

A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: a controlled 6-month intervention

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SUMMARY

Background: Irritable bowel syndrome is a gastrointestinal disorder of unknown aetiology. The effect of probiotics in this syndrome remains unclear.

Aim: To investigate whether a probiotic mixture containing *Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Bifidobacterium breve* Bb99 and *Propionibacterium freudenreichii* ssp. *shermanii* JS is effective in alleviating irritable bowel syndrome symptoms.

Methods: A total of 103 patients fulfilling the Rome I or II criteria took part in this 6-month, randomized, double-blind placebo-controlled trial. The patients received a probiotic capsule or a placebo capsule daily. Gastrointestinal symptoms and bowel habits were recorded.

Results: At the end the total symptom score (abdominal pain + distension + flatulence + borborygmi) was 7.7 (95% CI: –13.9 to –1.6) points lower in the probiotic group ($P = 0.015$). This represents a median reduction of 42% in the symptom score of the probiotic group compared with 6% in the placebo group. In individual symptoms, borborygmi was milder in the probiotic group ($P = 0.008$), and for the rest of the symptoms there was a non-significant trend.

Conclusions: The results indicate that this probiotic mixture is effective in alleviating irritable bowel syndrome symptoms. Considering the high prevalence of irritable bowel syndrome and the lack of effective therapies, even a slight reduction in symptoms could have positive public health consequences.

INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal (GI) disorders, and approximately 5–20% of the population is estimated to suffer from it.^{1, 2} The main clinical features of IBS include abdominal discomfort or pain, diarrhoea, constipation, bloating and flatulence. Existing therapies for IBS are considered to be only moderately effective and new therapies are being constantly sought.

The pathogenesis of IBS remains unknown, but current evidence suggests that altered gut motility, visceral

hypersensitivity and dysregulation of the brain–gut axis play a crucial role.¹ There is also evidence that, at least in a subgroup of patients, an imbalance in the intestinal microbiota may be associated with IBS.^{3, 4} An imbalanced microbiota may contribute to GI symptoms through altered colonic fermentation resulting in the increased formation of gas and an abnormal pattern of short chain fatty acids.^{5, 6} Evidence from inflammatory bowel disease also shows that enteric bacteria can trigger mucosal wall inflammation.⁷ The possible role of low-grade mucosal inflammation in IBS has recently attracted growing attention. A raised number of inflammatory cells in mucosal biopsies^{8–10} and an abnormal interleukin (IL)-10/IL-12 ratio¹¹ suggest the presence of inflammation in some IBS patients. Studies in animal models clearly

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indicate that a causal relationship exists between mucosal inflammation, altered GI motor function and visceral hypersensitivity.¹²

Some probiotic bacteria have been shown to be effective in the prevention and treatment of various GI disturbances, such as rotavirus diarrhoea.¹³ *Lactobacillus rhamnosus* GG (LGG) has also been shown to have immunomodulatory effects, e.g. the lowering of the proinflammatory cytokines, such as tumour necrosis factor (TNF)- α in allergic children¹⁴ and IL-6 and TNF- α in healthy volunteers.¹⁵ The effect of probiotics in IBS is, however, unclear.¹⁶ Some intervention studies have reported improvements in IBS symptoms,^{11, 17, 18} while others have found probiotics to be ineffective.^{19, 20} Taking into consideration the instability and heterogeneity of IBS symptoms, most of these studies have been of relatively short duration.

In previous studies LGG alone has not been successful in relieving IBS symptoms. IBS is a heterogeneous and most probably multiaetiological condition, and thus it can be hypothesized that a probiotic mixture may be more effective than a single strain. Therefore, the aim of the present study was to investigate whether a probiotic mixture containing LGG, *L. rhamnosus* LC705, *Bifidobacterium breve* Bb99 and *Propionibacterium freudenreichii* ssp. *shermanii* JS is effective in alleviating IBS symptoms during a controlled intervention study lasting 6 months. LGG is of human origin, and it is known to prevent and treat several GI disorders, especially diarrhoea.¹³ *L. rhamnosus* LC705 has been shown to have antimicrobial effects *in vitro*,²¹ and together with *P. freudenreichii* ssp. *shermanii* JS it inhibits yeasts and moulds in food and feed²² and alleviates constipation.²³

