Electronic Supplementary Material

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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1. Methods

1.1 Conventional therapy

Patients had access to usual standard of care as per general recommendations; i.e. an explanation of the benign nature of the disorder; nutritional orientation, for which a normal diet according to their age, based on The Eat well Plate of Mexico, was indicated; and use of symptomatic treatment at the discretion of the investigator (symptomatic treatment of constipation; if required, consideration of the use of polyethylene glycol in dust at a dose of 0.7–1.5 g/kg/day).

1.2 Calculation of sample size

Sample size was calculated based on the primary endpoint, i.e. the difference in the proportion of treatment responders between groups after 8 weeks of treatment. Response

rate was defined as patients with clinical improvement of symptoms in the Global Assessment Questions: '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 expected proportion of treatment success was 45% in the placebo group, at Week 4 [1]. The assumption was that a Week 8, a clinically meaningful difference between *B. clausii* and placebo groups of at least 20% (based on clinical expertise) would be achieved favorable to B. *clausii* treatment.

Sample size was calculated using:

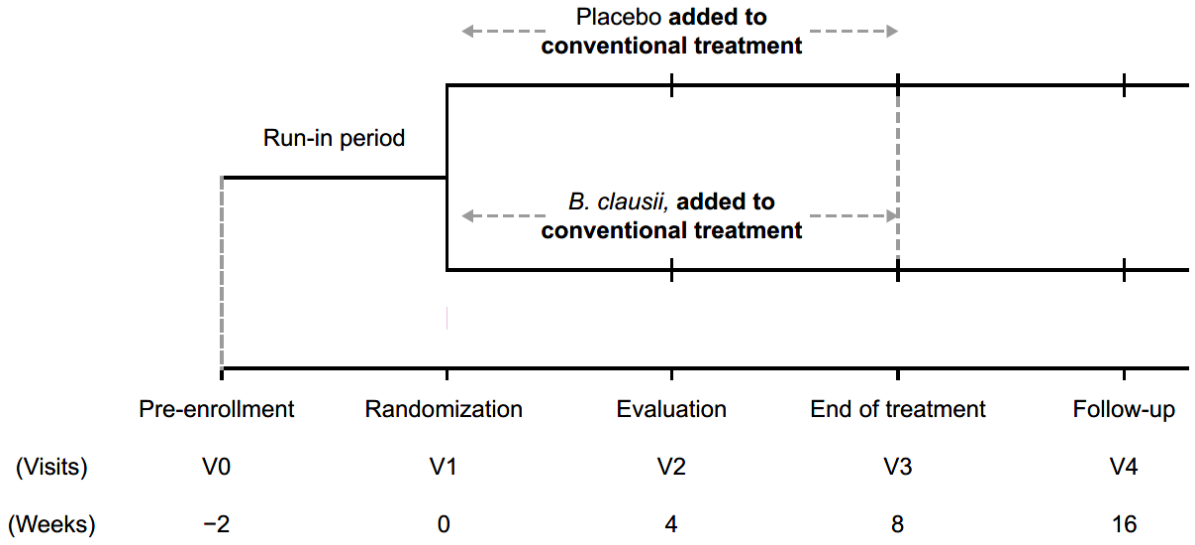
$$n = \frac{f(\alpha, \beta) \times [p_1 \times (100 - p_1) + p_2 \times (100 - p_2)]}{(p_2 - p_1)^2} = 105$$

where: α = 0.05 (one-sided); β = 0.10 (90% power); p_1 = success rate for placebo (45%); p_2 = success rate for B. clausii (65%).

2. Results

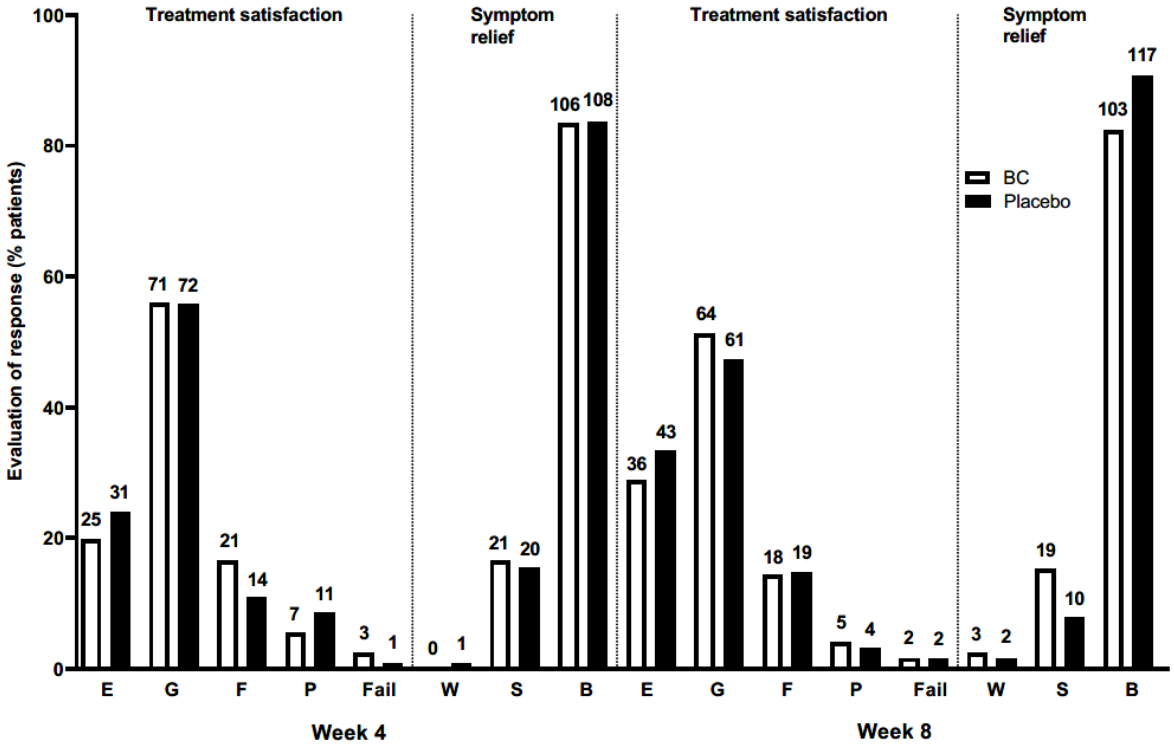
Laboratory parameters evaluated at Week –2 for pre-enrolment purposes were hematic biometry, C-reactive protein, anti-transglutaminase antibodies, anti-endomysium, urine, stool ova, parasite examination seriate, fecal calprotectin and fecal antigen of Giardia. Most of these laboratory results were either normal or abnormal without clinical significance. One patient (0.8%) per group had an abnormal result with clinical significance for parasite examination seriate.

Supplementary Fig 1. Study design.



V, visit.

Supplementary Fig 2. Evaluation of response to treatment (Global Assessment Questions) at Weeks 4 and 8 (ITT population).

