation (i.e., not functional constipation); after appropriate evaluation, the symptoms cannot be fully explained by another medical condition.

Key exclusion criteria included treatment with antibiotics or probiotics within 2 months prior to screening; growth failure or malnutrition; previous abdominal surgery; known gastrointestinal comorbidity (e.g., inflammatory bowel disease, celiac disease, *H. pylori* infection); lactose intolerance, without a diet eliminating lactose; history of bleeding from the low digestive tract in the 2 years prior or had abnormal endoscopic or histological studies; history of significant infections or inflammatory processes during pre-enrollment; any patient not suitable for participation, regardless of reason, as judged by the Investigator (including medical or clinical conditions, or patients potentially at risk of noncompliance to study procedures).

2.2 Ethics

The protocol was approved by Independent Ethics Committees and Research Committees. The study was conducted in accordance with all applicable laws, rules, and regulations, and with the Declaration of Helsinki and the International Council for Harmonisation guidelines for Good Clinical Practice. Parents/legal guardians provided written, informed consent on behalf of their child at the time of enrollment. An informed assent form was signed by the patient, if applicable (age of assent was determined by the Independent Ethics Committees and Research Committees).

2.3 Treatments

Patients were centrally randomized 1:1 to treatment with either *B. clausii* or placebo using an interactive web response system.

Bacillus clausii preparations consisted of spores at 2×10^9 colony forming units (CFU) in 5 mL of a ready-to-drink,

oral suspension (total dose 4×10^9 CFU/day), which was an odorless, colorless, and insipid liquid. Placebo preparations were developed to exactly mimic the physical characteristics of the *B. clausii* preparations. Patients, investigators, and other personnel were blinded to treatment; kits were only distinguishable by the randomization number. Treatment unblinding was only conducted in response to an adverse event (AE) requiring additional care.

Patients swallowed the contents of two appropriate 5-mL vials once daily every morning before a meal for 8 weeks. Both groups had access to the usual standard of care as per general recommendations (see ESM). On day 1 (study start) and day 28 (±3 days), a sufficient number of vials of either *B. clausii* or placebo were dispensed to each patient/parent/legal guardian to cover the time period until the next visit. Full instructions were given to patients/parents/legal guardians for study and conventional treatments (including storage conditions for study treatments, i.e., at room temperature [not more than 30 °C] away from direct light and moisture). Compliance with storage conditions was not monitored. There is evidence that *B. clausii* can survive in liquid media and even grow at temperatures of 20–45 °C [32]. Each patient had a diary for data recording by the patient/parent/legal guardian. These diaries were to capture the frequency and severity of their IBS symptoms, to meet recommendations that IBS clinical trials should account for fluctuations in symptoms and the potential for wide variations in bowel habits [29, 33, 34]. Evaluation visits were conducted at Weeks 4 and 8 (end of treatment). The follow-up period (no treatment) was for 8 weeks and patients were evaluated at the end of this period (Week 16) (Supplementary Fig. 1, see ESM).

2.4 Study Assessments

Treatment intake was recorded daily in the diary. The primary endpoint was the difference in the proportion of treatment responders between groups after 8 weeks of treatment. Response rate at Week 8 was defined as patients with clinical improvement of symptoms in the following Global Assessment Questions (GAQ): "How well did the medication relieve your symptoms? (satisfaction with treatment; excellent, good, fair, poor, failed)" rated as excellent or good and "Overall, how do you feel your problem is? (symptom relief; worse, same, better)" rated as better. The GAQ assesses the patient's overall relief of symptoms (wellbeing, symptoms of stomach discomfort, pain, and altered bowel habits) [34–37]. Several secondary endpoints were evaluated (i.e., response rate at Week 4; proportion of responders at Weeks 4 and 8; number of stools/day; stool consistency; abdominal distention/bloating; abdominal pain episodes by day; pain intensity; and IBS behavior). Response rate at Week 4 was assessed by GAQ. Proportion of treatment responders at Weeks 4 and 8 was evaluated by the Subject's Global Assessment of Relief for Children with IBS (SGARC) [38–40]; treatment response was defined as 0 = complete relief or 1 = considerable relief. At Weeks 4, 8, and 16, records were made in patient diaries for number of stools by day; consistency of stools (Bristol Stool Form Scale); abdominal distention/bloating (3-point scale); number of abdominal pain episodes by day; pain intensity (Face Pain Scale–Revised); and IBS behavior (Behavior Scale). These secondary endpoints are considered to be reliable measurements to evaluate the clinically important signs and symptoms associated with IBS [29, 33].