Both *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* JS originate from milk. *B. breve* is a normal gut microbe in infants, and there is a growing body of evidence that bifidobacteria have favourable clinical effects.²⁴

MATERIALS AND METHODS

Subjects

Experienced doctors in endoscopy units in the Helsinki metropolitan area recruited a total of 103 patients with a well-established IBS diagnosis (Table 1). In all, 86 patients completed the study. All the patients fulfilled the Rome I criteria,²⁵ and the majority (68%) also fulfilled the Rome II criteria.²⁶ Patients were divided into a

Table 1. Patient characteristics at baseline ($n = 103$)

	Probiotic group ($n = 52$)	Placebo group ($n = 51$)
Age [years; mean (range)]	46 (23–65)	45 (21–65)
Gender (F/M)	39 (75%)/13 (25%)	40 (78%)/11 (22%)
BMI [kg/m ²]; mean (range)]	25.7 (16.9–36.6)	24.4 (16.3–39.3)
Predominant bowel habit ^a		
Diarrhoea, n (%)	26 (50)	23 (45)
Constipation, n (%)	11 (21)	13 (25)
Alternating, n (%)	15 (29)	15 (29)
Fulfil Rome I criteria, n (%)	52 (100)	51 (100)
Fulfil Rome II criteria, n (%)	37 (71)	33 (65)
Regular IBS medication, n (%)	18 (34)	20 (39)

^a According to the Rome criteria.

BMI, body mass index; IBS, irritable bowel syndrome.

diarrhoea-predominant, a constipation-predominant and a mixed subgroup according to the supportive symptoms in the Rome II criteria. Additional inclusion criteria were: age between 20 and 65 years, and a clinical investigation with endoscopy or a barium enema of the GI tract performed during the year prior to the study. Patients who were pregnant, lactating, or had organic intestinal diseases or severe systemic diseases, previous major or complicated abdominal surgery, severe endometriosis, dementia or were otherwise incapable of adequate cooperation, were excluded. Subjects were also excluded if they had received antimicrobial medication during the previous 2 months. Patients with lactose intolerance were allowed to take part if they followed their previous low lactose or lactose-free diet.

Study design

The study was conducted as a 6-month, randomized, double-blind, placebo-controlled trial. The trial was preceded by a 1-week baseline period. During the intervention period the probiotic group received daily one capsule containing LGG, *L. rhamnosus* LC705, *B. breve* Bb99 and *P. freudenreichii* ssp. *shermanii* JS (Valio Ltd, Helsinki, Finland; total amount of bacteria $8\text{--}9 \times 10^9$ CFU/day; equal amount of each strain) and the control group received a placebo capsule. The placebo capsule consisted of microcrystalline cellulose, magnesium stearate and gelatine as encapsulating material. Some of the patients ($n = 55$) gave faecal samples at baseline, halfway through and at the end of

the study. The faecal samples were analysed for recovery of LGG in order to confirm compliance.

Other products containing probiotic bacteria were forbidden during the study. Because of the relatively long duration of the study, patients who were on previous IBS medication (mainly commercial fibre analogues, but also laxatives, antidiarrhoeals, antispasmodics, antiflatulence drugs and antidepressants) on an as-needed basis were allowed to continue this medication throughout the study. Any other regular medication was also allowed. The patients were advised not to change any medication or life style habits during the study, and to record antimicrobial treatments and other medication taken throughout the intervention. There were no differences between the groups in the use of IBS medication during the trial.

Symptom diaries and life style questionnaires

At baseline and throughout the 6-month trial the participants completed a symptom diary every month for a period of 1 week. The diary was developed for this study, based on the literature data on IBS diaries. Intensity of GI symptoms, frequency and form of stools and any IBS medication used were recorded in the symptom diary. The intensity of each symptom was measured on a scale of 0–4, where '0' represented absence of symptoms and '4' severe symptoms. The primary symptoms studied were: abdominal pain, distension, postmeal distension, distension following long periods of sitting, flatulence and borborygmi. In addition to these parameters the following secondary symptoms were recorded: urgency, feeling of incomplete evacuation, straining, belching, heartburn, nausea, postmeal nausea, postmeal fullness, vomiting and mucus or blood in stools.