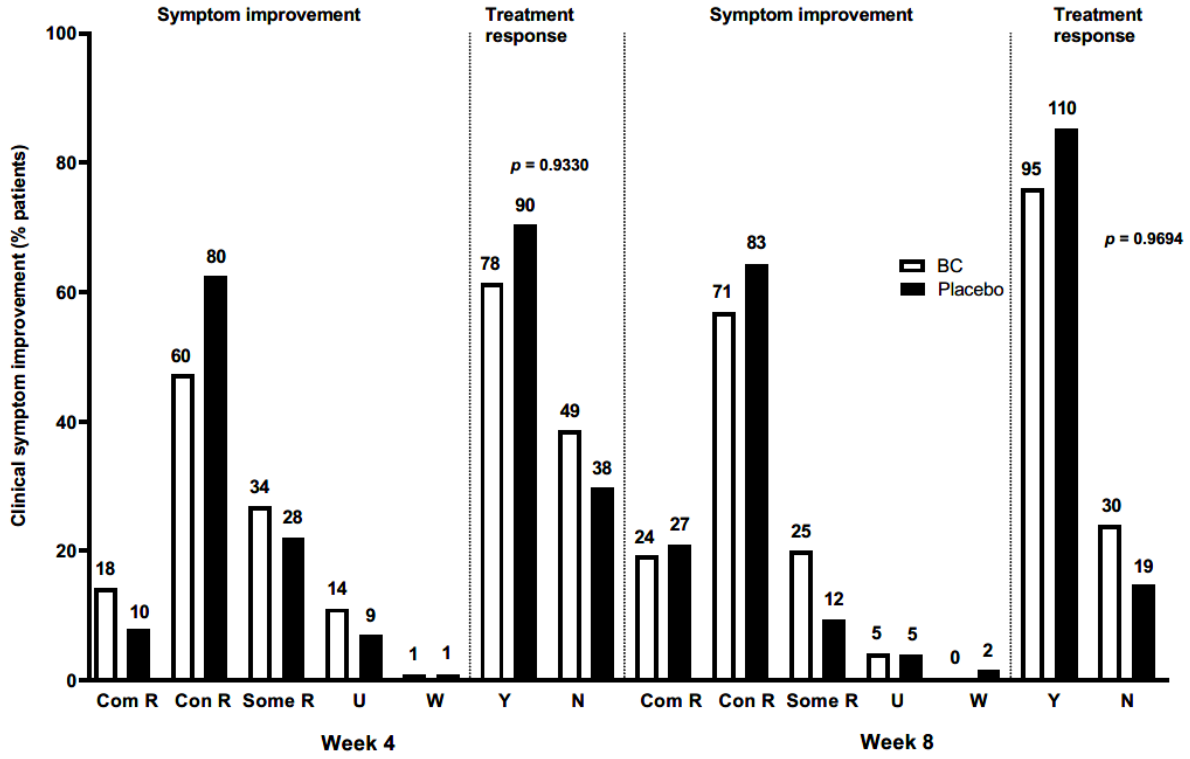


Treatment satisfaction is the response to the question 'How well did the medication relieve your symptoms (satisfaction with treatment)?' E = excellent; G = good; F = fair; P = poor; Fail = failed. Symptom relief is the response to the question 'Overall, how do you fell your problem is (symptom relief)?' W = worse; S = same; B = better.

Percentages were calculated based on non-missing values. The number above each bar is the number of patients.

BC, *Bacillus clausii*; ITT, intent-to-treat.

Supplementary Fig 3. Evaluation of clinical improvement of symptoms (SGARC) at Weeks 4 and 8 (ITT population).



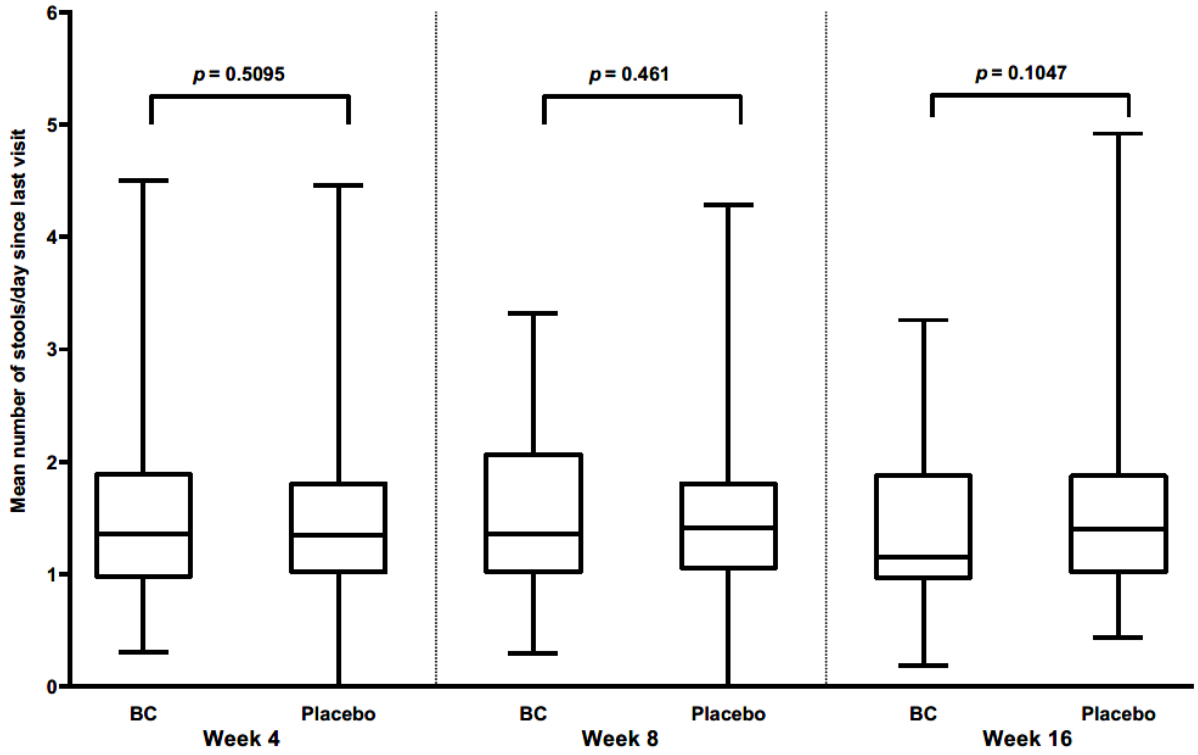
Symptom improvement = evaluation of clinical improvement of symptoms using SGARC. Com R = complete relief; Con R = considerable relief; Some R = somewhat relieved; U = unchanged; W = worse.

Treatment response = response to treatment defined as 'complete relied' or 'considerable relief' in SGARC. Y = yes; N = no.

Percentages were calculated based on non-missing values. The number above each bar is the number of patients. *p* values are from the Chi-Square test.

BC, Bacillus clausii; IBS, irritable bowel syndrome; ITT, intent-to-treat; SGARC, Subject's Global Assessment of Relief for Children with IBS.

