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Effect of a Symbiotic Mixture on Fecal Microbiota in Pediatric Patients Suffering of Functional Abdominal Pain Disorders

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Abstract: (1) Background: Functional abdominal pain disorders (FAPDs) represent one of the main etiologies of chronic abdominal pain in the pediatric population. A wide spectrum of probiotic or prebiotic mixtures has been evaluated in trials regarding benefits in patients with FAPDs, mainly in the adult population. (2) Methods: This study was interested in evaluating the effect of oral supplementation with a symbiotic mixture on intestinal microbiota in children with functional dyspepsia (FD), irritable bowel syndrome with diarrhea (IBS-D), and irritable bowel syndrome with constipation (IBS-C). A combination of six bacterial strains (*Lactobacillus rhamnosus* R0011, Lactibacillus casei R0215, Bifidobacterium lactis BI-04, Lactobacillus acidophilus La-14, Bifidobacterium longum BB536, Lactobacillus plantarum R1012) and 210 mg of fructo-oligosaccharides-inulin were administered orally, daily, for 12 weeks and patients were scored for severity of symptoms and fecal microbiota before and after the treatment. (3) Results: The proportion of patients with adequate symptom relief was higher in the IBS-D than in the IBS-C group; however, the difference was not statistically significant (74.4% vs. 61.9%, $p = 0.230$). There was an increasing proportion of bacterial genera associated with health benefits, for both IBS-C and IBS-D (IBS-C: 31.1 ± 16.7% vs. 47.7 ± 13.5%, $p = 0.01$; IBS-D: 35.8 ± 16.2% vs. 44.1 ± 15.1%, $p = 0.01$). (4) Conclusions: Administration of a symbiotic preparation resulted in significant changes to the microbiota and gastrointestinal symptoms in patients with FAPDs.

Keywords: microbiota; symbiotic; abdominal pain

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1. Introduction

Functional abdominal pain disorders (FAPDs), also referred to as functional gastrointestinal disorders (FGIDs), represent the one of the main etiologies of chronic abdominal pain in the pediatric population that involve interplay among regulatory factors in the enteric and central nervous systems [1]. FGIDs have a chronic evolution, usually a duration of more than 2 months, of abdominal pain in children who experience no alarming biomarkers, normal physical examinations, and stool samples negative for occult blood [2]. The on-going classification system, ROME IV, distinguishes several pain-predominant FGIDs based on their recognizable patterns of symptoms, such as functional dyspepsia (FD), irritable bowel syndrome (IBS), abdominal migraine, and FAP-not otherwise specified (FAP-NOS) [3]. Irritable bowel syndrome (IBS) has been affecting an increasing number of children in recent years, posing an important burden for healthcare practitioners as well as affecting quality of life for patients and their families [4,5]. In the pediatric population, IBS is defined, according to ROME IV criteria, as a gastrointestinal disorder characterized by abdominal pain/discomfort, bloating, and abnormal bowel habits [3]. As a part of functional gastrointestinal disorders (FGIDs), in the category of functional abdominal pain disorders, IBS and functional dyspepsia have crucial challenges in terms of diagnosis and

management. Considering the lack of specific biomarkers, these entities have symptoms-based diagnosis [3,6]. During the past two decades numerous studies researched possible causes and underlying mechanisms of appearance, but the clear physiopathology is yet to be revealed, despite pediatric neuro-gastroenterology findings in terms of intestinal motility, signaling molecules, changes in microbiota or epigenetic mechanisms [7].

There is a rationale to target microbiota while treating FGIDs, considering the dysbiosis proven in the patient's gut. The main patterns of a dysbiotic gut in IBS for example, is a low diversity of microbial strains compared to healthy controls.

Gut microbiota modifications, known as a dysbiotic gut, may play a role in functional abdominal pain disorders through gut immunity and integrity alteration [8]. Several studies have reported a lower level of microbial diversity in functional disorders microbiome [9], and species such as Lactobacilli and Bifidobacteria are heavily altered [10]. Thus, a growing body of clinical data have been gathered arround using probiotics in functional disorders' management, although study data are lacking on children [11].

A wide spectrum of probiotic/prebiotic mixtures or those individually administered have been evaluated in trials regarding benefits and clinical outcomes in patients with IBS, mainly in the adult population. Several clinical outcomes have been followed, including effects on IBS symptoms and/or quality of life, as well as microbiota changes after a period of treatment. Results have often been controversial and thus discouraging, although many methodological factors have contributed to this lack of consensus [12].

This study was interested in evaluating the effect of oral supplementation with a symbiotic mixture on the composition of intestinal microbiota in children with functional abdominal pain disorders (functional dyspepsia and irritable bowel syndrome), considering that most studies have shown decreased genus Bifidobacterium in IBS.