Before the intervention, halfway through, and at the end of the study the participants also filled in a health-related quality of life questionnaire and a food-frequency questionnaire. The quality of life questionnaire was the RAND 36-item health survey,²⁷ which assesses eight health concepts: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, general mental health, social functioning, energy/fatigue and general health perceptions. The food-frequency questionnaire assessed the frequency of use of the major foodstuffs (dairy products, cereals, meat, fruit and vegetables, fats and beverages) and the

consumption of foods generally considered to be symptom provoking, such as certain gas-producing fruit and vegetables, and spicy foods. The aim of the questionnaire was to obtain a general picture of the patients' diets, and to detect possible changes in the diets during the intervention. A large proportion of IBS patients feel that certain foods exacerbate their symptoms, and thus modifications in the diet could affect symptom severity.

Sample size and randomization

The sample size was calculated on the assumption that a clinically significant reduction in symptoms would occur in 70% of the patients in the probiotic group and 40% in the placebo group. It was calculated that, with a power of 80% and at a significance level of 0.05, the difference between the groups would be statistically significant with 42 patients per group. Altogether 103 patients were recruited in order to allow for possible dropouts. The subjects were randomized into the probiotic or the placebo group according to a computer-generated, blocked randomization list with a block size of 4.

Statistical analysis

The primary end points were the weekly sum of each GI symptom (abdominal pain, distension, flatulence and borborygmi; range: 0–28 for each), the total weekly symptom score calculated as the sum of all these symptoms (range: 0–112), and bowel habits. The weekly sums of other symptoms (urgency, feeling of incomplete evacuation, straining, belching, heartburn, nausea, postmeal nausea, postmeal fullness, vomiting and mucus or blood in stools; range: 0–28 for each) were considered as secondary end points.

The symptoms during the second half of the study period (months 4–6), and at the last month (month 6) were considered in the primary analyses. Because of the chronic and fluctuating nature of IBS, the mean scores for the second half of the study period were considered in most of the analyses. Differences between the groups were compared by ANCOVA, including baseline symptom as a covariate. Analyses were performed on the per-protocol (PP) population defined as those who fulfilled criteria for study compliance and who completed and returned all the symptom diaries ($n = 81$). The results are given for the PP population, unless otherwise stated.

A *t*-test for paired samples or a Mann–Whitney test was used for the quality of life data. The food-frequency data were tested by means of a chi-square test.

Ethics

All the participants gave their written informed consent and were told that they could withdraw from the study at any time. The Human Ethics Committee of the Joint Authority for the Hospital District of Helsinki and Uusimaa (HUS) approved the study protocol.

RESULTS

Gastrointestinal symptoms

During the last month of the study the treatment difference in the baseline-adjusted symptom score (abdominal pain + distension + flatulence + borborygmi) was -7.7 points (95% CI: -13.9 to -1.6) when the probiotic group was compared with placebo ($P = 0.015$; Figure 1). This is comparable with a median reduction of 42% in the symptom score for the probiotic group vs. a 6% reduction for the placebo group. During the second half of the trial the treatment difference in the total symptom score was -6.5 points (95% CI:

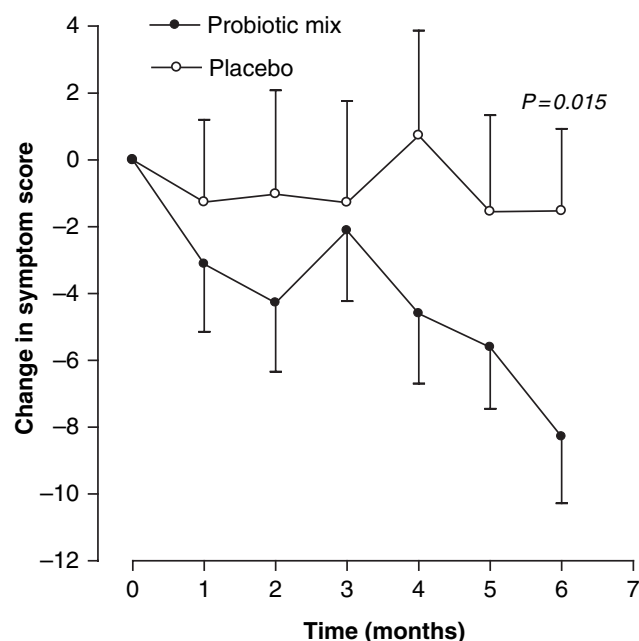


Figure 1. Change (mean \pm S.E.M.) in total symptom score (abdominal pain + distension + flatulence + borborygmi) during the 6-month intervention ($P = 0.015$ at 6 months; $n = 81$).