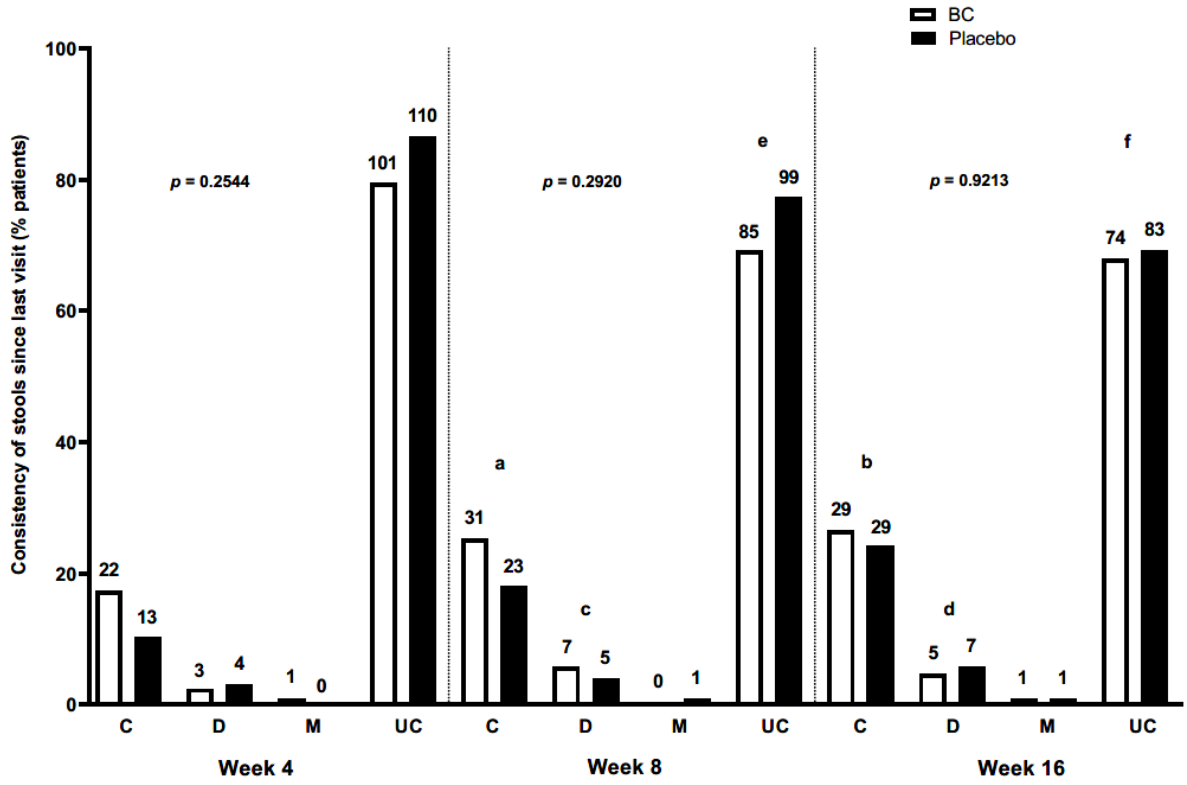
Supplementary Fig 4. Number of stools reported in the patient diary (ITT population).



Box and whisker plots show minimum, 25th quartile, median, 75th quartile and maximum values. *p* values are from the Mann-Whitney U test. Week 16 is at Week 8 of the follow-up period. Number of patients for BC and Placebo groups: Week 4 = 127 and 128; Week 8 = 123 and 129; Week 16 = 109 and 121.

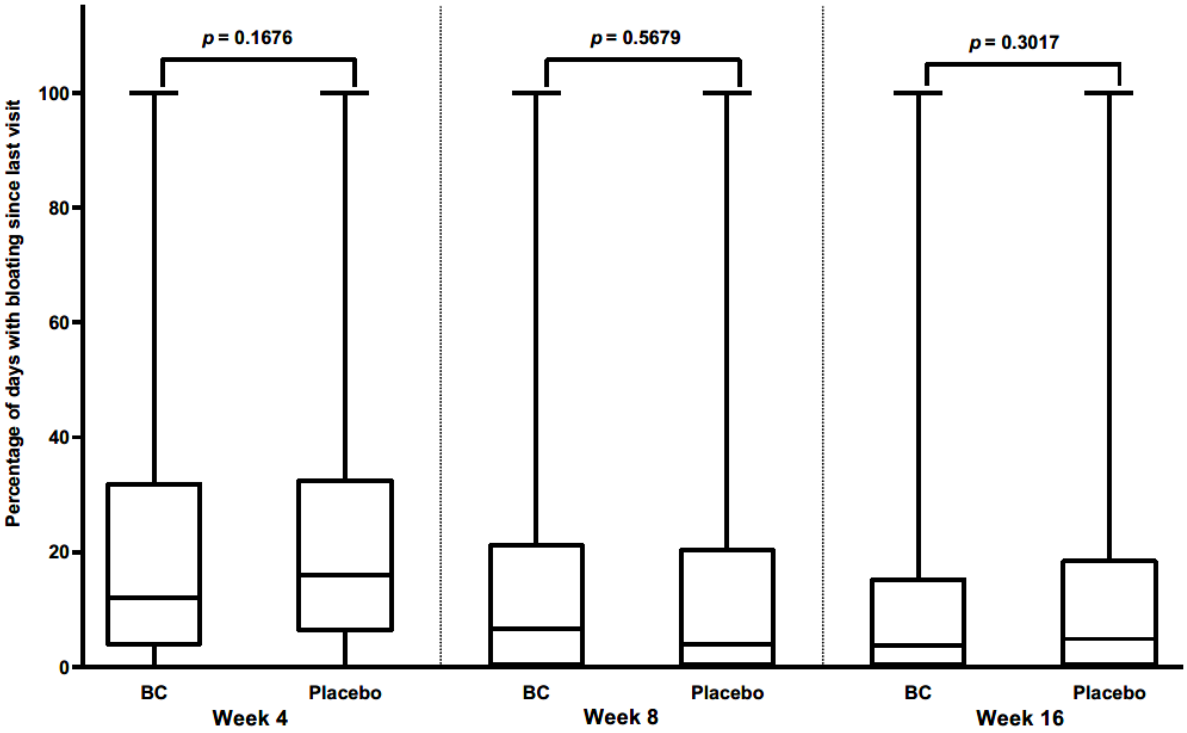
BC, *Bacillus clausii*; ITT, intent-to-treat.

Supplementary Fig 5. Categorical classification of consistency of stools reported in the patient diary (ITT population).



p values are from the Fisher Exact test. Week 16 is at Week 8 of the follow-up period.
Percentages were calculated based on non-missing values. The number above each bar is the number of patients.
p-values within group for BC and placebo groups:
Constipation vs Week 4: a = 0.0016 and 0.0016; b = 0.0047 and 0.0001.
Diarrhea vs Week 4: c = 0.0455 and 0.3173; d = 0.0833 and 0.0833.
Unclassified vs Week 4: e = 0.0003 and 0.0005; f = 0.0009 and <0.0001.
BC, *Bacillus clausii*; C, constipation; D, diarrhea; ITT, intent-to-treat; M, mix; UC, unclassified.

