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EFFECT OF DIETARY PROBIOTICS ON INTESTINAL MICROBIOTA IN PATIENTS WITH CROHN'S DISEASE

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Abstract

Introduction. Probiotics are well-known adjuvants, used as complementary therapeutic agents in health (e.g. gastrointestinal or metabolic) disorders, considering their beneficial role on gut microbiota, and their support in immunity.
The objective of the study was to evaluate the impact of probiotic supplementation on abundance of *Bacteroides spp.* in intestinal microbiome of patients with Crohn's disease (CD).
Materials and methods. The comparative evaluation was conducted over a 6-month period, on 49 subjects diagnosed with CD, who were separated into two groups, as follows: the study group (probiotics associated with allopathic treatment) and the control group (only allopathic treatment). All patients were evaluated at baseline and at 6 months. Demographic characteristics, associated pathology, and the evolution of intestinal microbiome and faecal pH were followed.
Results. In this research, the microbiome of patients with CD showed changes in the abundance of bacterial species. The combination of probiotic treatment led to the following changes: *Escherichia coli* (from $5.77x10^7$ to $4.15x10^7$, p=0.006) and *Enterobacter* spp. (from $1.92x10^4$ to $1.17x10^4$, p=0.009) values decreased

Résumé

Effet des probiotiques alimentaires sur le microbiote intestinal chez les patients atteints de la maladie de Crohn

Introduction. Les probiotiques sont des adjuvants bien connus, utilisés comme agents thérapeutiques complémentaires dans les troubles de santé (par exemple gastro-intestinaux ou métaboliques), compte tenu de leur rôle bénéfique sur le microbiote intestinal et de leur soutien à l'immunité.
L'objectif de l'étude était d'évaluer l'impact de la supplémentation en probiotiques sur l'abondance de Bacteria spp dans le microbiome intestinal des patients atteints de la maladie de Crohn (MC).
Matériels et méthodes. L'évaluation comparative a été menée sur une période de 6 mois, sur 49 sujets diagnostiqués avec la MC, qui ont été séparés en deux groupes, comme suit: le groupe d'étude (probiotiques associés au traitement allopathique) et le groupe témoin (uniquement traitement allopathique). Tous les patients ont été évalués au départ et à 6 mois. Les caractéristiques démographiques, la pathologie associée et l'évolution du microbiome intestinal et du pH fécal ont été suivies.

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significantly and *Faecalibacterium prausnitzii* (from $3.73x10^8$ to $4.55x10^8$, p=0.003), *Bifidobacterium* spp. (from $4.76x10^6$ to $4.92x10^6$, p<0.001 and *Bacteroides* spp. (from $4.68x10^7$ to $4.80x10^7$, p=0.012) values increased significantly; the pH value increased significantly at 6 months (from 6.30 to 6.59, p=0.043).

Conclusions. Treatment with the selected probiotic led to changes in the composition of beneficial microbial communities in patients with CD.

Keywords: gastrointestinal disorders, Crohn's disease, gut microbiome, inflammatory bowel diseases.

List of abbreviations:
CD – Crohn's disease
IBD – inflammatory bowel diseases
UC – ulcerative colitis
qPCR – quantitative polymerase chain reaction
ES – effect size
SCFAs – short-chain fatty acids

Résultats. Dans cette recherche, le microbiome des patients atteints de MC a montré des changements dans l'abondance des espèces bactériennes. La combinaison du traitement probiotique a entraîné les changements suivants: les valeurs d'*Escherichia coli* (de 5,77x107 à 4,15x107, p = 0,006) et d'*Enterobacter* spp. (de 1,92x104 à 1,17x104, p = 0,009) ont diminué de manière significative et *Faecalibacterium prausnitzii* (de 3,73x108 à 4,55x108, p = 0,003), les valeurs de *Bifidobacterium* spp. (de 4,76x106 à 4,92x106, p<0,001 et *Bacteroides* spp. (de 4,68x107 à 4,80x107, p = 0,012) ont augmenté significativement; la valeur du pH a augmenté significativement à 6 mois (de 6,30 à 6,59, p = 0,043).

Conclusions. Le traitement avec le probiotique sélectionné a entraîné des changements dans la composition des communautés microbiennes bénéfiques chez les patients atteints de MC.

