

## Original Research

### Comparative Study of Microbiota Profiles in Healthy Individuals Versus Patients with Inflammatory Bowel Disease

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#### ABSTRACT:

**Aim:** The aim of this study was to compare the gut microbiota profiles between healthy individuals and patients diagnosed with Inflammatory Bowel Disease (IBD), including both Crohn's disease and ulcerative colitis. This comparison aimed to identify microbial differences that may be associated with the pathogenesis of IBD. **Materials and Methods:** This study included 100 participants, divided into two groups: 50 healthy controls and 50 IBD patients. Fecal samples were collected from all participants and stored at -80°C until analysis. Microbiota profiles were assessed using 16S ribosomal RNA (rRNA) gene sequencing of the V3-V4 regions. DNA extraction, library preparation, and sequencing were performed using standard protocols on the Illumina MiSeq platform. Bioinformatics tools were used to process the sequencing data, with statistical analysis conducted to compare microbial diversity, richness, and composition between the two groups. **Results:** Significant differences were observed between IBD patients and healthy controls. IBD patients exhibited reduced microbial diversity, with significantly lower Shannon and Chao1 indices ( $p < 0.001$ ). A decrease in *Firmicutes* and an increase in *Proteobacteria* were noted in IBD patients compared to healthy controls ( $p = 0.023$  and  $p = 0.018$ , respectively). Key genera such as *Bacteroides*, *Escherichia*, and *Faecalibacterium* showed significant differences, with *Faecalibacterium* being reduced in IBD patients ( $p = 0.012$ ). Functional pathway analysis revealed altered metabolic processes, with increased lipid metabolism and xenobiotic biodegradation in IBD patients ( $p = 0.022$  and  $p = 0.012$ , respectively). **Conclusion:** This study demonstrates significant alterations in the gut microbiota composition of IBD patients compared to healthy controls, including reduced diversity and shifts in the abundance of key microbial phyla and genera. These findings emphasize the potential role of microbiota dysbiosis in IBD pathogenesis and suggest that modulating the microbiome could offer therapeutic benefits in the management of IBD.

**Keywords:** Inflammatory Bowel Disease, Gut Microbiota, Dysbiosis, 16S rRNA Sequencing, Microbial Diversity

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#### INTRODUCTION

Inflammatory Bowel Disease (IBD) represents a group of chronic disorders of the gastrointestinal tract, primarily characterized by inflammation of the intestines. The two most common forms of IBD are Crohn's disease and ulcerative colitis, both of which lead to symptoms such as abdominal pain, diarrhea, and weight loss. While the exact cause of IBD remains elusive, it is believed to arise due to a combination of genetic predisposition, environmental factors, and an abnormal immune response. One of the most recent and significant areas of research in understanding IBD is the role of the gut microbiota—the community of microorganisms residing in the human digestive tract.<sup>1</sup>

The human microbiota is a complex and diverse ecosystem that includes bacteria, archaea, fungi, viruses, and other microorganisms. These microbial communities are crucial in maintaining health by contributing to nutrient absorption, immune modulation, and the protection against harmful pathogens. Recent advancements in molecular techniques, particularly 16S rRNA sequencing and metagenomics, have provided deeper insights into the intricate relationship between the gut microbiota and various diseases, including IBD. As the understanding of the microbiome grows, it has become evident that alterations in microbiota composition and diversity play a significant role in the development and progression of IBD.<sup>2</sup>

A growing body of evidence suggests that the gut microbiota in individuals with IBD differs substantially from that of healthy individuals. This difference is typically characterized by a reduction in microbial diversity and the abundance of certain bacterial species. Healthy individuals maintain a balanced microbiota that supports a symbiotic relationship between the host and the microbial community. However, in patients with IBD, there appears to be a dysbiosis—a state of microbial imbalance—that exacerbates the inflammatory processes. This dysbiosis is thought to contribute to the pathogenesis of IBD by disrupting the normal immune response in the gut, increasing intestinal permeability, and promoting the development of inflammation.<sup>3</sup>

The composition of the gut microbiota is influenced by several factors, including diet, lifestyle, medication use, and geographical location. These factors are also believed to play a crucial role in the onset and exacerbation of IBD. For instance, the Western diet, characterized by high-fat and low-fiber intake, has been linked to alterations in gut microbiota that may predispose individuals to IBD. Additionally, antibiotic use, which can disrupt microbial communities, is considered a risk factor for the development of IBD. Given these findings, there has been increasing interest in exploring the potential for microbiota-based therapies to prevent or treat IBD.<sup>4</sup>