Safety was assessed by AE reporting in the diaries.

2.5 Data Analyses

Sample size was calculated for the primary endpoint. Based on certain assumptions (see ESM), 105 patients per group would be necessary to observe a success rate of at least 65% in the *B. clausii* arm (a 20% difference between groups), with 90% power and 5% significance level (one-sided). Considering a drop-out rate of ~ 20%, 260 randomized patients (130/group) were needed.

Demographics and baseline characteristics were summarized by using descriptive statistics (including *n*, %, mean and standard deviation [SD]) for each treatment group.

Treatment compliance (%) was defined as (administered doses/planned doses) × 100 and was summarized descriptively. A patient was considered treatment compliant if treatment intake was ≥ 80%. Treatment accountability (%) was defined as (used returned vials/planned doses) × 100.

The primary endpoint was evaluated by the Chi-square test or Fisher Exact test; 95% confidence intervals (CIs) were computed for the proportions in each treatment group and for the difference in the proportions between groups. The main analysis was conducted on the intent-to-treat (ITT) population with missing data considered as non-responders. A sensitivity analysis was repeated (no replacement of missing data) for the ITT population, and a supportive analysis was performed for the per protocol (PP) population.

Analyses of secondary endpoints were performed for the ITT population. In addition to descriptive statistics, several secondary endpoints were compared between treatment groups using the Chi-square test or Fisher Exact test (if the assumption of the Chi-square test was not verified). Percentage of bloating days and mean number of abdominal pain episodes were evaluated using The Mann-Whitney *U* test or Student's *t*-test for independent samples.

In a subgroup analysis, the primary endpoint was analyzed by concomitant medication use and by IBS type. In

an exploratory analysis, secondary endpoints were analyzed by IBS type.

AE incidences were summarized descriptively.

3 Results

3.1 Disposition and Baseline Characteristics

Overall, 311 patients were screened, and 259 (*B. clausii* $n = 129$; placebo $n = 130$) were randomized and treated (ITT population) (Fig. 1). In total, 253 patients (97.7%) (*B. clausii* $n = 124$, placebo $n = 129$) completed the study (Fig. 1). Reasons for discontinuation were withdrawal of consent (*B. clausii* $n = 3$); protocol violation (1 patient/group); and 'other' reasons (*B. clausii* $n = 1$).

Baseline demographic and clinical characteristics were generally well balanced between groups (Table 1). Of the laboratory parameters evaluated at Week −2 (see ESM), only one patient (0.8%) per group had an abnormal result with clinical significance for parasite examination seriate. IBS medication was taken before study start by 24% (31/129) and 26.2% (34/130) of patients in the *B. clausii* and placebo groups, respectively; the most frequent of which was in the alimentary tract and metabolism drug class (21.7% [28/129] vs 24.6% [32/130]). Within this class, drugs for functional gastrointestinal disorders were taken by 13.2% (17/129) and 13.8% (18/130) of patients, and drugs for constipation by 7.8% (10/129) and 6.2% (8/130) of patients in the *B. clausii* and placebo groups, respectively. Patients also took concomitant medication during the study (Table 1), the most common of which were drugs in the alimentary tract and metabolism drug class. Of these patients, 18.6% (24/129) and 25.4% (33/130) of patients in the *B. clausii* and placebo groups, respectively, took antispasmodics during the study period.

3.2 Treatment

In the safety population (patients receiving at least one dose of the appropriate formulation; *B. clausii* $n = 129$, placebo $n = 130$), mean (SD) of treatment exposure was 56.64 (6.23) and 57.67 (3.71) days for *B. clausii* and placebo, respectively. Treatment compliance was assessed in this population, and there was a total of 22 missing values related to product use (*B. clausii* 12 values missing, placebo 10 missing values). Thus, treatment compliance of > 80%, from diary cards, was seen in 81/117 (69.2%; *B. clausii*) and 77/120 (64.2%; placebo) of patients. In contrast, mean (SD) treatment accountability, from returns of used vials,