Mots-clés: troubles gastro-intestinaux, maladie de Crohn, microbiome intestinal, maladies inflammatoires de l'intestin

Introduction

Inflammatory bowel diseases (IBD) are chronic, immune-mediated conditions, of the gastrointestinal tract. The term IBD includes Crohn's disease (CD) and ulcerative colitis (UC), which both have a complex aetiology and pathogenesis that have been insufficiently described and acknowledged[1]. The IBD incidence has increased over time, because of industrial development and related lifestyle changes. The prevalence of these diseases is higher in developed countries, where it can reach up to 4 cases per 1000 inhabitants[2,3]. From the epidemiological point of view, the incidence of IBD varies by two criteria, that is, geographical region and age[4]. Thus, in the North American region, the incidence of UC varies between 2.2 and 19.2 cases per 100,000 inhabitants, and the incidence of CD between 3.1 and 20.2 per 200,000 inhabitants[5]. According to age, there is a bimodal distribution of IBD, with a first peak between 15 and 30 years, and the second peak after 60 years[5]. If approximately 25% of patients develop IBD in adolescence, 10–15% of them may have IBD onset after the age of 60 years[5,6].

CD can affect any segment of the gastrointestinal tract, from the oral cavity to the anus. About 30-40% of patients present only disease of the small intestine, 40-50% suffer from disease of the small and large intestine, and the other 15-20% only from colitis. Unlike UC, CD is a transmural process. From the endoscopic point of view, superficial small or aphthous ulcers are characteristics of a moderate disease. In more active disease, stellar ulcers merge longitudinally and transversely to demarcate the usually normal histologically mucosal islands. Active CD is characterized by focal inflammation and formation of fistular pathways. Thickened mesentery projections narrow the large intestine, and serous and mesenchymal inflammation support the appearance of adhesions and fistulas[7].

Although CD usually presents as a chronic or acute inflammation of the large intestine, the inflammatory process evolves into one of two models of the disease – fibrotic obstruction or penetrating fistula. The localization of the disease influences the clinical manifestations[8].

The pathogenesis of bowel diseases is correlated with the composition and diversity of microbiota and environmental factors[9]. The intestinal microbial flora is based on a high diversity of beneficial bacteria, fungi, and viruses. The human gut microbial flora includes over 1000 species of commensal bacterial species. There are four major phyla which predominate in a healthy human gut: Bacteroides, Firmicutes, Proteobacteria and Actinobacteria. These four bacterial species represent more than 90% of the microbiome's population[10,11].

The host immunity and inflammatory response can be highly influenced by the gut microbiota[12]. There are studies linking CD with gut microbiome dysbiosis[13]. Some studies show a difference between the composition and function of

intestinal microbiome in patients suffering from CD and healthy subjects[14,15].

The objective of the study was to determine the influence of dietary supplementation with probiotic yeast *Saccharomyces boulardii* on the abundance of bacterial species in the intestinal microbiome of patients with CD.

Materials and methods

A prospective 6-month comparative study was conducted in the Clinical County Emergency Hospital, Oradea, Romania, on 49 patients with CD, of whom 21 patients were administered probiotics (study group, SG) and 28 patients were not given probiotics (control group, CG). At the inclusion in the study, all patients were in clinical remission. Both groups received aminosalicylates in individualized doses; in SG, in addition to allopathic treatment, *Saccharomyces boulardii* 1 g was administered daily for six months. The repartition to one group or another was decided by the patients' option to supplement their treatment established by the gastroenterologist with probiotics. The profile of the intestinal microbiome has been determined for all patients and a comparative study has been made regarding the modification of bacterial species involved under treatment with *Saccharomyces boulardii*.

Faecal samples were taken and bacterial cultures evaluated by quantitative polymerase chain reaction (qPCR), using the Real Time PCR Equipment (deoxyribonucleic acid (DNA) Technology, Research and Production" LLC, JSC 2017, Russia), Multiplex kits (Immunodiagnostik Gmbh Germany), MutaPLEX AKM/FEAB PCR and MutaPLEX EU/BAC/BIF PCR tests[16].