A comparative study of the microbiota profiles in healthy individuals versus patients with IBD can provide valuable insights into the specific microbial changes that occur during the disease process. By analyzing the differences in microbial diversity, abundance, and composition between these two groups, researchers aim to identify specific microbial signatures associated with IBD. These signatures could potentially serve as biomarkers for diagnosing IBD, predicting disease activity, or monitoring therapeutic responses. Additionally, understanding the mechanisms by which microbiota alterations contribute to IBD pathogenesis could open new avenues for developing microbiota-targeted therapies, such as probiotics, prebiotics, and fecal microbiota transplantation.<sup>5</sup>

In healthy individuals, the microbiota is typically dominated by certain phyla of bacteria, including Firmicutes and Bacteroidetes. These bacteria are involved in the fermentation of dietary fibers, production of short-chain fatty acids, and regulation of the immune system. The presence of beneficial bacteria, such as *Faecalibacteriumprausnitzii*, *Bacteroides fragilis*, and *Akkermansiamuciniphila*, is often associated with gut health. In contrast, patients with IBD exhibit reduced diversity and an imbalance in these microbial populations. Studies have consistently shown a decrease in the abundance of Firmicutes and Bacteroidetes and an increase in potentially harmful microorganisms such as *Proteobacteria*, *Enterococcus*, and *Escherichia coli*.

These microbial shifts may not only promote inflammation directly but also create a favorable environment for pathogenic bacteria to thrive.<sup>6</sup>

Moreover, certain microbial species are thought to play a protective role in maintaining intestinal homeostasis and preventing inflammation. For example, *Faecalibacteriumprausnitzii* produces butyrate, a short-chain fatty acid that serves as an energy source for colonocytes and has anti-inflammatory effects. A decrease in this bacterium has been associated with increased inflammation in IBD patients. Similarly, *Akkermansiamuciniphila* has been shown to enhance intestinal barrier function, and its abundance is often reduced in IBD patients. These findings suggest that specific microbial species are critical in maintaining gut health and preventing the onset of IBD.

In addition to changes in microbial composition, the functional capacity of the microbiota in IBD patients may also be altered. The production of metabolites such as short-chain fatty acids, bile acids, and vitamins is influenced by the microbiota and can affect intestinal health. In IBD, the production of anti-inflammatory metabolites may be diminished, while pro-inflammatory molecules may be elevated. These functional changes can contribute to a cycle of inflammation, further exacerbating the symptoms of the disease.<sup>7</sup>

The study of the microbiota in IBD patients is not only crucial for understanding disease mechanisms but also for developing novel therapeutic strategies. Probiotics, prebiotics, and dietary interventions that aim to restore a healthy microbiota are currently being explored as potential treatments for IBD. However, more research is needed to determine the most effective microbiota-based therapies, as the gut microbiota is highly individualized and can vary widely between individuals.

## MATERIALS AND METHODS

This study was conducted on a cohort of 100 individuals, divided into two groups: 50 healthy controls and 50 patients diagnosed with Inflammatory Bowel Disease (IBD), including both Crohn's disease and ulcerative colitis. The participants were selected based on specific inclusion and exclusion criteria, ensuring that the healthy control group had no history of gastrointestinal disorders or significant comorbidities. The IBD group was diagnosed through clinical assessment, endoscopic examination, and histopathological analysis, according to the latest diagnostic guidelines. Fecal samples were collected from all participants after obtaining informed consent, and were stored at -80°C until analysis.

Microbiota profiles were assessed using 16S ribosomal RNA (rRNA) gene sequencing, focusing on the V3-V4 regions to capture a comprehensive view of the gut microbiome. DNA was extracted from the stool samples using a commercial kit, following the manufacturer's protocol. Library preparation was

performed using a standard protocol for amplicon-based sequencing, and sequencing was carried out on an Illumina MiSeq platform. The raw sequencing data were processed using bioinformatics tools, including quality control, filtering, and taxonomic classification, to generate microbial community profiles.

The results were analyzed to compare the diversity, richness, and composition of the gut microbiota between the healthy controls and IBD patients. Statistical analysis was performed using appropriate tests to evaluate differences in microbial abundances and diversity indices, with significance set at a p-value of less than 0.05. The study was approved by the institutional ethics committee, ensuring all procedures adhered to ethical guidelines for human research.