2. Materials and Methods

Study design. This was a prospective trial designed to investigate the efficacy of the *Jarro-Dophilus + FOS* in the treatment of children with IBS and functional dyspepsia. The investigation took place in the Grigore Alexandrescu Emergency Hospital for Children, from Bucharest, the capital of Romania, and participants were enrolled between August 2018 and February 2019. The study was approved by the Ethics Committee of the hospital under the registration numbered as 16634/03.07.2018. All parents of the patients who fulfilled the inclusion criteria, received, read, signed, and dated the informed consent before the study. Each proposed eligible patient was invited to discuss the study follow-up in detail (Visit 1). Parents had the opportunity to decide over a 72 h period if they agreed to enroll their child. At the same time, all patients were evaluated by a full investigation of medical history (Visit 1) and investigators allocated to one of the following categories, based on clinical evaluation, according to ROME IV criteria: diarrhea predominant (IBS-D), constipation predominant (IBS-C), or functional dyspepsia (FD). Data recorded for the selected patients at Visit 1 were: sex, age, weight, height, bowel movement assessment as number per day and consistency was recorded on the 7-point Bristol stool scale [13], and gastro-intestinal symptoms. Parents were instructed not to take any treatment throughout the study period and to inform the healthcare provider of any changes in the health state or acute episodes. Fecal samples were provided for dysbiosis analysis (Visit 1). The follow-up procedures were recorded every 4 weeks consisting of telephone contact with the parents who informed the doctors on symptoms and stool assessment on the Bristol scale, varying from the hardest (type 1) to the softest (type 7) with pictorial representations of each stool type. Types 1 and 2 are considered constipation, while types 6 and 7 are considered diarrhea (Visit 2 and Visit 3). After the 12 weeks of intervention, stool samples were collected in the same conditions as at the beginning of the study and data were noted upon Bristol stool evaluation and gastro-intestinal symptoms. Weekly telephone questioning assessed participant's compliance.

Participants. The study population was recruited from the patients presented in the Department of Pediatric Gastroenterology of the Grigore Alexandrescu Emergency

Hospital for Children. Individuals considered for study inclusion were required to fulfill the following criteria: age 4–14 years and diagnosis of functional abdominal pain disorders (functional dyspepsia and irritable bowel syndrome) according to ROME IV criteria [3]. Patients were excluded if they had abnormal hematological and biochemical markers and if they had abnormal findings on various procedures in the previous years, e.g., in barium enema or colonoscopy. We also excluded individuals if they had been treated with products containing prebiotics or probiotics in the 4 weeks preceding entry into the trial.

Intervention. Jarro-Dophilus + FOS is a combination of 6 bacterial strains (*Lactobacillus rhamnosus* R0011, Lactibacillus casei R0215, Bifidobacterium lactis BI-04, Lactobacillus acidophilus La-14, Bifidobacterium longum BB536, Lactobacillus plantarum R1012), and 210 mg of fructo-oligosaccharides-inulin. One capsule was administered orally, daily, for 12 weeks, and the medication was provided by the healthcare practitioners.

Clinical outcome. The patients were scored for severity of abdominal discomfort, dyspepsia, flatulence, and epigastric pain on a 10-point ordinate (numerical rating) scale.

Analysis of fecal microbiota. Fecal samples were collected from participants before and after treatment using a special laboratory kit with 2 sterile containers, which were then brought to the laboratory in conditions depending on the time spent from collection to laboratory delivery: if the interval was less than 24 h, both containers were stored and transported in cooled conditions at 4 °C; if the period between stool elimination and laboratory delivery was more than 24 h, 1 container was stored in a frozen condition at −80 °C until analysis, and the other one was cooled at 4 °C. Stool samples were analyzed using the test *Colonic dysbiosis—basic profile* (*SBY 1*) performed by Synlab-Germany. Microbiota composition was expressed as number of colony forming units (CFU) for various aerobic/anaerobic bacterial and fungal species. The analysis provided data on fecal pH, IgA in μg/mL (normal ranges 510–2040 μg/mL), lactoferine μg/mL (normal ranges <7.2), calprotectin in mg/kg (normal ranges <50.0 negative, 50–99 intermediary, >100 positive).

Statistical analysis. SPSS for Windows (ver. 18.0; SPSS) was used for statistical analysis. Continuous variables were analyzed using Student's *t*-tests and categorical variables were analyzed using Chi-square tests or Fisher's exact tests. The ANOVA comparisons were confirmed with Mann–Whitney U-tests. Correlations among the continuous variables were performed with Pearson and Spearman rank correlation coefficients. Results were considered statistically significant when the p values were <0.05.