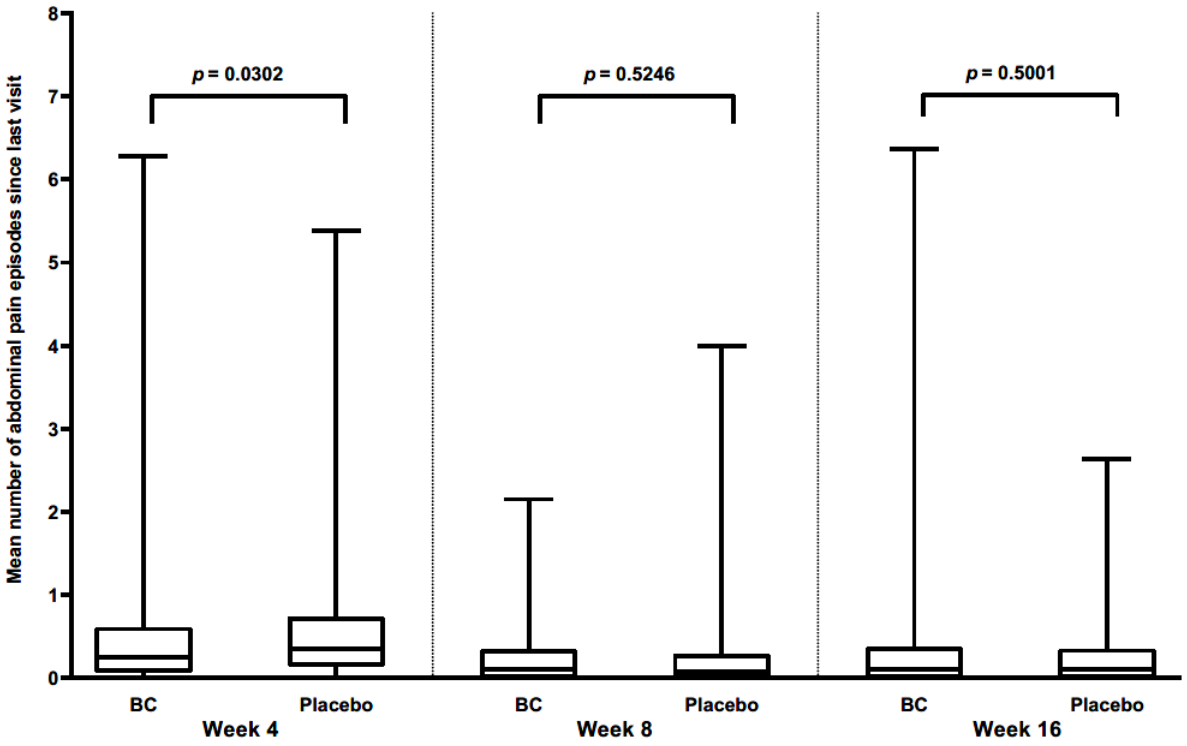
Supplementary Fig 6. Bloating recorded in the patient diary (ITT population).



Box and whisker plots show minimum, 25th quartile, median, 75th quartile and maximum values. *p* values are from the Mann-Whitney U test. Week 16 is at Week 8 of the follow-up period. Number of patients for BC and Placebo groups: Week 4 = 127 and 128; Week 8 = 123 and 129; Week 16 = 109 and 117.

BC, *Bacillus clausii*; ITT, intent-to-treat.

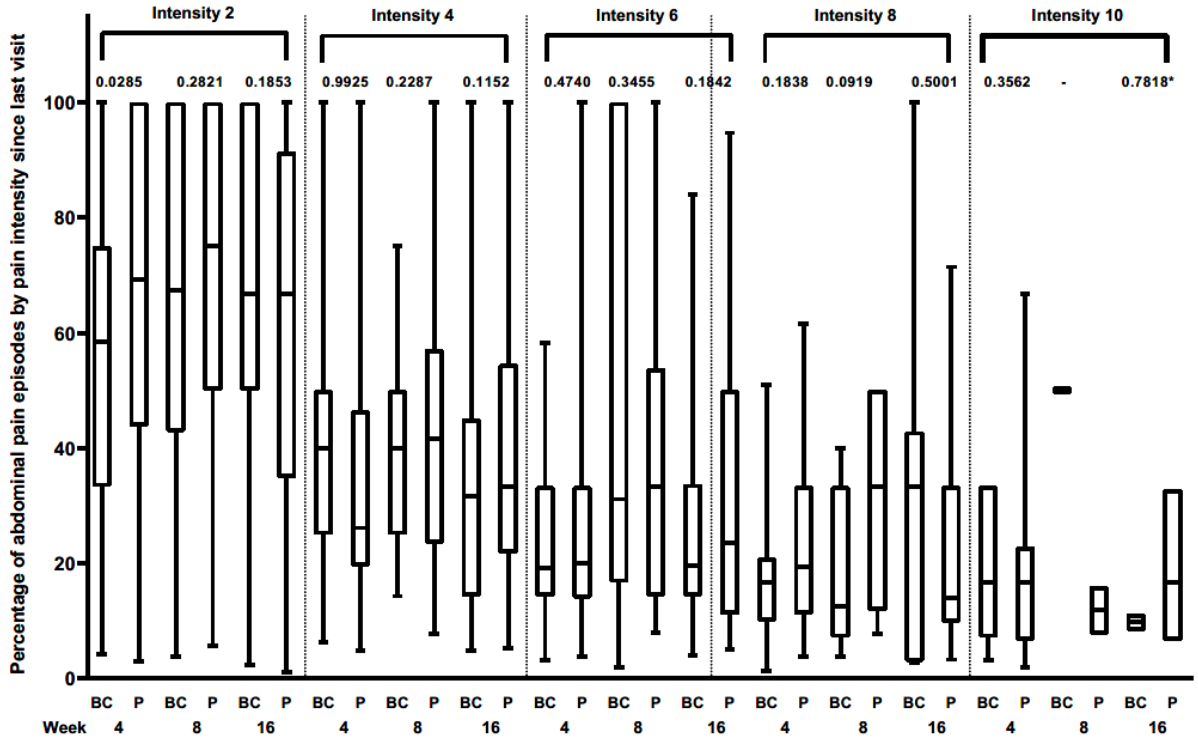
Supplementary Fig 7. Number of abdominal pain episodes recorded in the patient diary (ITT population)



Box and whisker plots show minimum, 25th quartile, median, 75th quartile and maximum values. *p* values are from the Mann-Whitney non-parametric test. Week 16 is at Week 8 of the follow-up period. Number of patients for BC and Placebo groups: Week 4 = 123 and 121; Week 8 = 113 and 115; Week 16 = 99 and 107.

BC, *Bacillus clausii*; ITT, intent-to-treat.

Supplementary Fig 8. Number of abdominal pain episodes recorded in the patient diary (ITT population).



Box and whisker plots show minimum, 25th quartile, median, 75th quartile and maximum values. *p* values are from the Mann-Whitney U test except for * which is a *t*-test for independent samples. Week 16 is at Week 8 of the follow-up period.