According to the protocol[16], the reference values are as follows: *Escherichia coli* $1x10^6$-$1x10^7$; *Proteus* spp. $<1x10^4$; *Klebsiella* spp. $<1x10^4$; *Enterobacter* spp. $<1x10^4$; *Serratia* spp. $<1x10^4$; *Morganella morganii* $<1x10^4$; *Citrobacter* spp. $<1x10^4$; *Pseudomonas* spp. $<1x10^4$; *Enteroccocus* spp. $1x10^6$-$1x10^7$; *Staphylococcus aureus* $<1x10^3$; *Akkermansia munciniphila* $>5x10^9$; *Faecalibacterium prausnitzii* $>2x10^{10}$; *Eubacterium* spp. $>1x10^8$; *Bifidobacterium* spp. $1x10^9$-$1x10^{11}$; *Bacteroides* spp. $>1x10^8$; ratio *Firmicutes* spp./*Bacteroides* spp. $1x10^{-1}$-$1x10^1$; *Candida albicans* $>1x10^2$; *Candida nonalbicans* $>1x10^2$; *Geotrichum* spp. $>1x10^3$. The reference values for faecal pH are 5.5-6.5.

Agreed by the Ethics Commission of the Clinical County Emergency Hospital, Oradea, Romania (no. 19/07.11.2019), this research was performed according to WMA Ethical Declaration of Helsinki. Each patient signed an informed consent form before inclusion in the study.

Data processing was performed using the SPSS 20 program. Average parameter values, frequency ranges, standard deviations, tests of statistical significance were calculated by the Student's method (t test) and c^2. ANOVA (Brown-Forsythe) was used to compare the means, and the level of statistical significance was 0.05. Figures were made using Harvard Graphics.

The statistical indicator sensitivity to change ("sensitivity to change") was also used, it was evaluated by calculating the effect size ("effect size" – ES). Sensitivity to change can be assessed in various types of clinical research or long-term observational studies. ES is a method of standardizing the magnitude of a change in a variable over a period. It is the average change for a variable expressed in units of standard deviation. This standardization allows the comparison of the values of the change of a variable in a study. ES can also be used to compare the same variables between different studies[17]. The values of ES can be interpretated like: ES <0.2 – minor change, ES between 0.2-0.49 – small change, ES between 0.5-0.79 – moderate change, and ES >0.8 – major change.

Results

Demographic data and clinical characteristics

In both groups, women predominated, without significant differences (57.14% vs 50.00%, p=0.624) and most patients were under 50 years of age (66.67% vs 57.15%). The mean age was insignificantly higher in the SG compared to the CG (41.43 vs 42.79 years, p=0.730). In the SG, 76.19% of the patients came from the urban environment, and in CG 57.14% (p=0.170), the urban/rural ratio being 3.2:1, respectively 1.3:1 (Table 1).

The most common associated diseases in patients included in the study were high blood pressure (38.10% vs 28.57%, p=0.485), and dyslipidaemia (23.81% vs 21.43%, p=0.845), followed by other cardiovascular diseases (23.81% vs. 14.29%, p=0.399) and obesity (19.05% vs. 25.00%, p=0.625). Diabetes mellitus was recorded in 4.76%, respectively 3.57% of the patients (p=0.837) (Table 2).

In both groups and all cases, regardless of the assessment, for *Proteus* spp., *Klebsiella* spp., *Serratia* spp., *Morganella morganii, Citrobacter* spp., *Pseudomonas* spp., a value of $1x10^3$ was recorded; $1x10^2$ was registered for *Staphylococcus aureus* and *Geotrichum* spp., and $1x10^1$ for *Candida albicans* and *Candida nonalbicans*; *Akkermansia muciniphila* was $>5x10^9$, and *Eubacterium* spp. was $1x10^7$.

Table 1. Distribution by demographic characteristics

Demographic characteristics	*Study group*		*Control group*	
	No.	%	*No.*	%
Gender				
Male	9	42.86	14	50.00
Female	12	57.14	14	50.00
Total	21	100.00	28	100.00
Age (years)				
<30 years	6	28.57	4	14.29
31-40 years	5	23.81	6	21.43
41-50 years	3	14.29	6	21.43
51-60 years	6	28.57	9	32.14
61-70 years	0	0.00	1	3.57
>70 years	1	4.76	2	7.14
Mean age (years)	41.43±14.57		42.79±11.93	
Environment				
Rural	5	23.81	12	42.86
Urban	16	76.19	16	57.14

Table 2. Distribution by associated diseases – Crohn's disease

Associated diseases	*Study group*		*Control group*		*Total*	
	No.	%	*No.*	%	*No.*	%
High blood pressure	8	38.10	8	28.57	16	32.65
Cardiovascular diseases	5	23.81	4	14.29	9	18.37
Diabetes mellitus	1	4.76	1	3.57	4	8.16
Obesity	4	19.05	7	25.00	8	16.33
Dyslipidaemia	5	23.81	6	21.43	11	22.45

Table 3. The evolution of pH – Crohn's disease

Groups	Baseline	At 6 months	p	ES
Study	6.30±0.41	6.58±0.44	0.043	0.67
Control	6.23±0.28	6.40±0.24	0.016	0.62
Total	6.26±0.34	6.48±0.35	0.002	0.64

The gastric pH was measured at baseline and the results were 6.30±0.41 for the SG and 6.23±0.28 for the CG (Table 3).