3. Results

- *Subjects and baseline characteristics*

A total of 30 patients were initially screened, six did not consent to following the study procedure and four patients dropped out before starting the intervention and two patients did not respond to the final recall, thus analysis was applied to 18 patients. Among this group, three (16.66%) patients were classified as FD, seven (38.88%) were classified as IBS-D, and eight (44.44%) were classified as IBS-C. Baseline characteristics of these patients are shown in Table 1.

Table 1. Basic characteristics of the study population.

	FD	IBS-D	IBS-C
Sex (M/F)	1/2	6/1	4/4
Age (y)	7.66 ± 1.52	6.42 ± 1.9	5.37 ± 2.19
Weight (kg)	25.66 ± 2.88	22.14 ± 6.38	21.38 ± 6.5
Length (cm)	125.33 ± 7.02	119.57 ± 13.42	112.12 ± 15.28

- *Assessment of symptoms.*

Overall, 14 (78%) patients reported treatment success (defined as no pain). The proportion of patients with adequate symptom relief was higher in the IBS-D than in the IBS-C group; however, the difference was not statistically significant (74.4% vs. 61.9%, p = 0.230).

In both IBS-C and IBS-D groups, scores on the Bristol scale improved significantly after intervention (baseline vs. after treatment; 2.8 ± 0.6 vs. 3.9 ± 0.9, $p = 0.03$, 6.1 ± 0.9 vs. 4.1 ± 1.0, $p = 0.01$, respectively). Abdominal distension and flatulence were significantly improved in both IBS-C and IBS-D groups (IBS-C: 6.5 ± 2.8 vs. 3.7 ± 1.8, $p = 0$ 01; IBS-D: 5.9 ± 2.2 vs. 2.9 ± 1.8, $p = 0.01$).

- *Analysis of fecal microbiota.*

In the fecal microbial analysis, there was an increasing proportion of bacterial genera associated with health benefits (e.g., Bifidobacterium and Lactobacillus), for both IBS-C and IBS-D (IBS-C: 31.1 ± 16.7% vs. 47.7 ± 13.5%, $p = 0.01$; IBS-D: 35.8 ± 16.2% vs. 44.1 ± 15.1%, $p = 0.01$). On the other hand, genera of harmful bacteria, including Escherichia, Clostridium, and Klebsiella were proven to decrease after treatment (21.3 ± 16.9% vs. 16.3 ± 9.6%, p =0.02) (Figure 1).

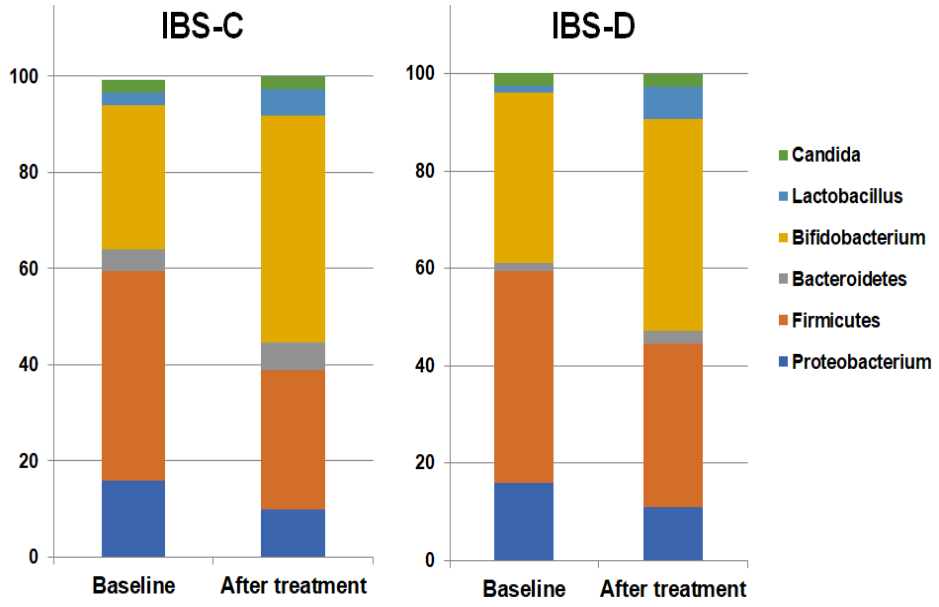

Figure 1. Effects of supplement and diagnosis on the microbiota.

At baseline, before any symbiotic intervention, Bifidobacterium profiles were significantly different between IBS-C and IBS-D (87.14 ± 23.19 vs. 71.37 ± 12.24; $p = 0.02$), with lower counts in IBS-D. The symbiotic administration had a significant effect on bacterial profiles from baseline to the end of treatment in both C-IBS and D-IBS groups (Table 2).

Table 2. Microbiota changes from baseline to endpoint.