## RESULTS

### Table 1: Demographic and Clinical Characteristics of Study Participants

This table summarizes the demographic and clinical characteristics of the study participants, with no significant differences observed between the healthy controls and IBD patients in terms of age, gender distribution, smoking status, and BMI. The average age of the healthy control group was  $34.5 \pm 10.3$  years, while the IBD group was slightly older with an average age of  $36.7 \pm 11.1$  years ( $p=0.372$ ), which is not statistically significant. Gender distribution was also balanced between the groups, with no significant differences in male to female ratio ( $p=0.824$ ). Smoking status showed no significant difference either, with 12 smokers in the control group and 18 smokers in the IBD group ( $p=0.415$ ). The BMI of both groups was similar, with the control group having an average BMI of  $24.3 \pm 4.5$  kg/m<sup>2</sup> compared to the IBD group's  $23.9 \pm 4.2$  kg/m<sup>2</sup> ( $p=0.578$ ). Disease duration for the IBD group averaged  $5.3 \pm 3.2$  years, and among IBD patients, 28 had Crohn's disease while 22 had ulcerative colitis. These results indicate that the IBD and healthy control groups were well-matched in terms of baseline characteristics, making them suitable for microbiota comparison.

### Table 2: Gut Microbiota Diversity Indices in Healthy Controls vs. IBD Patients

Table 2 shows the diversity indices of the gut microbiota in both groups. The Shannon Index, which measures microbial diversity, was significantly lower in IBD patients ( $3.11 \pm 0.61$ ) compared to healthy controls ( $4.35 \pm 0.52$ ), with a p-value of  $<0.001$ . This suggests a marked decrease in microbial diversity in IBD patients, indicative of dysbiosis. The Simpson Index, which reflects the probability that two randomly selected individuals from the community will belong to the same species, was also lower in IBD patients ( $0.81 \pm 0.11$ ) compared to healthy controls ( $0.91 \pm 0.08$ ), with a p-value of 0.013, further supporting reduced diversity in the IBD group. The Chao1 Richness Estimate, an index that predicts the

number of species in a sample, was also significantly lower in IBD patients ( $600 \pm 112$ ) than in the healthy controls ( $750 \pm 105$ ), with a p-value of  $<0.001$ . This further indicates that IBD patients have fewer microbial species compared to healthy individuals.

### Table 3: Abundance of Key Phyla in Healthy Controls and IBD Patients

Table 3 shows the relative abundance of major phyla in the gut microbiota of both groups. The results revealed significant differences between the two groups in the relative abundances of certain phyla. *Firmicutes*, which is generally considered beneficial to gut health, was significantly lower in IBD patients ( $43.1 \pm 7.9\%$ ) compared to healthy controls ( $50.2 \pm 5.4\%$ ) with a p-value of 0.023. *Bacteroidetes*, another major phylum, was slightly higher in IBD patients ( $36.2 \pm 6.5\%$ ) compared to healthy controls ( $30.4 \pm 4.8\%$ ), although the p-value of 0.054 suggests a trend towards significance but does not reach statistical significance. A significant increase in *Proteobacteria* was observed in IBD patients ( $10.5 \pm 4.2\%$ ) compared to healthy controls ( $7.2 \pm 3.4\%$ ) with a p-value of 0.018, indicating an imbalance in microbial composition in IBD patients. Other phyla such as *Actinobacteria* and *Verrucomicrobia* showed no significant differences between the groups, with p-values of 0.144 and 0.095, respectively.

### Table 4: Abundance of Key Genera in Healthy Controls and IBD Patients

Table 4 shows the relative abundance of specific genera within the gut microbiota. Notable differences were found in several genera. *Bacteroides* was significantly lower in healthy controls ( $20.5 \pm 3.1\%$ ) compared to IBD patients ( $24.2 \pm 4.3\%$ ) with a p-value of 0.038, indicating that IBD patients had a higher relative abundance of this genus. *Faecalibacterium*, which is associated with anti-inflammatory properties, was significantly reduced in IBD patients ( $6.3 \pm 2.4\%$ ) compared to healthy controls ( $9.8 \pm 1.7\%$ ) with a p-value of 0.012, suggesting a loss of beneficial bacteria in IBD. *Akkermansia*, a genus that plays a role in maintaining gut barrier function, was also significantly more abundant in IBD patients ( $4.5 \pm 1.2\%$ ) than in healthy controls ( $2.2 \pm 0.8\%$ ) with a p-value of 0.002, indicating a potential response to gut inflammation. Conversely, *Escherichia*, a genus that includes pathogenic species, was significantly higher in IBD patients ( $7.8 \pm 3.2\%$ ) compared to healthy controls ( $3.5 \pm 1.1\%$ ), with a p-value of  $<0.001$ , suggesting the presence of more pathogenic bacteria in the IBD group. Finally, *Clostridium* was less abundant in IBD patients ( $10.1 \pm 3.5\%$ ) than in healthy controls ( $15.1 \pm 4.3\%$ ) with a p-value of 0.030, indicating a decrease in this genus in IBD patients.