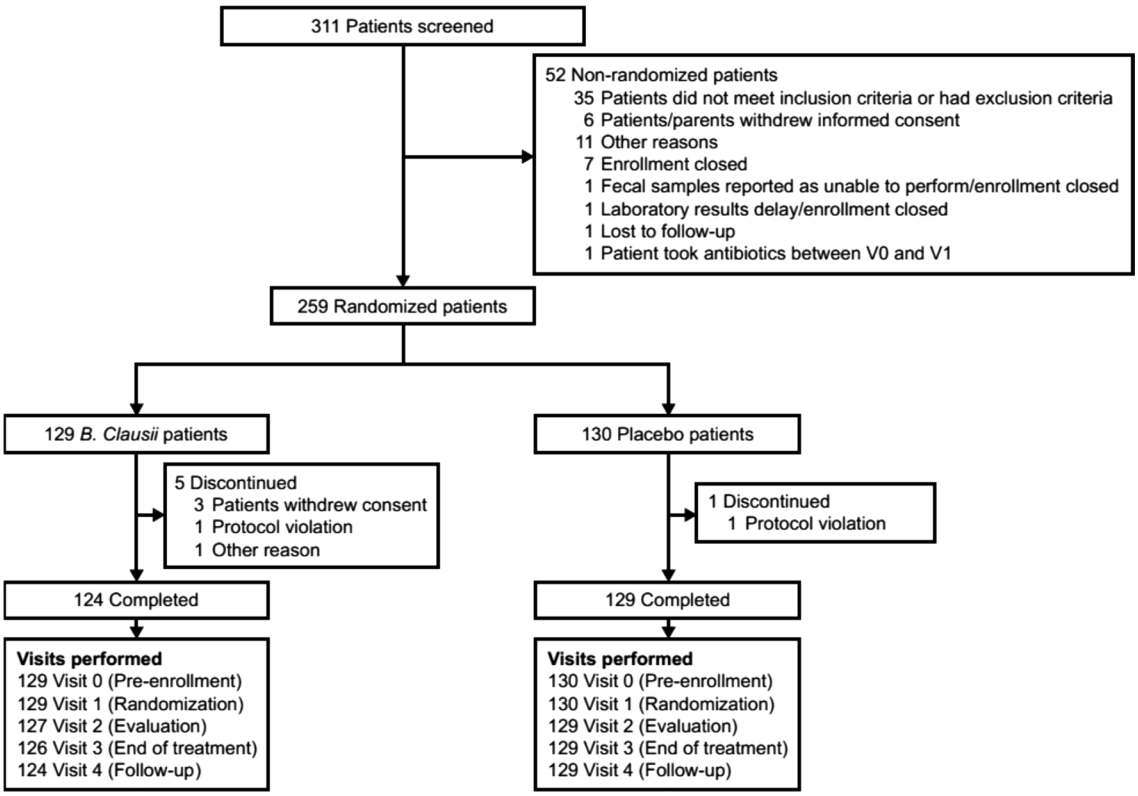

Fig. 1 Patient disposition. *B. clausii Bacillus clausii*, *V0* pre-enrollment visit, *V1* randomization visit. *Visit 2* = Week 4 of the study; *Visit 3* = Week 8 of the study; *Visit 4* = Week 16 of the study (8-week follow-up period)

Table 1 Baseline demographic and clinical characteristics

	B. clausii ($n = 129$)	Placebo ($n = 130$)
Gender, *n* (%), females	76 (58.9)	81 (62.3)
Age[a], years, mean (SD)	10.93 (3.30)	11.19 (3.12)
Origin, *n* (%)		
American Indian (Mestizo)	111 (86.0)	114 (87.7)
Caucasian/White	18 (14.0)	16 (12.3)
BMI (kg/m^2), mean (SD)	18.93 (4.31)	19.20 (4.06)
Any medical history, *n* (%), yes	34 (26.4)	28 (21.5)
Surgery	3 (8.8)	3 (10.7)
Other	31 (91.2)	26 (92.9)
IBS type, *n* (%)		
IBS-C	58 (45.0)	56 (43.1)
IBS-D	28 (21.7)	36 (27.7)
IBS-M	42 (32.6)	36 (27.7)
Unclassified IBS	1 (0.8)	2 (1.5)
Duration of IBS, months, mean (SD)	13.86 (22.85)	12.31 (16.13)
IBS symptoms[b], *n* (%)		
Abdominal pain	128 (99.2)	130 (100.0)
Abnormal form of stools	115 (89.1)	104 (80.0)
Abnormal passage of the bowel movements	52 (40.3)	55 (42.3)
Mucus in the stools	18 (14.0)	15 (11.5)
Symptoms of gas (flatulencies, perception of retention of gas and bloating)	94 (72.9)	84 (64.6)
Visceral hypersensitivity	4 (3.1)	5 (3.8)
Rectal or gastric hyperalgesia	5 (3.9)	7 (5.4)
Psychological distress (anxiety, depression, impulsiveness, anger)	37 (28.7)	43 (33.1)
Dyspepsia	17 (13.2)	16 (12.3)
Headache	19 (14.7)	23 (17.7)
Low back pain	8 (6.2)	8 (6.2)
Insomnia	3 (2.3)	3 (2.3)
Fatigue	10 (7.8)	7 (5.4)
Pollakiuria (urinary urgency)	0	1 (0.8)
Persistent vomiting	3 (2.3)	1 (0.8)
Nocturnal diarrhea	4 (3.1)	2 (1.5)
Dysphagia	0	1 (0.8)
Unintentional weight loss	1 (0.8)	0
Unexplained fever	0	1 (0.8)
Other	13 (10.1)	23 (17.7)
Any concomitant medication[c], *n* (%), yes	86 (66.7)	83 (63.8)
Alimentary tract and metabolism	58 (45.0)	58 (44.6)
Nervous system	40 (31.0)	33 (25.4)
Musculo-skeletal system	33 (25.6)	30 (23.1)
Respiratory system	29 (22.5)	23 (17.7)
Anti-infectives for systemic use	13 (10.1)	15 (11.5)
Dermatologicals	3 (2.3)	1 (0.8)
Cardiovascular system	1 (0.8)	2 (1.5)
Genito urinary system and sex hormone	0	3 (2.3)
Sensory organs	2 (1.6)	0
Systemic hormonal preparations, excl. sex hormones and insulins	2 (1.6)	0
Blood and blood forming organs	0	1 (0.8)