Bacteria	IBS-D		IBS-C	
	Mean Count ± SD		Mean Count ± SD	
	Baseline	Endpoint	Baseline	Endpoint
Bifidobacterium	87.14 ± 55.33 × 10^6	88.85 ± 35.87 × 10^6	71.37 ± 11.21 × 10^6	88.75 ± 43.78 × 10^6
Lactobacillus	35.85 ± 18.12 × 10^4	74.85 ± 29.78 × 10^4	39.25 ± 12.21 × 10^4	55.00 ± 22.89 × 10^4

IBS-D: diarrhea-predominant irritable bowel syndrome, IBS-C: constipation-predominant irritable bowel syndrome, SD: standard deviation.

4. Discussion

The present study is the first in our country that has been undertaken to investigate the potential efficacy of a symbiotic preparation regarding the management of intestinal dysbiosis in children with functional abdominal pain disorders, after the implementation of ROME IV criteria. GI microbiota alterations are being recognized as a crucial factor in the pathogenesis and pathophysiology of IBS [14]. Abdominal pain is a common cause

of parental concern, important enough to count for approximately 30% of medical visits in children ages 4 to 16 years [15]. Non-specific symptoms are sometimes attributed to components or deficiencies of the pediatric intestinal microbiome and researchers have focused on new therapies that ameliorate incremented symptoms in childhood and adulthood. As observed for other pathologies, such as autoimmune hepatitis [16], validation of scoring systems for abdominal pain disorders in children comparing those subjects with organic abdominal pain patients is required. Studies on the adult population point to variation of the human microbiome as a major co-factor in the IBS disease phenotype in adults [17]. Thus, adults having IBS with diarrhea were associated with diminished quantities of *Lactobacillus* spp., whereas patients with the phenotype of IBS with constipation were characterized by increased proportions of *Veillonella* spp. [18]. This observation was then followed by experimental treatments trying to modulate adult microbiota in order to improve symptoms. In terms of pediatric pathology, more studies are needed regarding microbiome changes in functional gastro-intestinal disease. Orally applied symbiotic preparations have been associated with microbiota changes and significant effects on clinical symptoms in patients with irritable bowel syndrome [19]. An interventional study focused on nonspecific diarrhea of infants has shown better outcomes in infants receiving oligofructose and inulin combination [20].

Our study has provided new data about intestinal dysbiosis in children with functional abdominal pain disorders, and its changes over 3 months of treatment with specific strains of probiotics and prebiotics. To our knowledge, this is the first study of its kind performed in our country. We identified specific microbial signatures associated with functional dyspepsia, IBS-D and IBS-C, respectively in children. Children with IBS-D yielded greater proportions of E.coli, while having IBS-C provided more species of Bacteroides; no particularities were found in children with FD. In adult studies, phylum Firmicutes was more abundant in patients with IBS-C and associated with increased quantities of fecal organic acids (acetic and propionic acids), and severity of pain [21]. Research by Saulnier et al. showed that the IBS-C and IBS-D subtypes were associated with differences in gut microbial composition encompassing at least 50–75 different taxa [15]. The clinical symptoms in study population were more diminished after treatment, with statistical significance, suggesting that the specific studied formula offering a particular combination and dose of Lactobacillus and Bifidobacterium species with fructo-oligosaccharides-inulin reduces patients' symptoms and improves clinical scores.

Behind our research is a growing body of evidence regarding the role of gut dysbiosis in several functional gastrointestinal disorders, including IBS. Studies such as that of Liu et al. indicated lower abundance of Bifidobacterium and Lactobacillus in IBS patients gut compared to healthy controls [22], and Herndon et al. stipulated that targeting gut microbiota may improve symptoms and provide health benefits [23].

We must conclude with some limitations of our study. First, this study included a relatively small number of patients, due to cost considerations and thus was underpowered for specific diagnoses. This might have failed to detect other significant changes in the microbiota. Although the duration of treatment was long, we did not follow-up with the subjects after the administration period. Secondly, we had no placebo control group, which is considered to be essential for interventional studies of functional gastrointestinal disorders [24]. Finally, we quantitatively analyzed only a few major groups of intestinal bacteria and this may lead to quantitative shifts between different subclasses that were not detected in this analysis.

5. Conclusions

A three month course of multiple strain of probiotic with prebiotic (fructo-oligosaccharides-inulin) combination resulted in significant changes in the gastrointestinal microflora and gastrointestinal symptoms in patients with functional abdominal pain disorders.

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I.F.Ţ.; resources, C.A.B.; data curation, C.A.B.; writing—original draft preparation, R.E.S. and C.A.B.; writing—review and editing, R.E.S. and I.F.Ţ.; visualization, R.E.S.; supervision, I.F.Ţ.; project administration, C.A.B. All authors have read and agreed to the published version of the manuscript.

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