A Mixture of 3 Bifidobacteria Decreases Abdominal Pain and Improves the Quality of Life in Children With Irritable Bowel Syndrome

A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Crossover Trial

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Goals: We assessed the efficacy of a probiotic mixture of *Bifidobacterium infantis M-63*, *breve M-16V*, and *longum BB536* in improving abdominal pain (AP) and quality of life (QoL) in children with irritable bowel syndrome (IBS) and functional dyspepsia (FD).

Background: AP-associated functional gastrointestinal disorders, particularly IBS and FD, are common in pediatrics, and no well-established treatment is currently available. Although probiotics have shown promising results in adults, data in children are heterogeneous.

Study: Forty-eight children with IBS (median age, 11.2 y; range, 8 to 17.9 y) and 25 with FD (age, 11.6 y; range, 8 to 16.6 y) were randomized to receive either a mixture of 3 *Bifidobacteria* or a placebo for 6 weeks. After a 2-week "washout" period, each patient was switched to the other group and followed up for further 6 weeks. At baseline and follow-up, patients completed a symptom diary and a QoL questionnaire. AP resolution represented the primary outcome parameter.

Results: In IBS, but not in FD, *Bifidobacteria* determined a complete resolution of AP in a significantly higher proportion of children, when compared with placebo ($P = 0.006$), and significantly improved AP frequency ($P = 0.02$). The proportion of IBS children with an improvement in QoL was significantly higher after probiotics than after placebo (48% vs. 17%, $P = 0.001$), but this finding was not confirmed in FD.

Conclusions: In children with IBS a mixture of *Bifidobacterium infantis M-63*, *breve M-16V*, and *longum BB536* is associated with improvement in AP and QoL. These findings were not confirmed in FD subjects. Trial identifier: NCT02566876 (http://www.clinicaltrial.gov).

Key Words: irritable bowel syndrome, functional dyspepsia, abdominal pain, probiotics

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Abdominal pain (AP)-associated functional gastrointestinal disorders (FGIDs), such as irritable bowel syndrome (IBS) and functional dyspepsia (FD), are common conditions in pediatrics with an estimated prevalence between 1% and 19%.[1,2] IBS and FD in children and adolescents account for a significant number (2% to 4%) of office visits to primary care physicians.[3–5]

Some studies suggest that the etiology and pathogenesis of FGIDs can be explained by an impaired communication between the brain and the gut, involving visceral hypersensitivity, sensory and gastrointestinal hypervigilance, and gastrointestinal dysmotility.[6] In IBS, alterations in the gut microbiota composition have been well described in a recent report by the Rome working team,[7] and recent evidence has elucidated the growing importance of the microbiota-gut-brain axis in the development of IBS.[8] Although the etiology of IBS and FD remains elusive, there is growing recognition of the role played by intestinal infections and disturbances of the colonic microflora in the development of these conditions.[9,10]

Conventional interventions include reassurance and general advice about managing pain, but no well-established treatment is currently available. The use of probiotics has been proposed with recent evidence of effectiveness in adults. Particularly, several meta-analyses evaluating the individual trials of probiotics in adults have concluded that both *Bifidobacteria* and the mixture of *Escherichia coli* (DSM 17252) and *Enterococcus fecalis* (DSM 16440) are effective in the treatment of IBS-associated AP, and probiotics in general seem to improve bloating and flatulence in IBS.[11–16] Available data in pediatric populations are heterogeneous, but there seems to be a benefit from the use of *Lactobacillus rhamnosus GG* and VSL#3 in children with pain-predominant FGIDs, especially in those with the diarrhea subtype.[17,18] In contrast, virtually no data are available on the effect of probiotics on FD-related AP in children, and the only published study including FD children has shown no effect of *Lactobacillus rhamnosus GG* on AP.[19]

Bifidobacteria are gram-positive, strictly anaerobic bacteria with a fermentative metabolism producing acetate and lactate. These probiotics, including the *infantis*, *breve*, and *longum* species, represent the most important helpful bacteria in children and account for 95% of the intestinal population in breastfed infants.[20] Their function is to digest carbohydrates and synthesize water-soluble vitamins. They also protect against infections by means of several antibacterial properties.