B. clausii Bacillus clausii, *BMI* body mass index, *IBS* irritable bowel syndrome, *IBS-C* IBS with constipation, *IBS-D* IBS with diarrhea, *IBS-M* mixed IBS, *SD* standard deviation

[a]Age at Week − 2

[b]Each patient could report more than one symptom

[c]By Anatomical Therapeutic Chemical Code

was 95.60% (13.8) and 98.05% (8.48) for *B. clausii* and placebo, respectively. The difference between treatment compliance and treatment accountability was due to a discrepancy between the diary card information registered by the patients/caregivers compared with the treatment accountability measured by counting the actual vials consumed.

The PP population included 146 patients (*B. clausii* $n = 75$, placebo $n = 71$). Compliance was the key reason for exclusion from the PP population (*B. clausii* 45/129 [34.9%]; placebo 52/130 [40.0%]). Data from the PP population were only analyzed for the supportive analysis of the primary endpoint.

3.3 Proportion of Patients with Clinical Improvements at Week 8

For the primary endpoint (ITT analysis), 73.6% (95% CI 67.3–80.0) of patients had clinical improvement of symptoms with *B. clausii* versus 78.5% (95% CI 72.5–84.4) with placebo; $p = 0.8182$ (Table 2). The percentage of patients reporting excellent or good satisfaction with treatment was similar between groups (80.0% *B. clausii;* 80.6% placebo) (Supplementary Fig. 2, see ESM). For *B. clausii*, 28.8% of patients rated treatment as excellent in relieving symptoms, and 51.2% as good; whilst, with placebo, 33.3% and 47.3% of patients rated the treatment excellent and good, respectively. Considering overall symptom relief, 82.4% (*B. clausii*) and 90.7% (placebo) of patients felt better (Supplementary Fig. 2, see ESM).

Results of the sensitivity analyses were consistent with the primary analyses, and there were no significant differences between the groups (Table 2). Supportive analyses of the primary endpoint (PP population) showed that 80.0% (95% CI 72.4–87.6) of patients had clinical improvement of symptoms with *B. clausii* versus 78.9% (95% CI 70.9–86.8) of patients in the placebo group; $p = 0.4332$. The difference in proportions between groups was 1.1% (95% CI − 9.9 to 12.1). The percentage of patients reporting an excellent or good treatment satisfaction was similar between groups (33.3% *B. clausii;* 31.0% placebo).

3.4 Key Secondary Efficacy Endpoints

As the primary endpoint was not statistically significant, inferential analyses were not performed for secondary endpoints. Hence, *p*-values are shown for descriptive purposes only.

For the response rate at Week 4, 72.4% (92/129 *B. clausii*) and 76.7% (99/130 placebo) of patients showed clinical improvement in symptoms; $p = 0.7855$. For the clinical improvement of symptoms, assessed with SGARC at Week 8, 19.2% (24/126 *B. clausii*) and 20.9% (27/129 placebo) reported complete relief of symptoms (Supplementary Fig. 3, see ESM). Most patients reported considerable relief (56.8%, *B. clausii*; 64.3%, placebo). Overall, 76.0% of patients had a positive response to *B. clausii*, and 85.3% to placebo ($p = 0.9694$).