Evolution at six months

Compared to the baseline values, in the SG, *Escherichia coli* decreased significantly at 6 months (from $5.77x10^7$ to $4.15x10^7$, p=0.006). In the CG, compared to the baseline values, *Escherichia coli* decreased insignificantly at 6 months (from $5.72x10^7$ to $4.76x10^7$, p=0.153). The baseline values are insignificantly higher in the SG than in the CG ($5.77x10^7$ vs $5.72x10^7$, p=0.937), and at 6 months the values were insignificantly lower ($4.15x10^7$ vs $4.76x10^7$, p=0.268). In patients from CD group, the *Escherichia coli* values were over the normal limit ($1x10^7$), and at 6 months they decreased after treatment, remaining high ($5.74x10^7$ vs $4.50x10^7$, p=0.006) (Figure 1a). In patients with CD, at 6 months, the effect of treatment on *Escherichia coli* was moderate (ES=0.51), major in SG (ES=0.81) and small in CG (ES=0.35) (Figure 2).

In the SG, *Enterobacter spp.* significantly decreased at 6 months (from $1.92x10^4$ to $1.17x10^4$, p=0.009). In the CG, compared to the baseline values, *Enterobacter spp.* decreased insignificantly at 6 months (from $2.04x10^4$ to $1.55x10^4$, p=0.101). The baseline values and at 6 months were insignificantly lower in the SG than in the CG ($1.92x10^4$ vs $2.04x10^4$, p=0.724, respectively $1.17x10^4$ vs $1.55x10^4$, p=0.080). In patients from the CD group, the *Enterobacter spp.* values are