-12.5 to -0.4) when the probiotic group was compared with the placebo group ($P = 0.037$), and the total symptom score had decreased from the baseline in 76% of the patients receiving probiotics and 43% of those receiving placebo ($P = 0.002$; Table 2). The use of antibiotics during the intervention period was greater in the control group (50% vs. 27%, $P = 0.032$). Because antibiotics are known to influence the composition of the microbiota and affect the severity of IBS symptoms in some patients, an analysis from which all antibiotic-treated subjects were excluded was performed. In this subsample of patients (probiotics $n = 30$, placebo $n = 20$) probiotics also appeared to be more effective in alleviating the symptoms during the last 3 months of the study: the treatment difference was -8.3 (95% CI: -17.3 to 0.4) points when the probiotic group was compared with the placebo ($P = 0.062$).

There was a trend towards milder symptoms in the probiotic group with all the primary symptoms (abdominal pain, distension, flatulence and borborygmi). However, the differences between the groups were non-significant with the exception of borborygmi (Table 2). During the last 3 months of the study the baseline-adjusted weekly score for borborygmi was -2.2 points (95% CI: -3.8 to -0.6) when the probiotic was compared with placebo ($P = 0.008$). When the proportion of patients in whom the symptom score had decreased was considered, the probiotic treatment appeared to have a beneficial effect in all symptoms. During the second half of the trial flatulence had decreased in 68% of the patients in the probiotic group and 40% of the patients in the placebo group ($P = 0.011$). The corresponding figures for the other symptoms and the total symptom score are shown in Table 2.

Defecation

The defecation data were analysed for all the patients, and separately for the diarrhoea-predominant, the constipation-predominant and the mixed subgroup, according to the Rome II criteria. The defecation data are presented in Table 3. A trend towards increased weekly frequency in the probiotic group was noted in the constipation and mixed subgroups during the last 3 months of the study. There were no significant differences between the treatment groups, but an almost significant difference could be seen for the mixed subgroup (12.2. vs. 10.2; $P = 0.076$). When the

Table 2. Baseline-adjusted gastrointestinal symptoms during the second half of the study (4–6 months) and proportion of patients in whom the symptom was alleviated ($n = 81$)

Symptom score per week	Baseline-adjusted symptom score during the second half of the study				Symptoms alleviated from baseline to the second half of the study (% of patients)		
	Probiotic [$n = 41$; mean (95% CI)]	Placebo [$n = 40$; mean (95% CI)]	Probiotic vs. placebo ^a , mean (95% CI)	P -value ^a	Probiotic (%; $n = 41$)	Placebo (%; $n = 40$)	P -value ^b
Abdominal pain	4.2 (2.9–5.6)	5.8 (4.4–7.1)	-1.5 (-3.5 to 0.4)	0.110	66	43	0.035
Distension	5.1 (3.8–6.4)	6.7 (5.4–8.0)	-1.6 (-3.4 to 0.2)	0.083	56	43	0.221
Flatulence	8.2 (6.8–9.7)	9.5 (8.0–10.9)	-1.2 (-3.3 to 0.8)	0.232	68	40	0.011
Borborygmi	2.8 (1.6–3.9)	5.0 (3.8–6.2)	-2.2 (-3.8 to -0.6)	0.008	61	38	0.035
Total symptom score	20.4 (16.1–24.6)	26.8 (22.5–31.1)	-6.5 (-12.5 to -0.4)	0.037	76	43	0.002

The possible range for each weekly symptom score is 0–28, and for the total symptom score 0–112.

Statistically significant values ($P < 0.05$) have been written in bold.

^a ANCOVA, baseline symptom as covariate.

^b Chi-square test.