Therefore, as *Bifidobacteria*, in combination with other probiotic strains, have proven to be useful in IBS children,[18] we hypothesized that these probiotics, even used alone, could be beneficial in children with IBS and FD. Thus, our aim was to assess the efficacy of a mixture of 3 *Bifidobacteria* (*infantis M-63*, *breve M-16V*, and *longum BB536*) in reducing gastrointestinal symptoms and improving the quality of life (QoL) in children affected by FD and IBS.

METHODS

The study was a randomized, double-blind, placebo-controlled, crossover trial conducted at 2 pediatric tertiary care centers in Naples and Foggia. All children aged 8 to 17 years referred for IBS or FD to the Pediatric Clinics of the 2 participating centers between January and December 2014 were eligible for the study. IBS and FD were diagnosed using the Rome III criteria for pediatric FGIDs.[2] The main exclusion criterion was the presence of chronic organic gastrointestinal diseases, assessed by full clinical history and examination, and laboratory investigations including complete blood cell count, erythrocyte sedimentation rate, C-reactive protein, serum amylase and lipase, tissue transglutaminase antibodies, total serum IgA, and fecal calprotectin. Abnormalities in any of these tests resulted in the patient's exclusion from the study. Further exclusion criteria were previous abdominal surgery, diseases affecting bowel motility, or concomitant psychiatric, neurological, metabolic, renal, hepatic, infectious, hematological, cardiovascular, or pulmonary disorders. Finally, patients treated with antibiotics, proton-pump inhibitors, H2 antagonists, or receiving any commercial preparation of probiotics during the previous 3 months were also excluded.

The study was articulated in 16 weeks (Table 1). After recruitment, patients entered a 2-week run-in phase (baseline period), during which evacuative frequency, stool features, and gastrointestinal symptoms were recorded on a daily basis using a questionnaire/diary provided at study entry by the physician. At the end of the baseline period, patients returned to the center where information regarding AP characteristics, bowel habits, and associated symptoms was recorded using a previously validated interviewer-administered questionnaire for pediatric FGIDs.[21] The "Functional Disability Inventory" (FDI), a second interviewer-administered validated questionnaire,[22] was used to assess physical and psychosocial functions and investigate patients' QoL. The instrument consists of 15 items concerning perceptions of activity limitations during the past 2 weeks. Total score is computed by summing the ratings for each item and ranges from 0 to 60; higher scores indicate greater disability. After completing these questionnaires, patients were assigned in a double-blinded manner to the placebo or intervention group according to a computer-generated randomization allocation table. An independent physician not directly involved in the study had the responsibility of the computer program generating the randomization. Participants were randomized to receive either 1 sachet per day of a mixture of 3 *Bifidobacteria* (namely, 3 billions of *Bifidobacterium longum BB536*, 1 billion of *Bifidobacterium infantis M-63*, and 1 billion of *Bifidobacterium breve M-16V*) or an identical looking and tasting placebo for 6 weeks. The probiotics used are actually commercialized in Italy by Valeas S.p.A. (Milan, Italy), which also provided the placebo. The company did not provide any additional resources for this investigator-initiated study. No further medication other than analgesics was allowed for the whole duration of the study.

After completing the 6 weeks of treatment, no preparation was administered for a 2-week "washout" period. Afterwards, each patient was switched to the other group and treated with placebo or probiotics for a further period of 6 weeks.

At each follow-up visit subjects underwent a complete physical examination, data recorded on the daily diaries were collected, and compliance to treatment was verified by collection of empty medication packages. Furthermore, the questionnaire of symptoms and the FDI were administered and answers were recorded.