Results of stool evaluations and bloating were similar between groups with no significant differences (Supplementary Figs. 4–6, see ESM).

At Week 4, the median number of abdominal pain episodes (mean by day) was higher with placebo (0.55) versus *B. clausii* (0.47) ($p = 0.0302$). At Weeks 8 and 16, the

Table 2 Clinical improvement of symptoms at Week 8 (primary endpoint)

	B. clausii ($n = 129$)	Placebo ($n = 130$)
Clinical improvement of symptoms at Week 8 with missing values replacement[a], *n* (%)		
Yes (responders)	95 (73.6)	102 (78.5)
No (non-responders)	34 (26.4)	28 (21.5)
95% CI for proportion of responders	67.3–80.0	72.5–84.4
Difference in proportions between groups (*B. clausii* – placebo), % (95% CI)	− 4.8 (− 13.5 to 3.9)	
p-value	0.8182	
Clinical improvement of symptoms at Week 8 without missing values replacement[a], *n* (%)		
Yes (responders)	95 (76.0)	102 (79.1)
No (non-responders)	30 (24.0)	27 (20.9)
95% CI for proportion of responders	69.7–82.3	73.2–85.0
Difference in proportions between groups (*B. clausii*—placebo), % (95% CI)	− 3.1 (− 11.7 to 5.5)	
p-value	0.7212	

B. clausii Bacillus clausii; *CI* confidence interval

[a]Clinical improvement of symptoms was defined as 'Excellent' or 'Good' to the questions "How well did the medication relieve your symptoms?" and 'Better' in "Overall how do you feel your problem is?" Missing values are assumed as non-responders

p-values are from the Chi-square test

median number of episodes was similar between groups (Supplementary Fig. 7, see ESM).

Percentage of abdominal pain episodes with a given pain intensity are shown in Supplementary Fig. 8 (see ESM). The median percentage with pain intensity 2 at Week 4 was 56.4% (*B. clausii*) and 69.2% (placebo; $p = 0.0285$). At Weeks 8 and 16, pain intensity 2 was the most frequently experienced pain intensity level in both groups. As pain intensity increased, the median percentage decreased at all timepoints (Supplementary Fig. 8, see ESM).

The categorical assessment of IBS behavior was similar between groups, with no significant differences at any timepoint. For all categories, most patients in both groups throughout the study considered that there were no disturbances in daily activities (Supplementary Fig. 9, see ESM).

3.5 Subgroup Analyses

For the subgroup analysis of the primary efficacy endpoint according to concomitant medication class (data not shown) and IBS type (Supplementary Table 1, see ESM), there were no statistically significant differences between the groups.

3.6 Exploratory Analyses

For the exploratory analyses of secondary endpoints, only three comparisons were statistically significant between the groups.

For abdominal distention/bloating by IBS type, the overall results were similar between groups and most patients reported feeling 'better' throughout the study. At Week 8, 70.4% of patients with IBS with diarrhea (IBS-D) in the *B. clausii* group rated their abdominal distention/bloating as 'better', which was significantly lower ($p = 0.0432$) than the placebo group (88.9%) (Supplementary Fig. 10, see ESM).

For patients with IBS with constipation (IBS-C), at Weeks 4 and 16 the median percentage of days with bloating was significantly lower in patients receiving *B. clausii* versus those receiving placebo: 8.1 versus 18.4 (Week 4; $p = 0.0448$) and 3.6 versus 9.5 (Week 16; $p = 0.0224$) (Supplementary Fig. 11, see ESM). No differences in bloating were seen at Week 8 in patients with IBS-C, or in patients with IBS-D or mixed IBS (IBS-M) at any timepoint (Supplementary Fig. 11, see ESM).

3.7 Safety and Tolerability

Overall, 52.7% (68/129, *B. clausii*) and 50.8% (66/130, placebo) of patients had at least one AE (Table 3). One serious AE was reported in the *B. clausii* group, comprising a case of febrile multisystem inflammatory syndrome of moderate severity lasting 2 days, which was not related to *B. clausii* and did not require medical intervention. The cause of this inflammatory syndrome was unknown, and COVID-19 testing was not performed in this study.