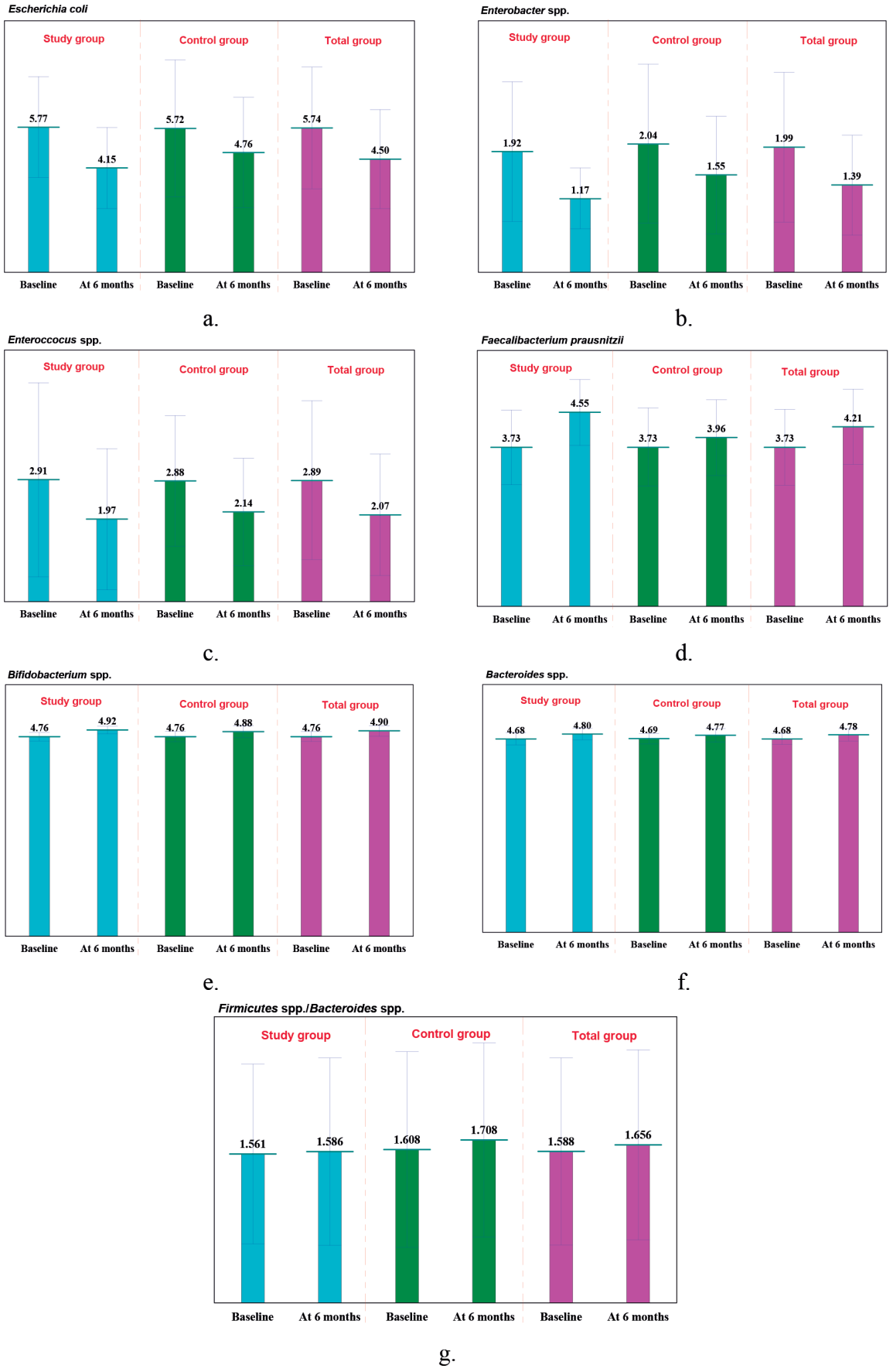

Figure 1. The evolution of intestinal bacteria abundance: a. *Escherichia coli*, b. *Enterobacter* spp., c. *Enteroccocus* spp., d. *Faecalibacterium prausnitzii,* e. *Bifidobacterium* spp., f. *Bacteroides* spp., g. *Firmicutes* spp./*bacteroides* spp.