**Table 5: Microbial Functional Pathways Predicted in Healthy Controls and IBD Patients**

Table 5 presents the predicted functional pathways of the gut microbiota in both groups. The analysis revealed significant differences in the predicted metabolic pathways between healthy controls and IBD patients. Carbohydrate metabolism was slightly reduced in IBD patients ( $19.2 \pm 4.1\%$ ) compared to healthy controls ( $22.1 \pm 3.8\%$ ) with a p-value of 0.056, indicating a trend toward reduced carbohydrate metabolism in IBD. Lipid metabolism was significantly higher in IBD patients ( $15.3 \pm 3.2\%$ )

compared to healthy controls ( $12.5 \pm 1.9\%$ ) with a p-value of 0.022, reflecting potential alterations in fat metabolism in the context of IBD. The biosynthesis of secondary metabolites was also significantly lower in IBD patients ( $7.5 \pm 2.1\%$ ) compared to healthy controls ( $9.7 \pm 1.5\%$ ) with a p-value of 0.042, possibly indicating reduced production of beneficial metabolites. Finally, xenobiotics biodegradation was significantly higher in IBD patients ( $6.9 \pm 1.7\%$ ) compared to healthy controls ( $5.2 \pm 0.8\%$ ) with a p-value of 0.012, suggesting that IBD patients may have altered microbial metabolism of foreign compounds.

**Table 1: Demographic and Clinical Characteristics of Study Participants**

Characteristic	Healthy Controls (n=50)	IBD Patients (n=50)	p-value
Age (years)	34.5 ± 10.3	36.7 ± 11.1	0.372
Gender (Male/Female)	25/25	26/24	0.824
Smoking Status (Smoker/Non-smoker)	12/38	18/32	0.415
BMI (kg/m <sup>2</sup> )	24.3 ± 4.5	23.9 ± 4.2	0.578
Disease Duration (years)	-	5.3 ± 3.2	-
Disease Type (Crohn's/Ulcerative Colitis)	-	28/22	-

**Table 2: Gut Microbiota Diversity Indices in Healthy Controls vs. IBD Patients**

Diversity Index	Healthy Controls (n=50)	IBD Patients (n=50)	p-value
Shannon Index	4.35 ± 0.52	3.11 ± 0.61	<0.001
Simpson Index	0.91 ± 0.08	0.81 ± 0.11	0.013
Chao1 Richness Estimate	750 ± 105	600 ± 112	<0.001

**Table 3: Abundance of Key Phyla in Healthy Controls and IBD Patients**

Phylum	Healthy Controls (%)	IBD Patients (%)	p-value
Firmicutes	50.2 ± 5.4	43.1 ± 7.9	0.023
Bacteroidetes	30.4 ± 4.8	36.2 ± 6.5	0.054
Actinobacteria	6.1 ± 2.3	5.2 ± 1.9	0.144
Proteobacteria	7.2 ± 3.4	10.5 ± 4.2	0.018
Verrucomicrobia	2.5 ± 1.1	3.4 ± 1.5	0.095

**Table 4: Abundance of Key Genera in Healthy Controls and IBD Patients**

Genus	Healthy Controls (%)	IBD Patients (%)	p-value
Bacteroides	20.5 ± 3.1	24.2 ± 4.3	0.038
Faecalibacterium	9.8 ± 1.7	6.3 ± 2.4	0.012
Akkermansia	2.2 ± 0.8	4.5 ± 1.2	0.002
Escherichia	3.5 ± 1.1	7.8 ± 3.2	<0.001
Clostridium	15.1 ± 4.3	10.1 ± 3.5	0.030

**Table 5: Microbial Functional Pathways Predicted in Healthy Controls and IBD Patients**

Functional Pathway	Healthy Controls (%)	IBD Patients (%)	p-value
Carbohydrate Metabolism	22.1 ± 3.8	19.2 ± 4.1	0.056
Amino Acid Metabolism	16.3 ± 2.2	18.1 ± 3.4	0.200
Lipid Metabolism	12.5 ± 1.9	15.3 ± 3.2	0.022
Biosynthesis of Secondary Metabolites	9.7 ± 1.5	7.5 ± 2.1	0.042
Xenobiotics Biodegradation	5.2 ± 0.8	6.9 ± 1.7	0.012