Table 3. Baseline-adjusted weekly defecation frequency during the second half of the study (4–6 months)

IBS subgroup	Probiotic [$n = 41$; mean (95% CI)]	Placebo [$n = 40$; mean (95% CI)]	Probiotic vs. placebo	
			Mean (95% CI)	P -value ^a
Diarrhoea	14.0 (12.1–15.9)	13.5 (11.3–15.6)	0.5 (-2.4 to 3.4)	0.712
Constipation	9.8 (7.1–12.4)	7.3 (5.2–9.4)	2.4 (-1.0 to 5.9)	0.152
Mixed	12.2 (10.7–13.6)	10.2 (8.7–11.8)	1.9 (-0.2 to 4.1)	0.076
All ($n = 81$)	12.4 (11.3–13.5)	11.1 (10.0–12.2)	1.3 (-0.3 to 2.9)	0.102

Results are given separately for the diarrhoea-predominant, the constipation-predominant and the mixed subgroup according to Rome II criteria ($n = 81$).

^a ANCOVA, baseline defecation frequency as covariate.

IBS, irritable bowel syndrome; CI, confidence interval.

probiotic group was compared with the placebo in the constipation subgroup, the proportion of hard stools had decreased. The treatment difference between the groups was -25% (95% CI: -52.5 to 2.0; $P = 0.067$). There were no differences between the groups in the weekly bowel frequency (14.0 vs. 13.5; $P = 0.712$) or the faecal consistency for the diarrhoea-predominant patients. Looking only at the diarrhoea-predominant patients ($n = 38$), we also analysed the incidence of diarrhoea (at least 1 day with a minimum of three loose or watery stools) during the study. In the probiotic group nine patients (43%) suffered from diarrhoea at least once during the second half of the study, while the corresponding number for the placebo was four (24%).

Secondary symptoms

In the case of the secondary symptoms the probiotic treatment had a positive effect on urgency and the feeling of incomplete evacuation. During the last

3 months of the study the baseline-adjusted urgency score was 5.2 for the placebo and 3.3 for the probiotic, resulting in a treatment difference of -1.9 (95% CI: -3.5 to -0.3; $P = 0.021$). The treatment difference for the feeling of incomplete evacuation score was -2.1 points (95% CI: -4.0 to -0.1; $P = 0.039$). There were no other significant differences between the groups to do with the secondary symptoms.

Quality of life

At baseline the mean quality of life score was somewhat higher in the probiotic group, but the difference between the groups was non-significant (72 of 100 vs. 64 of 100 points; $P = 0.094$). Compared with the baseline, there was no change in the mean score at 3 months or at 6 months in either group. Looking at the change in the eight individual concepts during the intervention, there was a difference between the groups in only one. The score describing the patients' general

health perceptions decreased slightly in the probiotic group (3.5 points) while it increased with the placebo subjects (2.2 points; $P = 0.053$).

Food intake

There were no major differences between the groups regarding change in overall food consumption or in the consumption of foodstuffs generally considered as symptom provoking.

Study compliance and dropouts

Study compliance was estimated by counting the number of capsules remaining in each returned carton, and by analysing LGG from faecal samples. The mean study compliance was considered good with the returned capsules (mean compliance 96%, range: 74–100). The recovery figures for faecal LGG in the probiotic group and in the placebo group were 89.5% vs. 4.8% in the middle of the study and 78.9% vs. 14.3% at the end of the study. However, a larger proportion of patients in the probiotic group already had LGG in the faeces at baseline (31.6% vs. 9.5%).

In all, 17 patients (eight probiotic, nine placebo) withdrew from the study. The reasons for withdrawal were the following: illness or hospitalization for other causes than IBS (one probiotic, three placebo), a sensation of increased GI symptoms (three probiotic, one placebo), a desire to use other probiotic products during antimicrobial treatment for other causes than IBS (two probiotic), pregnancy (two placebo), non-compliance (one placebo) and other reasons (two probiotic, two placebo).

DISCUSSION

This study examined whether probiotic therapy with LGG, *L. rhamnosus* LC705, *B. breve* Bb99 and *P. freudenreichii* ssp. *shermanii* JS is effective in alleviating IBS symptoms. The results indicate that the probiotic mixture is helpful in treating IBS symptoms. This is the first long-term study (6 months) reporting the effects of probiotic treatment in IBS. Earlier interventions on probiotics in IBS have been of shorter duration, lasting from 10 days to 8 weeks.^{11, 17–20} Given the chronicity of functional GI disorders, a minimum treatment duration of 8–12 weeks is generally recommended for IBS trials.²⁸

The study was conducted as a 6-month, randomized, double-blind, parallel-group, placebo-controlled trial. According to current recommendations, this is the optimum study design.²⁸ All the patients fulfilled the Rome I criteria for IBS, and most of them (68%) also fulfilled the Rome II criteria. Overall, the study population is considered to represent IBS patients in primary care relatively well. All other published studies on probiotic therapy in IBS have been conducted with smaller numbers of patients than in this study. In contrast to most other studies, the patients were allowed to use their regular IBS medication throughout the study. Because of the long duration of the intervention, it was felt that it would be unethical to ask the patients to withhold their usual medication. The proportion of patients on IBS medication was the same in both treatment groups and remained stable during the study.