Overall, 44.2% (57/129, *B. clausii*) and 35.4% (46/130, placebo) of patients had at least one treatment-emergent AE (TEAE) (Table 3). Nine TEAEs in seven patients in the *B. clausii* group (2 patients discontinued treatment due to rhinopharyngitis [$n = 1$], and testing positive for pathogen parasites) and six TEAEs in five patients in the placebo group were treatment related. Incidences of TEAEs were similar between the groups (Table 3). The most frequently reported TEAEs by system organ class were upper respiratory infections (influenza was the most frequent in both groups), and nervous system disorders (headache was the most frequent in both groups). Vital signs (systolic blood pressure, diastolic blood pressure, heart rate, temperature) were similar between both groups throughout the study (data not shown).

4 Discussion

IBS symptom relief in children was found to be very high in both *B. clausii* and placebo groups, which exceeded expectations. There were no significant differences between groups for proportion of patients with clinical improvements at Week 8 or any of the key secondary endpoints. The AE profile was similar between groups.

More IBS treatments are needed to support conventional therapies [4–9]. In a meta-analysis of 20 IBS randomized clinical trials (RCTs), probiotics improved global IBS symptoms versus placebo (pooled relative risk 0.77, 95% CI 0.62–0.94) [41]. Probiotics significantly reduced abdominal pain (mean difference −1.15, 95% CI −2.05 to −0.24) in an analysis of nine RCTs in children with IBS [40]. Thus, gut microbiota remain as a good IBS therapeutic target [1, 10, 42].

Enterogermina® (four *B. clausii* strains) is indicated for treating disturbances of intestinal bacterial microbiota. *B. clausii* significantly improved symptoms in children with diarrhea [13, 14, 18–27]. In a study in children (6–12 years, $n = 15$) with IBS, *B. clausii* plus conventional therapy resulted in significantly more patients with overall symptom improvement and bowel movement normalization, significantly fewer patients with abdominal bloating, and significantly lower pain intensity and number of pain events versus conventional treatment [28]. This larger RCT, BaclauSII, aimed to fill a data gap for children with IBS.

There are several potential reasons why BaclauSII was not able to demonstrate an overall positive adjuvant effect with *B. clausii* versus placebo on IBS symptoms. BaclauSII was powered to detect a 20% difference between groups, thus, the very high placebo responses may have masked any additional benefit of *B. clausii*. Placebo effects occur

Table 3 Summary of AEs and TEAEs

	B. clausii ($n = 129$)		Placebo ($n = 130$)	
	n (%)	AEs/TEAEs	*n* (%)	AEs/TEAEs
Any AE	68 (52.7)	137	66 (50.8)	144
Any TEAE	57 (44.2)	97	46 (35.4)	91
Any TEAE of special interest	0	0	0	0
Any SAE	1 (0.8)[a]	1	0	0
Any serious TEAE	0	0	0	0
Any TEAE related to the study medication	7 (5.4)	9	5 (3.8)	6
Any serious TEAE related to the study medication	0	0	0	0
Any TEAE leading to discontinuation of study medication	2 (1.6)	2	0	0
Any serious TEAE leading to discontinuation of study medication	0	0	0	0
AE leading to death	0	0	0	0
TEAE leading to death	0	0	0	0
TEAEs by SOC and PT (≥3% of patients in either group)				
Infections and infestations	26 (20.2)	31	24 (18.5)	29
Nasopharyngitis	4 (3.1)	4	4 (3.1)	4
Pharyngitis	4 (3.1)	5	4 (3.1)	4
Influenza	7 (5.4)	7	8 (6.2)	8
Nervous system disorders	11 (8.5)	15	17 (13.1)	19
Headache	11 (8.5)	15	15 (11.5)	17
Gastrointestinal disorders	9 (7.0)	11	11 (8.5)	12
Injury, poisoning and procedural complications	10 (7.8)	12	5 (3.8)	6
Overdose	5 (3.9)	7	3 (2.3)	4
Respiratory, thoracic and mediastinal disorders	8 (6.2)	9	7 (5.4)	8
Musculoskeletal and connective tissue disorders	4 (3.1)	4	2 (1.5)	2
Reproductive system and breast disorders	2 (1.6)	4	4 (3.1)	6

AE adverse event, *B. clausii Bacillus clausii*, *PT* preferred term, *SAE* serious adverse event, *SOC* system organ class, *TEAE* treatment-emergent adverse event

[a]This SAE was a case of febrile multisystem inflammatory syndrome of moderate severity lasting 2 days, which was not related to *B. clausii* and no medical intervention was required

in gastroenterology RCTs more than in other diseases [43]. Moreover, the placebo response in gastroenterology (visceral pain, nausea) has neuro- and psychobiological properties along the gut–brain axis [43], a key target for probiotics in IBS [1, 42]. In addition, BaclauSII was conducted under strictly controlled social conditions during the COVID-19 pandemic. Isolation at home is likely to have resulted in more diet control and greater family stability. A recent survey of adults with IBS reported a significant decrease in severe IBS symptoms during lockdown in the COVID-19 pandemic compared with pre-pandemic data, possibly due to reduced external stressors [44]. Diet directly modifies the composition of the intestinal microbiota [45]; thus, recommendations to follow a healthy diet according to age as part of the conventional treatment could have led to the improvement seen in the placebo group. Indeed, better adherence to the conventional therapy may also have occurred during social isolation in the COVID-19 pandemic, thereby also contributing to improved IBS symptoms in both groups; although adherence to conventional therapy was not assessed in this study.