**DISCUSSION**

The demographic and clinical characteristics of our study population, as shown in Table 1, demonstrated no significant differences between healthy controls and IBD patients in terms of age, gender distribution, smoking status, and BMI. These findings are consistent with the study by Gevers et al. (2014), who

found no substantial differences in these baseline characteristics between Crohn's disease patients and healthy individuals, ensuring that differences in microbiota could be attributed to the disease itself rather than confounding factors. This absence of significant demographic variations between groups strengthens the validity of our microbiota analysis,

providing a well-matched comparison group for the investigation of gut microbial differences.<sup>8</sup>

In contrast, a notable difference was observed in gut microbiota diversity, as shown in Table 2. The Shannon Index and Chao1 Richness Estimate were significantly lower in IBD patients compared to healthy controls ( $p < 0.001$ ). These findings are in agreement with a study by Frank et al. (2007), who reported a decreased microbial diversity in patients with IBD, particularly in those with Crohn's disease, compared to healthy controls. The reduction in microbial diversity is a hallmark of dysbiosis, which is commonly associated with chronic inflammatory conditions like IBD (Frank et al., 2007). Our results further confirm the importance of microbial diversity in maintaining gut health and suggest that its reduction may contribute to the pathogenesis of IBD.<sup>9</sup> Regarding the abundance of key microbial phyla (Table 3), we observed a significant decrease in *Firmicutes* in IBD patients, along with an increase in *Proteobacteria*, which is considered a marker of dysbiosis. These findings are in line with those of Lozupone et al. (2012), who also observed a decrease in *Firmicutes* and an increase in *Proteobacteria* in individuals with IBD. *Firmicutes* are important for maintaining gut barrier function and producing short-chain fatty acids (SCFAs), which play a protective role against inflammation. The increase in *Proteobacteria* suggests a shift toward a more pathogenic microbiome in IBD patients, consistent with findings from previous studies that highlighted the role of *Proteobacteria* in inflammatory diseases.<sup>10</sup> When examining the genus-level microbiota (Table 4), we found a significant increase in *Bacteroides* and *Escherichia* and a decrease in *Faecalibacterium* and *Clostridium* in IBD patients compared to healthy controls. These results are comparable to those of Walker et al. (2011), who found an overabundance of *Bacteroides* and *Escherichia* in IBD patients, along with a decrease in beneficial genera like *Faecalibacterium* and *Clostridium* that are associated with anti-inflammatory properties. The increase in *Escherichia*, which includes pathogenic species such as *Escherichia coli*, suggests an imbalance that could contribute to the inflammatory response in IBD patients (Walker et al., 2011). Furthermore, the reduction in *Faecalibacterium* and *Clostridium* aligns with studies showing that these genera play a critical role in maintaining gut homeostasis and reducing inflammation.<sup>11</sup>

The functional pathways of the gut microbiota, as shown in Table 5, revealed several significant differences between IBD patients and healthy controls. We found a marked increase in lipid metabolism and a decrease in secondary metabolite biosynthesis in IBD patients. These findings are similar to those reported by Morgan et al. (2012), who found alterations in metabolic pathways related to lipid metabolism and reduced production of SCFAs in IBD patients. The increased lipid metabolism

observed in our study could be indicative of an altered microbial metabolism in response to gut inflammation, which is consistent with the findings of Morgan et al. (2012), who suggested that such metabolic shifts may influence disease progression in IBD.<sup>12</sup>

In addition, the increase in xenobiotics biodegradation pathways in IBD patients, as shown in Table 5, suggests an altered capacity of the gut microbiota to process foreign compounds. This finding is consistent with the work of Qiu et al. (2017), who reported that IBD patients exhibited increased microbial degradation of xenobiotics, potentially influencing drug metabolism and response to therapy. The altered microbial ability to degrade xenobiotics may have implications for the pharmacokinetics of medications used to treat IBD, warranting further investigation in future studies.<sup>13</sup>

## CONCLUSION

In conclusion, this study highlights significant alterations in the gut microbiota composition of patients with Inflammatory Bowel Disease (IBD) compared to healthy controls. We observed reduced microbial diversity, shifts in the relative abundance of key phyla and genera, and alterations in microbial functional pathways, which are consistent with dysbiosis associated with IBD. These findings underscore the role of gut microbiota in the pathogenesis of IBD and suggest potential therapeutic targets for modulating the microbiome.

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