Number of patients for BC and Placebo groups:

Pain intensity 2: Week 4 = 90 and 103; Week 8 = 61 and 63; Week 16 = 58 and 60.

Pain intensity 4: Week 4 = 73 and 66; Week 8 = 37 and 49; Week 16 = 44 and 45.

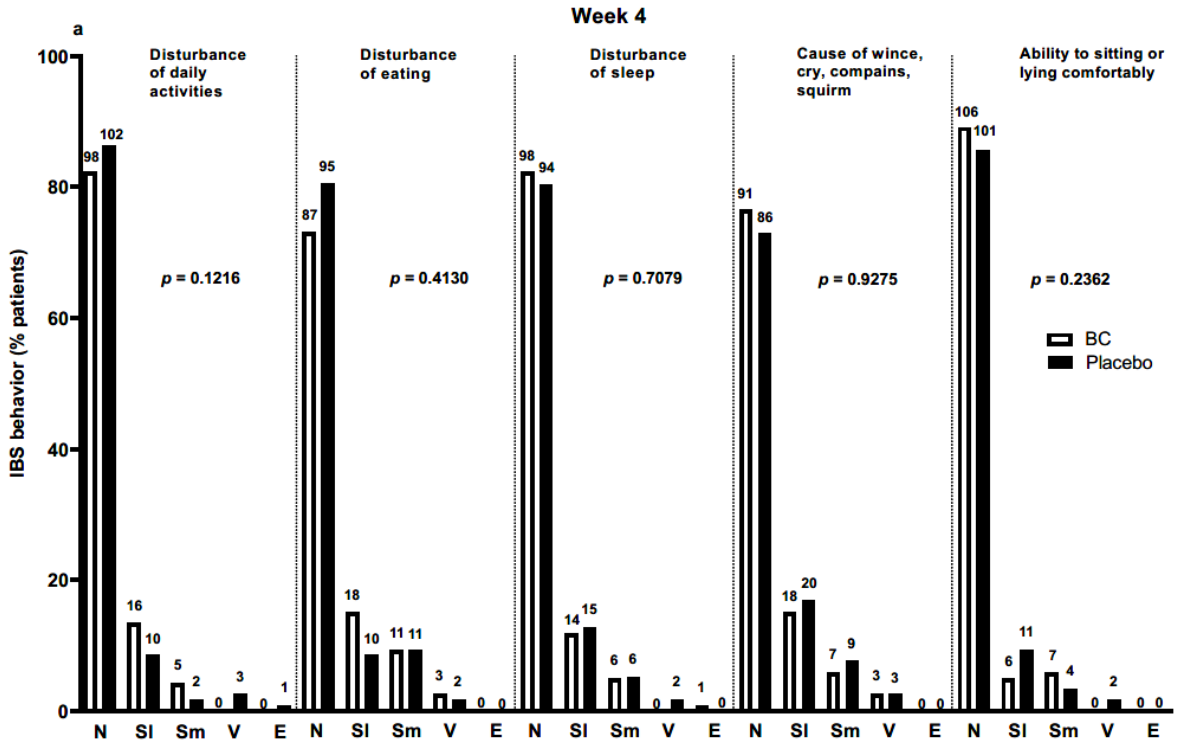
Pain intensity 6: Week 4 = 40 and 38; Week 8 = 17 and 19; Week 16 = 22 and 21.

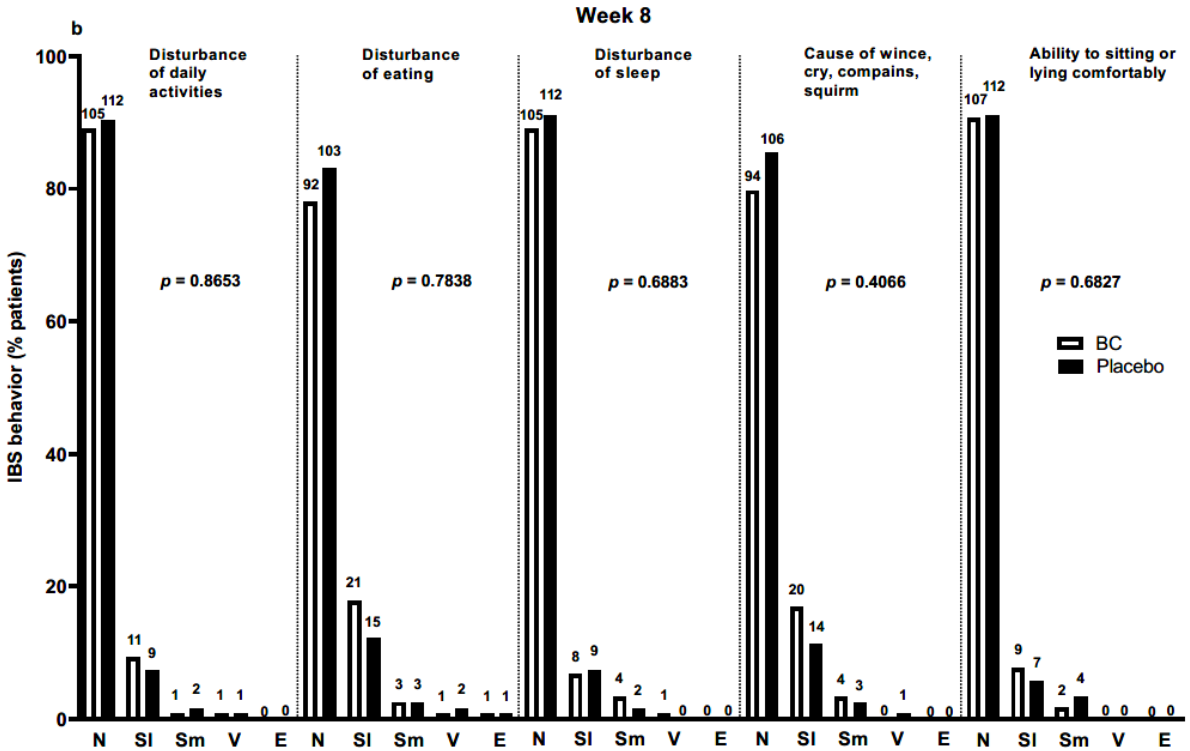
Pain intensity 8: Week 4 = 13 and 17; Week 8 = 9 and 7; Week 16 = 7 and 8.