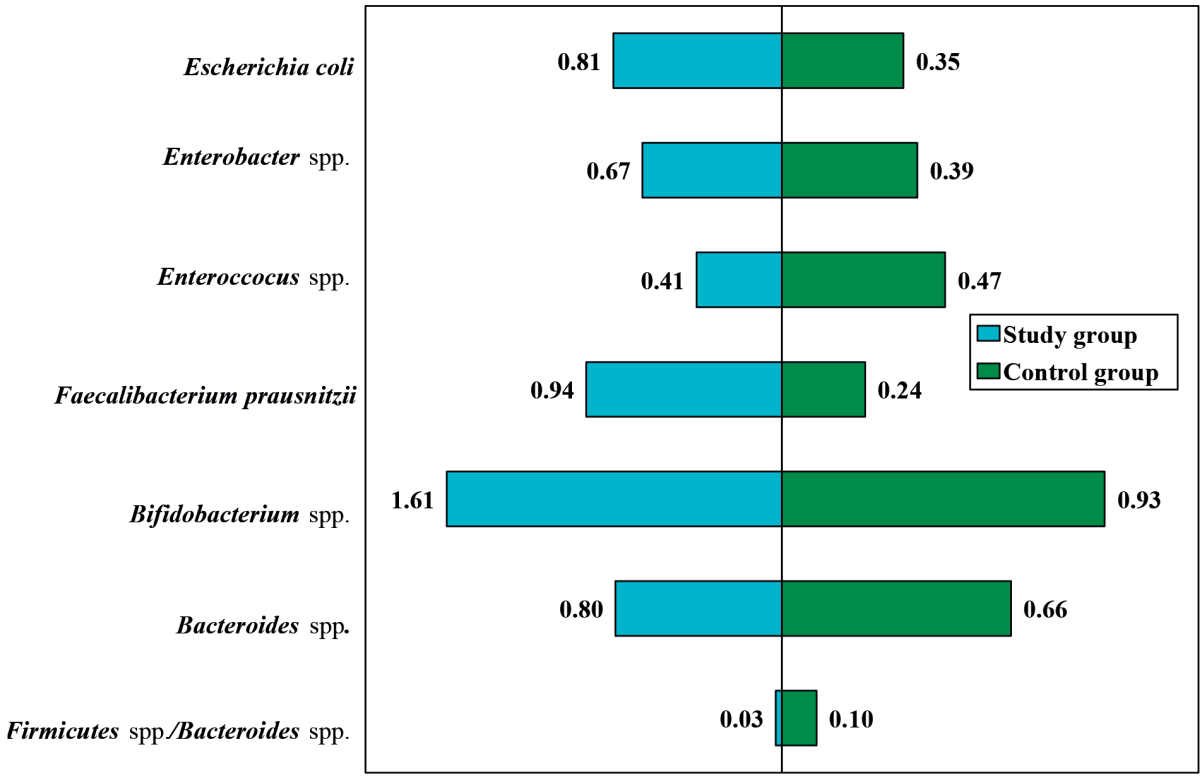

Figure 2. The effect of probiotics at 6 months – Crohn's disease

at the upper normal limit ($1x10^4$), and at 6 months they decreased after treatment, remaining still high ($1.99x10^4$ vs $1.39x10^4$, p=0.004) (Figure 1b). The treatment effect at 6 months on *Enterobacter spp.* in patients with CD was moderate (ES=0.51), moderate in SG (ES=0.67) and small in CG (ES=0.39) (Figure 2).

The average values of *Enterococcus spp.* insignificantly decreased at 6 months in SG and CG (from $2.91x10^7$ to $1.97x10^7$, p=0.140, respectively from $2.88x10^7$ to $2.14x10^7$, p=0.068), without significant differences between the two groups, regardless of the time of evaluation ($2.91x10^7$ vs $2.88x10^7$, p=0.967, respectively $1.97x10^7$ vs $2.14x10^7$, p=0.687). In patients from the CD group, the *Enterococcus spp.* values were at the upper normal limit ($1x10^7$), and at 6 months they decreased after treatment, remaining lower ($2.89x10^7$ vs $2.07x10^7$, p=0.018) (Figure 1c). The effect on *Enterococcus spp.* after 6 months of treatment was small (ES=0.44), in both SG and CG (ES=0.41, respectively ES=0.47) (Figure 2).

In the SG, *Faecalibacterium prausnitzii* values increased significantly at 6 months (from $3.73x10^8$ to $4.55x10^8$, p=0.003), and increased insignificantly in CG (from $3.73x10^8$ to $3.96x10^8$, p=0.357). Comparing the two groups, it was found that, at baseline, the mean values of *Faecalibacterium prausnitzii* were equal ($3.73x10^8$, p=0.977), and at 6 months the values were significantly higher in the SG ($4.55x10^8$ vs $3.96x10^8$, p=0.017). In SG, the *Faecalibacterium prausnitzii* values were below the lower normal limit ($2x10^{10}$), and at 6 months they increased after treatment, remaining lower ($3.73x10^8$ vs $4.21x10^8$, p=0.009) (Figure 1d). The effect of treatment at 6 months on *Faecalibacterium prausnitzii* in patients with CD was moderate (ES=0.54), major in SG (ES=0.94) and small in CG (ES=0.24) (Figure 2).

In both groups, the values of *Bifidobacterium* spp. increased significantly at 6 months (from $4.76x10^6$ to $4.92x10^6$, p<0.001, respectively from $4.76x10^6$ to $4.88x10^6$, p=0.002). At baseline, the average values of *Bifidobacterium* spp. were equal ($4.76x10^6$, p=0.906), and at 6 months the values were insignificantly higher in the SG ($4.92x10^6$ vs $4.88x10^6$, p=0.213). In patients with CD, the *Bifidobacterium* spp. values were below the low normal limit ($1x10^9$), and at 6 months they increased after treatment, remaining still lower ($4.76x10^6$ vs $4.90x10^6$, p<0.001) (Figure 1e). The effect at 6 months of treatment on *Bifidobacterium* spp. in patients with CD was major (ES=1.19), major in SG (ES=1.61) and in CG (ES=0.93) (Figure 2).

Bacteroides spp. values increased significantly at 6 months (from $4.68x10^7$ to $4.80x10^7$, p=0.012, respectively from $4.69x10^7$ to $4.77x10^7$, p=0.031). At baseline, the average values of *Bacteroides* spp. were insignificantly lower in the probiotics group ($4.68x10^7$