The main outcome parameter considered was AP resolution, defined as no episodes of pain during the treatment period, as reported in the questionnaire of symptoms. Secondary outcome parameters were reduction in AP frequency, patient-reported QoL, changes in bowel habit for IBS patients, and improvement in nausea for FD subjects. In the absence of longitudinal data indicating the required reduction in FDI score to define a relevant improvement in QoL, we considered significant a decrease of at least 75% from the baseline score.

The investigators involved in the recruitment and follow-up of patients, those coordinating the study and analyzing the data, patients themselves, and their caregivers were all unaware of the randomization group at each phase of the study.

The institutional ethical review boards of both participating centers approved the study protocol. Written informed consent was obtained from the parents or legal guardians before enrollment. The study was registered at Clinical Trials.gov (NCT02566876).

Statistical Analysis

The study sample was calculated from the percentage of patients reaching the primary outcome measure—namely, resolution of AP. We calculated the sample size assuming that the tested probiotics would result in at least a 40% and 30% increase in treatment success for FD and

TABLE 1. Study Timeline

Visit No	1	2	3	4	5	6	7	8	9
Time	Day 1	Day 15	Day 29	Day 43	Day 57	Day 71	Day 85	Day 99	Day 113
Inclusion/exclusion criteria	X	X							
Informed consent	X								
Physical examination	X	X			X	X			X
Randomization		X							
Drug delivery		X	X	X		X	X	X	
Laboratory tests	X								
Assessment of treatment adherence			X	X	X		X	X	X

X indicates action performed.

IBS, respectively. We estimated that, with a power of 80% and a significance level of 0.05, a sample size of 25 FD subjects and 48 IBS subjects was appropriate. The Wilcoxon signed-rank test was used to assess changes in variables after placebo and probiotics. The Fisher exact test and the χ^2 test for categorical variables were used where appropriate. Statistical significance was set at a *P*-value <0.05. Data were analyzed using a statistical software package for Windows (SPSS-PC, version 13.0; Chicago, IL).

RESULTS

Ninety-one patients with a new diagnosis of AP-associated FGID based on the Rome III criteria were eligible for the study, but only 78 of them (50 subjects with IBS and 28 with FD) eventually met all inclusion criteria and were enrolled. Four subjects were lost at follow-up during the washout period and 1 was excluded because of the need for administration of antibiotic therapy (Fig. 1). A total of 73 children completed the study. Of them, 48 were diagnosed with IBS (median age, 11.2 y; range, 8 to 17.9 y) and 25 with FD (median age, 11.6 y; range, 8 to 16.6 y). Compliance to prescribed medications was excellent for both placebo and probiotics, with only 4 subjects (3 with IBS and 1 with FD) failing to deliver back 2 of the 6 medication packages used and 2 subjects (both with FD) failing to deliver 1 placebo package. Table 2 summarizes the baseline characteristics of the enrolled patients. Both placebo and *Bifidobacteria* mixture were well tolerated, and no adverse events were recorded throughout the study.

Before starting placebo, 15 of 25 FD patients (60%) and 39 of 48 IBS patients (81%) reported AP, whereas this symptom was present in 12 FD patients (48%) and 44 IBS patients (92%) before starting treatment with the *Bifidobacteria* mixture. By per-protocol analysis, the comparison between the 2 treatment groups showed that AP completely disappeared in a significantly higher proportion of IBS children receiving probiotics (42% vs. 14.5%, $P = 0.006$; Fig. 2), but this finding was not confirmed in FD subjects (20% vs. 36%, $P = 0.3$). Findings deriving from intention-to-treat analysis were comparable, with complete AP resolution occurring in 42% versus 14% of IBS children and in 21% versus 32% of FD subjects after probiotics and placebo, respectively ($P = 0.003$ and 0.5). Similarly, in IBS patients, administration of *Bifidobacteria* mixture significantly improved AP frequency ($P = 0.02$) when compared with placebo ($P = 0.1$), whereas this finding was not confirmed in FD subjects ($P = 0.06$ and 0.09 after probiotics and placebo, respectively).