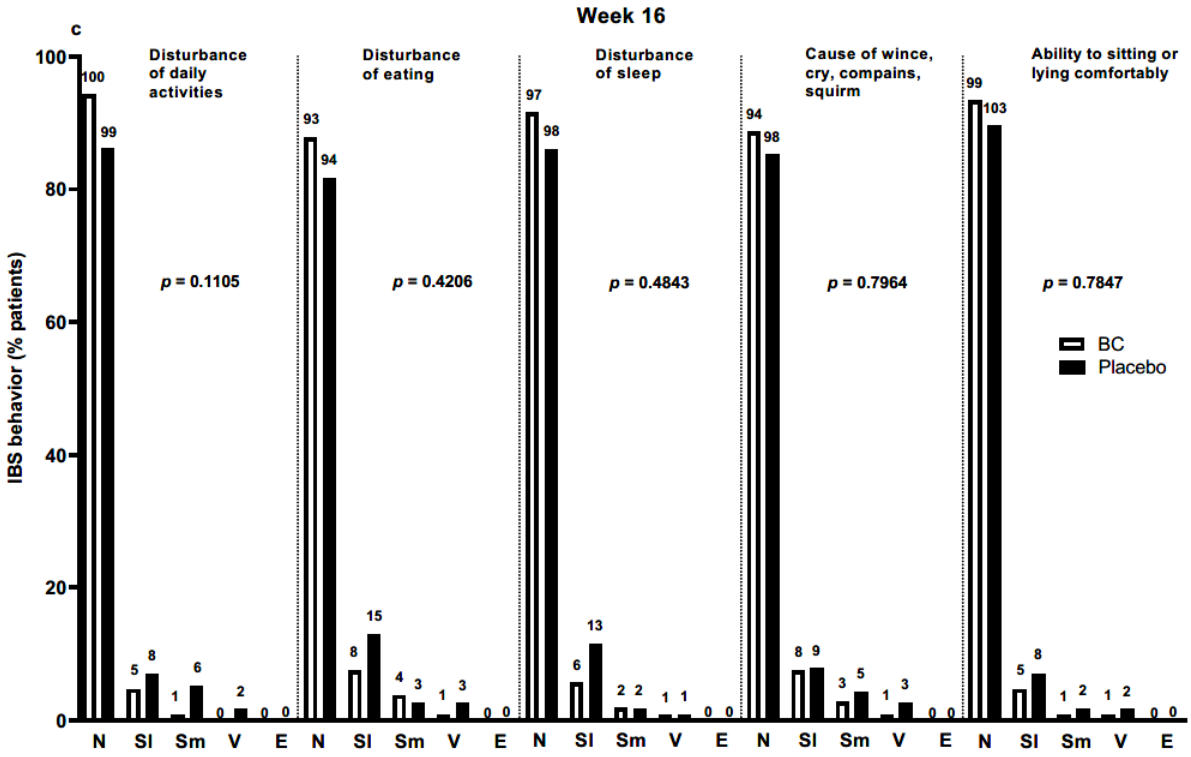
Pain intensity 10: Week 4 = 10 and 9; Week 8 = 1 and 2; Week 16 = 2 and 3.

BC, *Bacillus clausii*; ITT, intent-to-treat; P, placebo.

Supplementary Fig 9. Categorical assessment of IBS behavior reported in the patient diary (ITT population).







p values are from the Fisher Exact test. Week 16 is at Week 8 of the follow-up period.

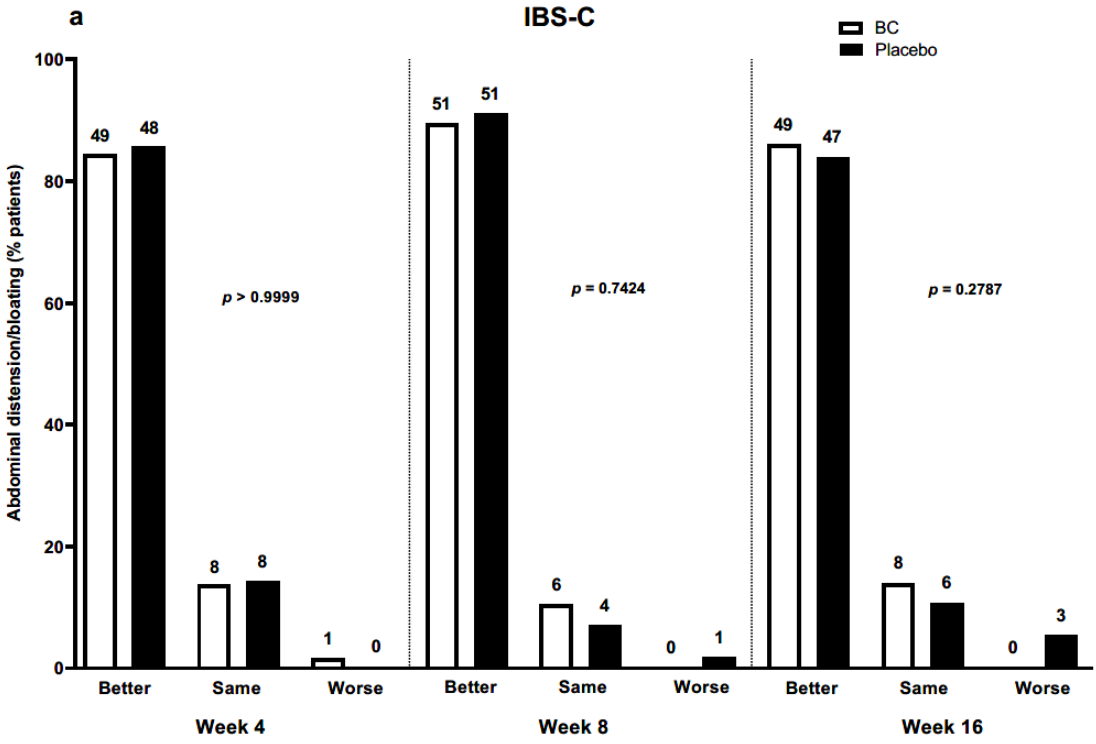
Percentages were calculated based on non-missing values. The number above each bar is the number of patients.

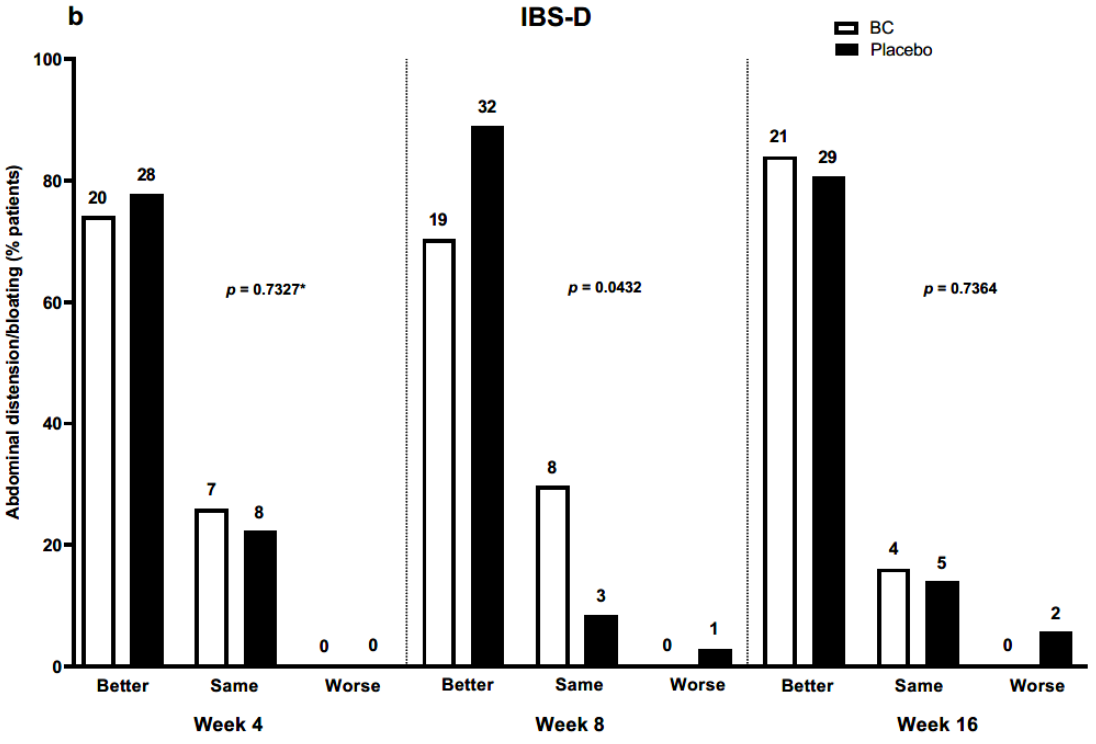
For categorical assessment of IBS the responses were: N = not at all; Sl = slightly; Sm = somewhat; V = very; E = extremely.