Table 4. The analysis of the evolution of the microbiome – Crohn's disease

Microbiome	Baseline	At 6 months	p-value	ES
Study group				
Escherichia coli ($x10^7$)	5.77±2.01	4.15±1.61	0.006	0.81
Enterobacter spp. ($x10^4$)	1.92±1.11	1.17±0.49	0.009	0.67
Enteroccocus spp. ($x10^7$)	2.91±2.31	1.97±1.68	0.140	0.41
Faecalibacterium prausnitzii ($x10^8$)	3.73±0.88	4.55±0.78	0.003	0.94
Bifidobacterium spp. ($x10^6$)	4.76±0.10	4.92±0.09	<0.001	1.61
Bacteroides spp. ($x10^7$)	4.68±0.15	4.80±0.14	0.012	0.80
Firmicutes spp./*Bacteroides* spp. ($x10^0$)	1.561±0.943	1.586±0.983	0.933	0.03
Control group				
Escherichia coli ($x10^7$)	5.72±2.72	4.76±2.19	0.153	0.35
Enterobacter spp. ($x10^4$)	2.04±1.26	1.55±0.94	0.101	0.39
Enteroccocus spp. ($x10^7$)	2.88±1.56	2.14±1.28	0.058	0.47
Faecalibacterium prausnitzii ($x10^8$)	3.73±0.92	3.96±0.88	0.357	0.24
Bifidobacterium spp. ($x10^6$)	4.76±0.13	4.88±0.14	0.002	0.93
Bacteroides spp. ($x10^7$)	4.69±0.13	4.77±0.16	0.031	0.66
Firmicutes spp./*Bacteroides* spp. ($x10^0$)	1.608±1.025	1.708±1.018	0.716	0.10
Total				
Escherichia coli ($x10^7$)	5.74±2.42	4.50±1.97	0.006	0.51
Enterobacter spp. ($x10^4$)	1.99±1.19	1.39±0.79	0.004	0.51
Enteroccocus spp. ($x10^7$)	2.89±1.90	2.07±1.45	0.018	0.44
Faecalibacterium prausnitzii ($x10^8$)	3.73±0.89	4.21±0.88	0.009	0.54
Bifidobacterium spp. ($x10^6$)	4.76±0.12	4.90±0.12	<0.001	1.19
Bacteroides spp. ($x10^7$)	4.68±0.14	4.78±0.15	0.001	0.73
Firmicutes spp./*Bacteroides* spp. ($x10^0$)	1.588±0.981	1.656±0.995	0.734	0.07

vs $4.49x10^7$, p=0.899), and at 6 months the values were insignificantly higher in the probiotics group ($4.80x10^7$ vs $4.77x10^7$, p=0.557). In patients from the CD group, the *Bacteroides* spp. values were below the low normal limit ($1x10^8$), and at 6 months they increased after treatment, remaining lower ($4.68x10^7$ vs $4.78x10^7$, p=0.001) (Figure 1f). The effect at 6 months of treatment on *Bacteroides* spp. in patients with CD was moderate (ES=0.73), major in SG (ES=0.80) and moderate in CG (ES=0.66) (Figure 2).

In both groups, the values of *Firmicutes* spp./*Bacteroides* spp. increased insignificantly at 6 months (from $1.561x10^0$ to $1.586x10^0$, p=0.933, respectively from $1.608x10^0$ to $1.708x10^0$, p=0.734). At baseline, at 6 months, the average values of *Firmicutes* spp./*Bacteroides* spp. were insignificantly lower in the probiotics group ($1.561x10^0$ vs $1.608x10^0$, p=0.868, respectively $1.586x10^0$ vs $1.708x10^0$, p=0.674). In patients from the CD group, the *Firmicutes* spp./*Bacteroides* spp. values were within the normal range ($1x10^{-1}$-$1x10^1$), at baseline and at 6 months ($1.588x10^0$ vs $1.656x10^0$, p=0.734) (Figure 1g). At 6 months of treatment, the effect on *Firmicutes* spp./*Bacteroides* spp. in patients with CD was minor (ES=0.07), being also minor in the SG and CG (ES=0.03, respectively ES=0.10) (Table 4).

In both groups, the pH value increased significantly at 6 months (from 6.30 to 6.59, p=0.043, respectively from 6.23 to 6.40, p=0.016). At baseline, and at 6 months, the average pH values were insignificantly higher in SG compared to CG (6.05 vs 6.23, p=0.478, respectively 6.58 vs 6.40, p=0.101).

In patients from CD group, the pH values were within the normal range, and at 6 months they increased after treatment, remaining within the normal range (6.26 vs 6.48, p=0.002). The effect at 6 months of treatment on pH in patients with CD was moderate (ES=0.64), both in SG (ES=0.67) and in CG (ES=0.62) (Table 3).

Discussion

Intestinal microbiome has a very important role in the evolution of CD, both in experimental animals and in humans[18,19]. Recently, some studies have revealed that patients with CD have a relative lower abundance of short-chain fatty acids (SCFAs) producing bacteria. This fact was shown by studying patients'

faecal samples[20,21]. In patients with active IBD, it has been demonstrated that the most repetitive decrease is the decrease of *Faecalibacterium prausnitzii*[22].