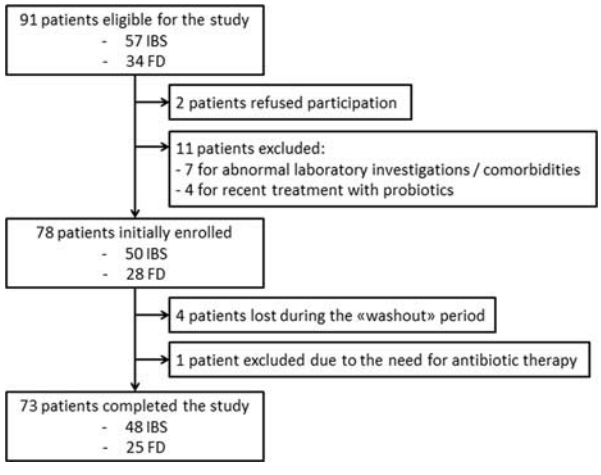

FIGURE 1. Flow chart of patient recruitment. FD indicates functional dyspepsia; IBS, irritable bowel syndrome.

Pre-placebo median FDI scores were 8 (range, 0 to 40) and 5 (range, 0 to 40) in FD and IBS patients, respectively. Before starting treatment with the *Bifidobacteria* mixture, median FDI scores were 4 (range, 0 to 35) and 4.5 (range, 0 to 40) in FD and IBS subjects, respectively.

Per-protocol analysis showed that the proportion of IBS children who reported an improvement in QoL was significantly higher after probiotics than after placebo (48% vs. 17%, $P = 0.002$; Fig. 3). Because of the questionable clinical significance of a decrease in FDI from a score of 1 to 0, we repeated the analysis after the exclusion of these patients (n = 3 after probiotics, n = 0 after placebo), and we still found a statistically significant difference ($P = 0.006$). Such a finding was confirmed at the intention-to-treat analysis, which showed that an improved QoL was reported by 46% of IBS patients after probiotics versus 16% after placebo ($P = 0.002$). In contrast, no difference in the percentages of FD children reporting a significant reduction in FDI score was observed between the treatment and placebo groups (28% vs. 24%, $P = 1$).

In patients with IBS, prevalence of constipation was 21% before starting treatment with the *Bifidobacteria* mixture and 17% before starting placebo. After treatment, the proportion of subjects who reported resolution of this symptom was not significantly different between the 2 groups (60% and 37.5% of subjects, respectively, $P = 0.6$). Pre-probiotic and pre-placebo prevalence of diarrhea in IBS children was 23% and 29%, respectively. In both probiotic and placebo groups resolution of diarrhea after treatment was observed in 36% of patients ($P = 1$).

In FD subjects the prevalence of nausea, vomiting, regurgitation, fullness, and heartburn before starting probiotics was 48%, 16%, 36%, 56%, and 24%, respectively, whereas it was 48%, 12%, 36%, 52%, and 8%, respectively, before starting placebo. For all of these parameters, the comparison between the 2 groups showed no significant differences in the proportion of subjects reporting resolution of symptoms after treatment.

DISCUSSION

In the current study we investigated the effectiveness of a mixture of 3 *Bifidobacteria* on AP and QoL of children with IBS or FD. Our main finding was the significant decrease in both prevalence and frequency of AP observed in IBS patients treated with probiotics when compared with the placebo group. Furthermore, we found that QoL, assessed by an interviewer-administered validated questionnaire, improved in a significantly higher proportion of IBS patients treated with the *Bifidobacteria* mixture tested. Nevertheless, such findings were not confirmed in FD subjects, who showed no significant improvement in AP and no improvement in QoL.