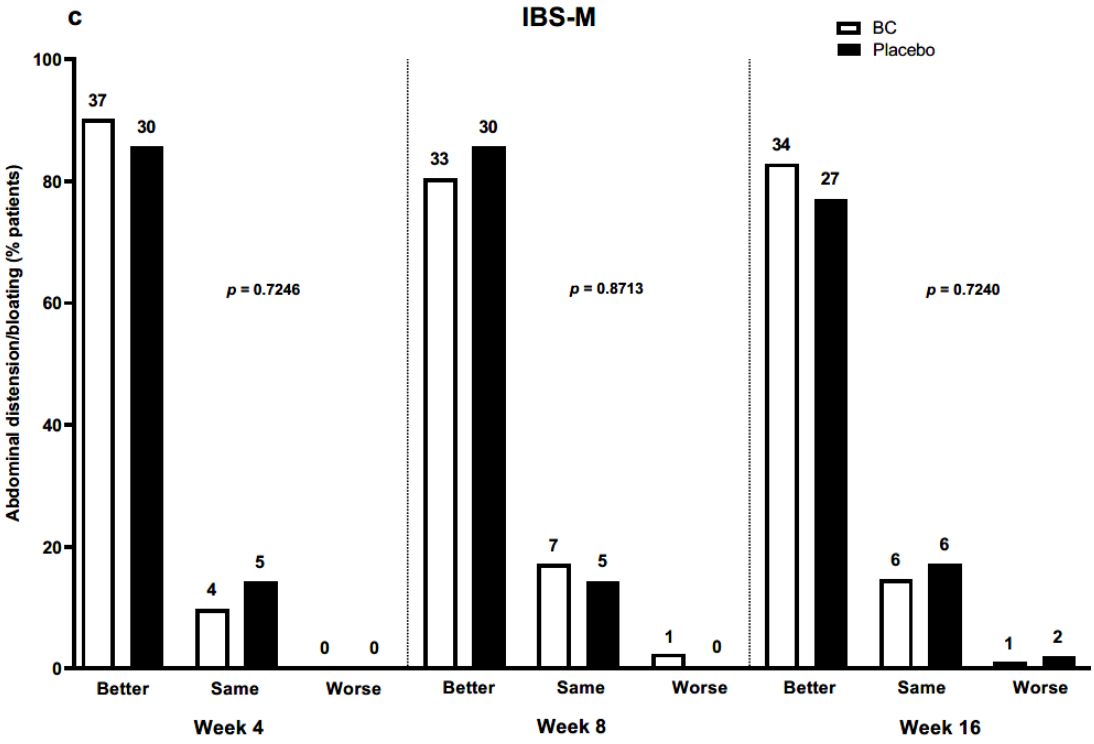
Only the last Week collected in the patient diary was considered for this analysis.

BC, *Bacillus clausii*; IBS, irritable bowel syndrome; ITT, intent-to-treat.

Supplementary Fig 10. Abdominal distention/bloating by IBS type (ITT population).