Furthermore, the children were not exposed to school pressure, and had a lower risk of infections that could reduce IBS symptoms, including gastrointestinal diarrhea. Such favorable conditions may explain the excellent response to conventional therapy. Although these considerations are speculative, it remains to be defined whether the placebo results in BaclauSII would be reproducible in normal everyday life. Recent probiotic studies in functional gastrointestinal disorders are conducted in the context of improving global health outcomes, and demonstrating global improvements with probiotics is becoming more challenging.

Some significant differences were seen in BaclauSII. Median percentage of days with bloating was lower in patients with IBS-C in the *B. clausii* group versus placebo. Bloating is common in IBS [46]. However, two RCTs showed that other probiotics did not impact the rate of bloating versus placebo (relative risk 0.32; 95% CI 0.04–2.56)

[40]. In addition, median number of abdominal pain episodes was lower with *B. clausii* compared with placebo at Week 4.

Various probiotic strains have been shown to have beneficial effects in children with IBS [47]. *Escherichia coli* strains significantly improved symptoms in children with chronic IBS [48]. *Bacillus coagulans* with prebiotics significantly improved response rate versus placebo in children with functional abdominal pain [49]. Trials with *Bifidobacterium* strains have demonstrated significant abdominal pain reduction [50], and significantly improved belching, abdominal fullness, bloating, and constipation [51] in children with IBS. Various *Lactobacillus* strains had positive benefits in children with IBS in several RCTs [52–56]. An RCT with a probiotic mixture of *Lactobacillus* and *Bifidobacterium* strains demonstrated superiority versus placebo for symptom relief and had significant effects on abdominal pain/discomfort, abdominal bloating/gassiness, and life disruption [57]. In a meta-analysis of RCTs of probiotics in children, abdominal pain score, abdominal pain treatment success, frequency of abdominal pain, and standard abdominal pain were significantly reduced compared with placebo, although abdominal pain relief was not significant between probiotics and placebo [40]. BaclauSII had similar observations on reductions in abdominal pain and bloating among IBS patients taking *B. clausii*; although these were secondary endpoints in this study, and the primary endpoint was not significant. Thus, evidence is growing on the positive impact of probiotics in children with IBS. Evidence-based global guidelines from the World Gastroenterology Organization recommend specific probiotic strains for certain gastrointestinal disorders, including IBS [58].

A key challenge in understanding probiotic effects is study heterogeneity, making it difficult to compare effects of different strains and mixtures. Probiotic beneficial effects in IBS may be strain-specific [59]. Such strain-specificity may reflect different mechanisms impacting the gut–brain axis [59, 60].

No unexpected safety findings were observed in BaclauSII. The type and incidence of TEAEs observed were similar between groups. *B. clausii* is well tolerated in studies in children with diarrhea [13, 19–22, 61].

Moreover, the BaclauSII study also has some limitations. Duration of treatment is important in assessing outcomes in IBS. Certain treatments, such as the antispasmodic otilonium bromide, have beneficial effects within 10–15 weeks of treatment [62] whereas low FODMAP diets can reduce IBS symptoms within 11 days to 3 weeks [63]. As the patients received their ongoing IBS treatment during the 2-week run-in period, beneficial effects of such treatments may have shown up during the 8-week study period with *B. clausii* or placebo with conventional treatment, thereby potentially masking the potential benefit of *B. clausii* treatment. Although treatment was close to 8 weeks and treatment compliance was > 80% in most patients, the PP population only included 146 patients, mainly due to treatment non-compliance. However, this low treatment compliance was due to some diary cards being completed incorrectly as the treatment accountability (based on actual number of vials consumed) was high. This sizeable non-compliance might have impacted detection of treatment differences. IBS is heterogenous and may result in different symptoms across a population and diet may contribute to this heterogeneity [64]. Indeed, in BaclauSII, heterogeneity was noted as evidenced by different drug classes used to manage IBS symptoms. Thus, BaclauSII may not have been sufficiently powered to account for these factors. Further targeted investigations are recommended.