A similar discrepancy between the 2 conditions has been highlighted in a previous meta-analysis assessing the efficacy of another probiotic, *Lactobacillus rhamnosus GG*, on FGIDs,[17] even though only 1 study involving FD patients was included.[19] In this setting, the significant improvement in both AP and QoL detected in IBS patients

TABLE 2. Baseline Characteristics of the Study Population

General Data	IBS	FD
N	48	25
Median age (range) (y)	11.2 (8-17.9)	11.6 (8-16.6)
Male:Female	21:27	11:14

	Pre-probiotics		Pre-placebo	
Symptoms (%)	IBS	FD	IBS	FD
Abdominal pain	92	48	81	60
Constipation	21	—	17	—
Diarrhea	23	—	29	—
Nausea	—	48	—	48
Vomiting	—	16	—	12
Regurgitation	—	36	—	36
Fullness	—	56	—	52
Heartburn	—	24	—	8

FD indicates functional dyspepsia; IBS, irritable bowel syndrome.

adds further evidence to the emerging crucial role of probiotics in the therapeutic management of this condition, demonstrating the effectiveness and safety of the 3 used probiotic strains. In contrast, the absence of benefit that we found for the use of *Bifidobacteria* on FD-related AP is in line with previous reports highlighting the poor efficacy of other probiotic strains in this condition.

FGIDs, particularly those in which AP is the predominant clinical manifestation, have a significant impact on children's and adolescents' QoL, may importantly limit daily activities, and sometimes determine long-term psychological implications.[23,24] Furthermore, recurrent symptoms have a major impact on health and social costs, particularly when they continue throughout adolescence into adulthood.[25,26]

Although a complete pathophysiologic understanding of these conditions has not been achieved, a general consensus in the field is that many FGIDs represent disorders of the brain-gut axis. This bidirectional connection between the central and enteric nervous systems links emotional and cognitive centers of the brain with peripheral functions. Among these, intestinal motility, the entero-endocrine system, and the immune system are likely the main determinants of the clinical expression of most FGIDs.[27]

So far, the therapeutic approach to pediatric FGIDs has been generally focused on reassurance and behavioral advice for the management of pain.[28] In addition, despite the absence of good-quality evidence, dietary therapies, such as fiber supplementation and reduction in the consumption of fermentable carbohydrates, are often prescribed.[29] Moreover, as depression, anxiety, and stress likely have a relevant role in the pathogenesis of these conditions, behavioral therapy and hypnotherapy have been shown to be partially beneficial.[30]

In this setting, probiotics play an emerging role as new therapeutic tools in FGIDs, because of the growing recognition of the importance of gut microbiota and intestinal infections in influencing brain-gut interactions.[31] Recent preclinical data suggest that changes in the gut microbiota can affect brain signaling systems related to pain and associated emotional behavior.[32] In rodents, the probiotics-induced modulation of gut microbiota has been shown to interfere with affective behavior, pain response, and gene expression in the brain.[33] Furthermore, the identification of neuroactive molecules produced by bacterial components of the microbiota represents additional evidence of the effects in the central nervous system determined by signals generated in the gut.[34] Therefore, probiotics are likely to have a relevant role in the management of FGIDs, by affecting the gut microbiota or by altering brain function and pain perception centrally.[34]

So far, several adult studies have shown the effectiveness of probiotics in the control of symptoms such as bloating and pain,[35–37] and some evidence, with conflicting

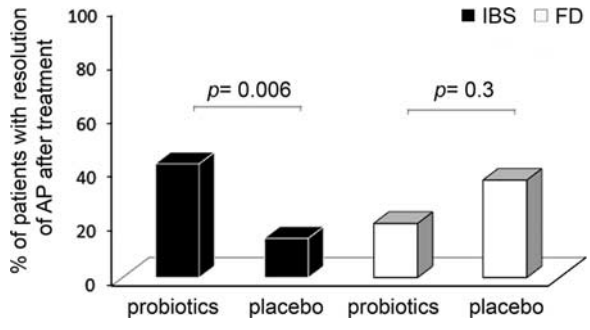

FIGURE 2. Differences in the proportions of irritable bowel syndrome (IBS) and functional dyspepsia (FD) subjects with abdominal pain (AP) resolution after placebo and probiotics.