Similar to the results of previous studies[23], in our study the microbiome of patients with CD showed changes in the abundance of bacterial species. Thus, the *Faecalibacterium prausnitzii, Bifidobacterium* spp., and *Bacteroides* spp. values were below the low normal limit, while the *Escherichia coli, Enterobacter* spp. and *Enterococcus* spp. values were over the upper limit of the normal.

Adherent-invasive *Escherichia coli* is involved in the pathogenesis of IBD, and recent evidence shows that its presence not only indicates the appearance of IBD, but also seems to predict relapses in affected patients. *Enterobacteriaceae* and *Streptococcus* seem to play, as well, an important role in the microbiome dysbiosis and further diagnosis of IBD[23]. At 6 months, after treatment, associated or not with probiotics, the *Escherichia coli, Enterobacter* spp., *Enteroccocus* spp. values decreased significantly in CD group and in SG ($p<0.05$), but they remained high. In CG, without probiotics, the values also decreased, but insignificantly ($p>0.05$). The values of *Bifidobacterium* spp. and *Bacteroides* spp. increased significantly at 6 months ($p<0.05$). Also, *Faecalibacterium prausnitzii* values significantly increased in CD group and in SG, but insignificantly in CG ($p>0.05$). At 6 months the values remained low.

In a meta-analysis of 38 studies, Zhang et al. explored the clinical effects and intestinal microbiota changes determined by the treatment with probiotics, prebiotics and synbiotics in patients with IBD[24,25]. These authors concluded that the use of probiotic supplements leads to an increase in the number of beneficial bacteria, especially *Bifidobacteria*, in the intestinal microbiome in patients with IBD[24].

Depending on bicarbonate secretion by colonic epithelial cells, the quantity and type of fermentation products, assimilation of microbial metabolites by the host, along the human bowel, the pH may fluctuate from 5 to 7[26].

The changes in the composition of microbiota and metabolism can affect the bowel function; for example, at pH = 5.5, butyrogenic *Faecalibacterium* and *Roseburia* grow better and produce more butyrate than at an approximately neutral pH of 6.7, and therefore they change their colonic function by nourishing colonocytes and protecting against DNA damage induced by hydrogen peroxide. The production of propionate can be easily inhibited by an acidic pH (5.5), due to the limited growth of propionate-producing species such as *Bacteroides*[27].

After butyrate, the second important source of energy for colonocytes is propionate[28], which has anti-inflammatory properties and has an important role in the IBD treatment[29]. In this study, the pH was 6.30±0.41 in the SG and 6.23±0.28 in CG and increased significantly at 6 months (from 6.30 to 6.59, $p=0.043$, respectively from 6.23 to 6.40, $p=0.016$).

The main limitations of this research are the small number of patients and the short evaluation period. In addition, in the absence of a healthy control group, imbalances in the intestinal flora cannot be correlated only with CD. Several studies conducted under more controlled conditions and including a higher number of patients are needed to evaluate the effects of probiotic treatment in patients with CD.

Conclusions

Patients with CD included in this study showed imbalances in their intestinal flora, characterized by a low abundance of *Faecalibacterium prausnitzii*, *Bifidobacterium* spp., and *Bacteroides* spp. The treatment with the selected probiotic led to changes in the composition of beneficial microbial communities in patients with CD, including an increase in *Faecalibacterium prausnitzii*, *Bifidobacterium* spp., and *Bacteroides* spp. and a significant decrease in *Escherichia coli*, *Enteroccocus* spp. and *Enterobacter* spp. The treatment with *Saccharomyces boulardii* would be an option for intestinal microbiome imbalances in patients with CD, but further studies are needed to establish the changes of gut microbiota in different phases of the disease and to provide more information about the probiotics' effects in these patients.

Authors Contribution:

Conceptualization, F.M.P, D.M.T., T.B. and S.B.; methodology, F.M.P and S.B.; software, T.B, and A.F.B; validation, D.M.T and S.B..; formal analysis, T.B. and S.B.; investigation, F.M.P, A.F.B, and A.G.T.; writing–original draft preparation, F.M.P. and D.M.T.; writing–review and editing , F.M.P, D.M.T. and S.B.; visualization, S.B.; supervision, D.M.T. and S.B.; project administration, S.B. All authors have read and agreed with the final version of this article.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study"

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