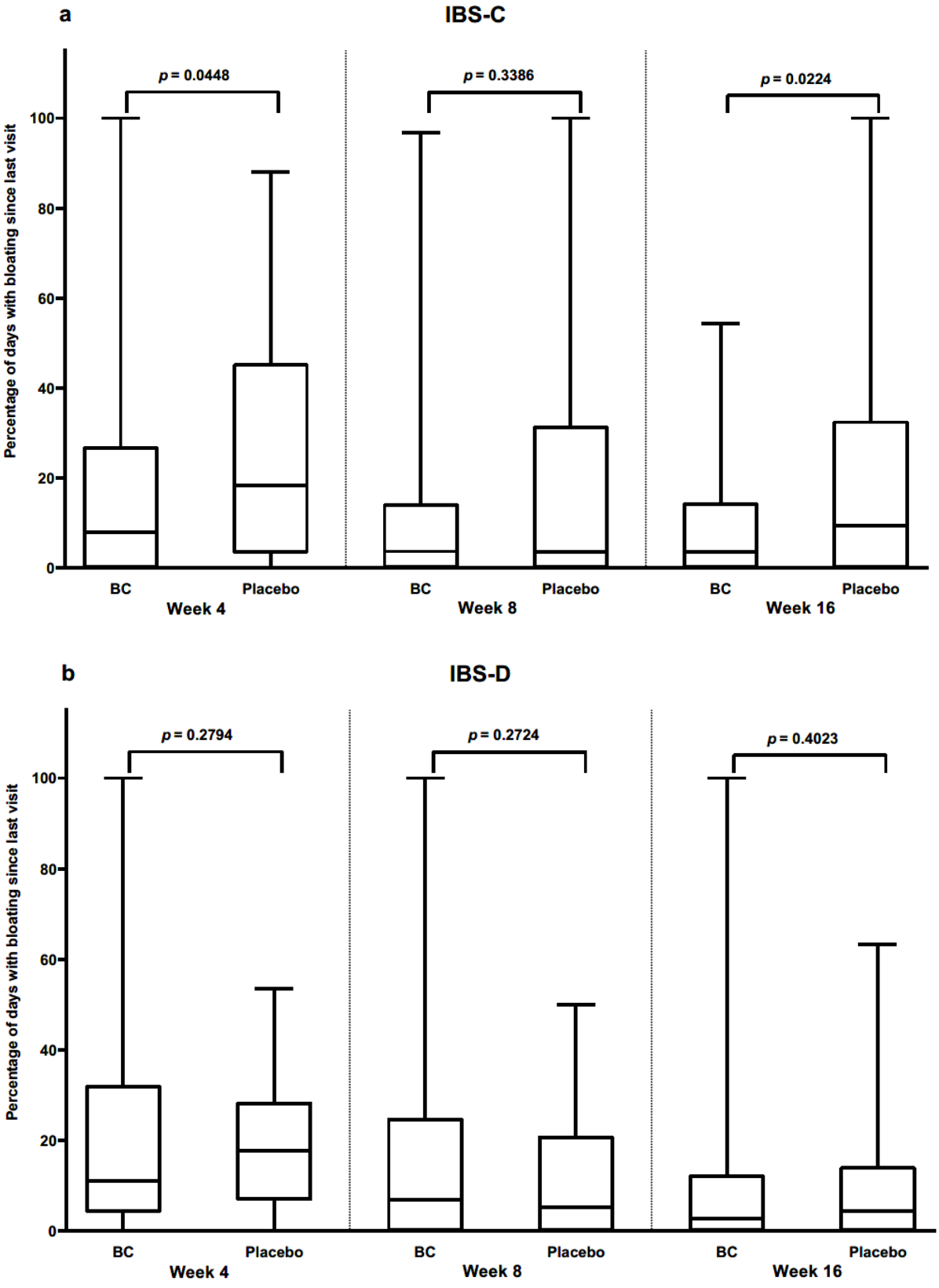


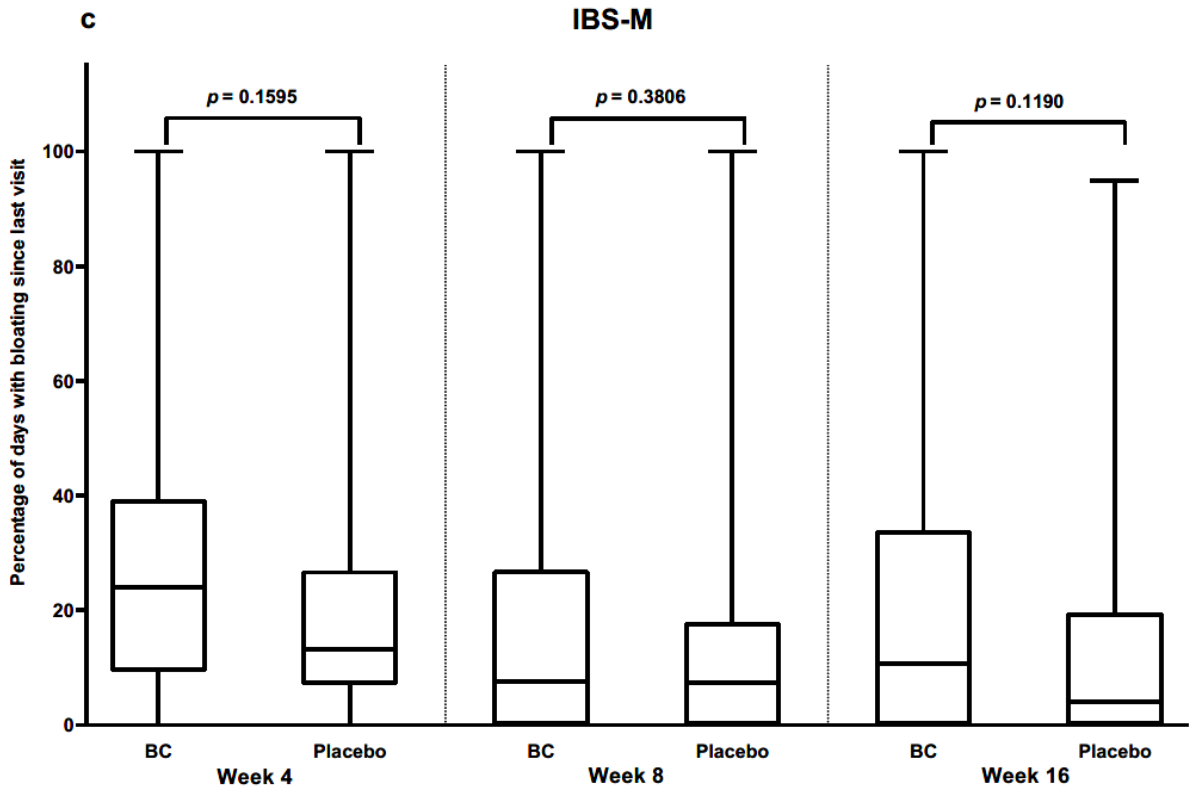
Percentages were calculated on non-missing values. The number above each bar is the number of patients.

p values are from the Fisher Exact test except for * which was the Chi-Square test. Week 16 is at Week 8 of the follow-up period.

BC, *Bacillus clausii*; IBS-C, irritable bowel syndrome with predominant constipation; IBS-D, irritable bowel syndrome with predominant diarrhea; IBS-M, irritable bowel syndrome with mixed bowel habits; ITT, intent-to-treat.

Supplementary Fig 11. Bloating by IBS type (ITT population)





Box and whisker plots show minimum, 25th quartile, median, 75th quartile and maximum values. p values are from the Mann-Whitney U test. Week 16 is at Week 8 of the follow-up period. Number of patients for BC and Placebo groups:

IBS-C Week 4 = 58 and 56; Week 8 = 55 and 56; Week 16 = 51 and 50.

IBS-D Week 4 = 27 and 36; Week 8 = 27 and 36; Week 16 = 22 and 34.

IBS-M Week 4 = 41 and 34; Week 8 = 40 and 35; Week 16 = 35 and 31.

BC: *Bacillus clausii*; IBS-C: irritable bowel syndrome with predominant constipation; IBS-D: irritable bowel syndrome with predominant diarrhea; IBS-M: irritable bowel syndrome with mixed bowel habits ITT: intent-to-treat.

Supplementary Table 1 Clinical improvement of symptoms at Week 8 by IBS type (ITT population)

	IBS–C		IBS–D		IBS–M	
	B. clausii (*n* = 58)	Placebo (*n* = 56)	*B. clausii* (*n* = 28)	Placebo (*n* = 36)	*B. clausii* (*n* = 42)	Placebo (*n* = 36)
Clinical improvement of symptoms[a], *n* (%)						
Yes (responders)	44 (75.9)	42 (75.0)	17 (60.7)	30 (83.3)	33 (78.6)	28 (77.8)
No (non-responders)	14 (24.1)	14 (25.0)	11 (39.3)	6 (16.7)	9 (21.4)	8 (22.2)
95% CI for proportion of responders	66.6–85.1	65.5–84.5	45.5–75.9	73.1–93.6	68.2–89.0	66.4–89.2
Difference in proportions between groups (*B. clausii* – placebo), % (95% CI)	0.9 (–12.4 to 14.1)		–22.6 (–40.9 to –4.3)		0.8 (–14.6 to 16.2)	
p value	0.4575		0.9790		0.4663	

B. clausii Bacillus clausii, *CI* confidence interval, *IBS-C* irritable bowel syndrome with predominant constipation, *IBS-D* irritable bowel syndrome with predominant diarrhea, *IBS-M* irritable bowel syndrome with mixed bowel habits, *ITT* intent to treat

[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

Reference

1. Saps M, Youssef N, Miranda A, Nurko S, Hyman P, Cocjin J, et al. Multicenter, randomized, placebo-controlled trial of amitriptyline in children with functional gastrointestinal disorders. Gastroenterology. 2009;137(4):1261–9.