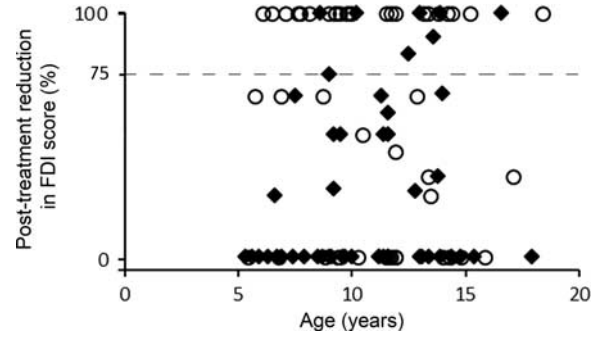

FIGURE 3. Distribution of posttreatment reduction in Functional Disability Inventory (FDI) score in IBS patients according to age. The proportion of subjects reporting a reduction in FDI score was higher after probiotics (empty circles) than after placebo (black diamonds); $P = 0.002$. Dotted line, FDI score reduction = 75% of pretreatment score.

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results, has been published in children as well.[18,19,38] Among the available pediatric studies, no investigation has been carried out with the mixture of 3 *Bifidobacteria* (*infantis M-63*, *breve M-16V*, and *longum BB536*) used in the present study. These 3 probiotics were only evaluated as part of a preparation (VSL#3) consisting of 8 different strains of *Bifidobacteria*, *Lactobacilli*, and *Streptococcus thermophilus*, which was recently demonstrated to have some benefits in IBS children.[18]

One of the strengths of our study is the design. To evaluate our probiotics combination, we opted for a crossover trial to minimize the variability between subjects. Such variability represents a known limit of simple double-blind studies, particularly those involving FGID patients, in whom the placebo effect may be a relevant issue.[39] Interestingly, our data showed resolution of AP after administration of placebo in 36% of FD patients, but only in 14.5% of IBS subjects. For both diseases the relevance of the placebo effect has been highlighted previously,[19,39] even though few data are available for FD, and the reported percentages of placebo response in IBS subjects range widely from 16% to 71%, with an average of approximately 40%.[39] Likely explanations for the low placebo response observed in our IBS patients are the stringent Rome III criteria used during enrollment, the presence of a run-in phase in our study design, and the several office visits performed throughout the study, which are all elements that have been associated with decreased placebo response in IBS.[39]

The evaluation of QoL as primary outcome represents another strength of our study, particularly because it was assessed by means of a specific and validated questionnaire. However, although our patients were unselected, their baseline FDI score was in the "no/minimal disability range" (FDI $\leq$ 12) in both FD and IBS groups,[40] making it difficult to assess a relevant QoL improvement. Therefore, in the absence of established criteria to define the clinical significance of FDI changes over time, we arbitrarily considered significant a 75% drop in the baseline score.

A limitation of the present study is represented by the possible "carryover" effect between treatments, which may affect crossover trials. To obviate this, a washout period was used between the end of the first treatment period and the beginning of the second. No widely accepted guidelines indicating the appropriate duration of intervals between treatment periods in crossover studies dealing with probiotics are currently available. Therefore, despite this limitation, we considered reasonable a 2-week washout period, which was chosen on the basis of a previous similarly designed trial evaluating the efficacy of a probiotic product including the *Bifidobacteria* strains tested in the present study.[41] Other study drawbacks are the short follow-up period and the limited sample size, particularly for the FD group, which may at least partially explain the negative findings that emerged in these subjects, and warrants further research in larger FD populations to verify the generalizability of our results. Finally, our study lacks an assessment of different doses of the *Bifidobacteria* mixture, and a comparison with other probiotic strains previously tested in IBS, such as *Lactobacillus rhamnosus GG*. These evaluations would have probably strengthened the power of our observations, but were beyond the purpose of our study.

In summary, our study is the first to address the possible use of the probiotic mixture of *Bifidobacteria infantis M-63*, *breve M-16V*, and *longum BB536* in children affected by IBS. Further research is needed on larger populations to confirm and extend our findings. Management of FD-related AP still represents an unsolved challenge, in which the role of probiotics is controversial. In this scenario, the identification of effective therapeutic tools represents a research priority.

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