Whatever the reason(s) why recent RCTs have not identified *B. clausii* positive benefits in children with gastrointestinal disorders, which is reflected by the paucity of top-level evidence from probiotic trials [59], more studies with larger patient numbers are required with various probiotic doses and longer treatments to better determine the benefits of *B. clausii*, particularly in detecting any efficacy differences that might be related to different societies and healthcare environments. The current BaclauSII trial did not report many significant improvements in IBS-related parameters, most likely due to the high placebo effect, as described above. An average placebo effect of 40–50% is typically expected in IBS studies [65, 66]. Indeed, the sample size calculation for BaclauSII was based on a treatment success of 45% in the placebo group [35] and an expected difference of 20% between the groups. However, much higher placebo effects of 70–80% have been reported [66, 67], a finding which is supported by BaclauSII. Therefore, the sample size should have been much larger in BaclauSII to show a favorable effect of *B. clausii*. In addition, due to IBS heterogeneity, data from real-world studies may also be valuable in identifying *B. clausii* benefits in IBS.

5 Conclusion

This study between the *B. clausii* and placebo treatment groups was not able to demonstrate the efficacy of *B. clausii* as an adjuvant to conventional treatment of patients with IBS. There were favorable observations at some time points in the *B. clausii* group, particularly for abdominal pain and bloating. Overall, any potential benefit of *B. clausii* in this trial may have been masked by the high placebo effect, the controlled environment under a COVID-19 pandemic lockdown, education received by patients/guardians during enrollment about IBS and the importance of diet, and the understanding of the mechanisms of pain and how to deal

with symptoms related to IBS. Thus, further investigations in larger and more targeted controlled trials are necessary.

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Declarations

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Conflicts of interest/Competing interests Rodrigo Vázquez-Frias has received research grants from Sanofi; received consulting fees from BioGaia, Carnot, Nestle, and Nestle Nutrition Institute; and has received speaker honorarium from Abbot Pharmaceuticals, BioGaia, Carnot, Ferrer, Nestle, Nestle Nutrition Institute, and Schwabe Pharma. Alejandra Consuelo-Sánchez has participated in advisory boards for Sanofi and academic events organized by Sanofi; received some payment for conducting procedures in this study (as per his previous contract with his hospital); and has assisted in National and International Congresses (pre-2019). Carlos Patricio Acosta-Rodríguez-Bueno declares no conflict of interest. Andrés Blanco-Montero received a payment as part of the research team that conducted this study. Daniel Casas Robles received payment for participating in this study research. Vanessa Cohen, Daniel Márquez, and Marcos Perez III are current employees of Sanofi and may hold shares and/or stock options in the company.

Ethics The protocol was approved by Independent Ethics Committees and Research Committees. The study was conducted in accordance with all applicable laws, rules, and regulations and with the Declaration of Helsinki and the International Council for Harmonisation guidelines for Good Clinical Practice.

Consent to participate Parents/legal guardians provided written, informed consent on behalf of their child at the time of enrollment. An informed assent form was signed by the patient, if applicable (age of assent was determined by the Independent Ethics Committees and Research Committees).

Consent to publication Not applicable.

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 our trial participants. Further details on Sanofi's data sharing criteria, eligible studies, and process for requesting access can be found at https://www.vivli.org/.

Code availability Not applicable.

Author contributions Rodrigo Vázquez-Frias, Alejandra Consuelo-Sánchez, Carlos Patricio Acosta-Rodríguez-Bueno, Vanessa Cohen, Daniel Márquez, and Marcos Perez III contributed to study conception and methodology. Validation was performed by Rodrigo Vázquez-Frias. Investigations were conducted by Rodrigo Vázquez-Frias, Alejandra Consuelo-Sánchez, Carlos Patricio Acosta-Rodríguez-Bueno, Andrés Blanco-Montero, and Daniel Casas Robles. Carlos Patricio Acosta-Rodríguez-Bueno, Vanessa Cohen, Daniel Márquez, and Marcos Perez III contributed resources. Data curation was provided by Alejandra Consuelo-Sánchez, and Daniel Casas Robles. Supervision was provided by Rodrigo Vázquez-Frias, Vanessa Cohen, Daniel Márquez, and Marcos Perez III. Vanessa Cohen, Daniel Márquez, and Marcos Perez III contributed to project administration and funding acquisition. All authors contributed to writing (review and edit) of the paper, and read and approved the final manuscript.

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