During the last month of the study the treatment difference in the symptom score was -7.7 points when the probiotic group was compared with placebo. The differences for the weekly scores for all primary symptoms were also in the favour of probiotic therapy. In contrast to earlier studies where LGG on its own has been shown to prevent and treat certain diarrhoeas,¹³ no effect on defecation frequency, consistency or incidence of diarrhoea in diarrhoea-predominant patients could be seen. There is evidence that two of the strains in the mixture, *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* JS alleviate constipation, and thus it could be that the mixture used in this trial is not optimal for diarrhoea. Probiotics could influence IBS symptoms by balancing the microbiota. There is some evidence of an imbalanced microbiota in IBS,^{3, 4} but the data are not entirely consistent. An inflammatory component has also been suggested in IBS, especially in postinfectious IBS. When they were asked, only two of the study patients reported having had a gastroenteritis episode during the previous year, and thus this study population cannot be considered as suffering from postinfectious IBS. LGG, one of the probiotics in the mixture, has been shown to have immunomodulatory properties,^{14, 15} and could hence alleviate a possible low-grade inflammation. On the contrary, LGG alone was not effective in treating IBS symptoms in an 8-week crossover study.²⁰ Recent trials on atopic children indicate that the probiotic mixture used in this study appears to modulate the immune responses in a different way from LGG alone.^{29–31} In addition to the

balancing effect on the microbiota and the immunomodulatory effects, recent studies also suggest that probiotics may influence intestinal motility. *In vitro* studies on isolated intestines of guinea-pigs have shown that probiotics, especially bifidobacteria, have a relaxing effect on the colon.³² *Lactobacillus paracasei* seems also to attenuate postinfective dysmotility in an animal model of IBS.³³ Besides alleviating IBS symptoms, the data from a pilot study also suggest that the probiotic supplementation used in this study improves tolerance to *Helicobacter pylori* eradication therapy.³⁴

Previous studies have found another probiotic mixture, VSL-3, to be effective in treating IBS in one placebo-controlled trial³⁵ and in an open, uncontrolled 20-day study.¹⁸ *Lactobacillus plantarum* 299v, too, has been shown to be effective in alleviating flatulence,¹⁷ and in reducing abdominal pain and the total symptom score.³⁶ A very recent trial shows that *B. infantis* 35624 feeding alleviates IBS symptoms and normalizes an abnormal IL-10/IL-12 ratio.¹¹ However, the lack of a common symptom questionnaire makes it difficult to compare the results and the size of the symptom improvement with other studies.

The use of validated quality of life instruments in treatment interventions is strongly recommended.²⁸ Even though the probiotic treatment had a positive effect on the IBS symptoms, no increase in the quality of life could be seen. In one recent trial on IBS and probiotics, slight improvements in quality of life could be seen,¹¹ but no other reported trials on IBS and probiotics have included quality of life measurements. It is possible that the reduction in symptoms, which we observed, was of such magnitude that there was no effect on the quality of life measurements. The RAND-36 survey used in this study is not IBS-specific, and thus it could also be that it was unable to detect possible changes in IBS-related quality of life.

Lactobacilli and bifidobacteria are considered to be safe for healthy and immunosuppressed individuals.³⁷ Three subjects in the probiotic group and one subject in the placebo group experienced an increase in GI symptoms following the administration of the capsules. However, no apparent adverse effects were observed. Considering the high prevalence of IBS and the ineffectiveness of current therapies, even a slight reduction in symptoms could have positive public health consequences. In the treatment of a non-malignant syndrome such as IBS, the safety of the therapy is of particular importance, and hence probiotics may be a relevant option.

In conclusion, a probiotic supplementation with LGG, *L. rhamnosus* LC705, *B. breve* Bb99 and *P. freudenreichii* ssp. *shermanii* JS could be a safe and easy way of alleviating IBS symptoms.

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