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Original Research

First Middle Eastern-Based Gut Microbiota Study: Implications for Inflammatory Bowel Disease Microbiota-Based Therapies

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Abstract

Objective: To investigate the differences in gut microbiota composition between healthy individuals and those with inflammatory bowel disease (IBD) in Jordan and to understand how these differences may contribute to the pathogenesis of IBD. **Methods:** This study involved the comprehensive analysis of 20 gut microbiota samples from diverse populations in Jordan. Using 16S rRNA sequencing, the gut microbiome composition of healthy individuals was compared to that of individuals with IBD. The analysis focused on microbial diversity and the relative abundance of specific bacterial taxa. **Results:** The study revealed significant disparities in the gut microbiota composition between healthy individuals and those with IBD. IBD patients exhibited reduced microbial diversity. There was a notable decrease in the abundance of beneficial taxa such as Firmicutes and Bacteroidetes, specifically the families Erysipelotrichaceae, Ruminococcaceae, and Lachnospiraceae. IBD patients showed elevated levels of potentially harmful bacteria, particularly the Proteobacteria phylum. Three bacterial classes, Pseudomonas, Clostridia, and Escherichia, were significantly increased in IBD patients. Beneficial genera such as Faecalibacterium prausnitzii, Bifidobacterium, Blautia, Coprococcus, and Bacteroides dorei were more prominent in healthy controls, highlighting their roles in maintaining gut health and anti-inflammatory properties. **Conclusion:** The findings underscore a significant association between gut microbiota dysbiosis and IBD. IBD patients in Jordan exhibited lower microbial diversity and an imbalance favoring harmful bacterial taxa. These insights into the gut microbiota composition offer a valuable understanding of IBD pathogenesis and suggest that therapeutic strategies targeting the gut microbiome could be promising for managing the disease. Further research into the interplay between gut microbiota and the immune system is essential for developing novel IBD treatments.

KEYWORDS: IBD; Crohn's Disease, Ulcerative Colitis; gut; biopsy; microbiome; Roseburia; Faecalibacterium prausnitzii; Blautia; Coprococcus; Bacteroides dorei

INTRODUCTION

The gut microbiome is the collective genome of symbiotic and pathogenic microorganisms living in the gut, including bacteria, archaea, viruses, and fungi. Human intestines harbor many bacteria, which collectively have more genomes than all human cells[1]. Microbes are distributed spatially in the gut, where the colon has the highest diversity and abundance of microorganisms, primarily aerobic due to its proximity to the outer environment. In contrast, the small intestine has mainly anaerobic commensals. Due to this heterogeneity in the composition of the microbial ecosystem, it is hard to study many of these microorganisms, especially the anaerobes, by culture. However, omics-based approaches will help understand the intestinal ecosystem and the factors that may play a role in shaping this ecosystem using culture-independent approaches[2].

Humans and their microbiomes have co-developed in commensalism or mutualistic relationships, shaping some phenotypes [3,4]. Due to some changes and shifts in the microbial community, this human-microbiome relationship may become pathological, such as obesity, diabetes, atherosclerosis, and inflammatory bowel disease (IBD)[3,4]. Three predominant enterotypes were identified while studying the gut microbiome; these enterotypes are dominated by Bacteroides, Prevotella, and Ruminococcus[5]. These enterotypes cluster in an unknown way but appear independent of the place of origin, gender, age, or body mass index (BMI)[6]. The concept of enterotypes can significantly simplify the study of microbial communities. Dysbiosis, an imbalance in these microbial communities, particularly in the gut's microbiota, is linked to various health issues. For instance, IBD is associated with notable shifts in the GI microbiome's structure, characterized by reduced diversity, decreased Firmicutes, and increased Proteobacteria[7].

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This state of dysbiosis is thought to contribute to a range of conditions, including obesity, diabetes, autoimmune disorders, skin diseases, dementia, and IBD itself, highlighting the critical role that a balanced microbiome plays in maintaining overall health.

The association between dysbiosis and IBD has been repeatedly suggested by both clinical and experimental data, indicating that dysbiosis may be a contributing factor to the rise of IBD. However, researchers have no consensus on whether dysbiosis is a cause or consequence of IBD[8,9]. IBD is one of the disorders linked directly to the microbial community in the gut, as some studies indicate[11,12]. In these cases, the contribution of the host genetic makeup to the disease risk is less than 50%[10,11].

Metagenomics is a culture-independent approach that helps identify the composition of the gut microbial community (diversity and abundance), thus addressing and overcoming the challenges posed by the heterogeneity of the gut microbiota encountered with conventional microbiological techniques[12–14]. There is a lack of research that studies the gut microbiota in the Middle East, an area peculiar in its genetics and dietary habits. To our knowledge, no previous study addressed the changes in the gut microbiota in IBD patients in our region. The primary aim of our project is to study the gut mucosal microbiota among normal Jordanian patients and compare it with IBD.

MATERIALS AND METHODS

Study Design and Data Collection

OThe study included ten IBD patients and ten age- and gender-matched healthy controls recruited from medical records at Jordan University Hospital (JUH). Personal interviews were utilized to acquire participants' sociodemographic and anthropometric characteristics (age, gender, education, weight, and length, etc.), health-related factors (IBD type and status, family history of IBD, and family history of other autoimmune disorders), and environmental exposures (smoking status, birth mode of delivery, breastfeeding status, antibiotics use and awareness, along with childhood conditions). Clinical laboratory results were acquired from the individuals' health records such as (C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR) levels, complete blood count (CBC), and white cell count with differential (WBC)[15].

Inclusion/Exclusion Criteria

Our study included patients diagnosed with IBD (whether Crohn's disease or ulcerative colitis) who followed at Jordan University Hospital. Exclusion criteria included recurring infections with *H. pylori*, recurring or invasive amoebiasis, infection with cytomegalovirus, diagnosis with Familial Mediterranean Fever, chronic obstructive pulmonary disease (COPD), colorectal cancer, celiac disease, peptic ulcer disease, diverticulosis, and current use of biologic therapy (such as Tumor Necrosis Factor [TNF] inhibitors, Anti integrins, IL23/12 inhibitors or JAK inhibitors, or has taken antibiotics anytime during the three months preceding the ileocolonoscopy.

Sample Collection

Human gut biopsies were collected from participants (n=20) undergoing ileocolonoscopic procedures. For each participating individual, an ileocolonoscopy was performed, and biopsies were obtained from the terminal ileum and ascending, transverse, and descending colon regions (4 biopsy sets from each participant with a total of 80 biopsy sets). This was done to have a representative microbial community structure of the gut microbiota for all individuals. The healthy control group was matched to the IBD group by age, gender, and BMI, as these factors are known to affect the nature of the gut microbiome. Biopsy samples were stored in cryovials containing aliquots of DNA/RNA shield (Zymo Research, USA) to prevent changes in the microbial community. They were stored within 1-2 hours in the -20°C freezer in the lab.

DNA Extraction and 16S rDNA metagenomic sequencing

The samples were processed and analyzed with the Microbiome Analysis Service: Targeted Metagenomic Sequencing (Zymo Research Europe, Freiburg, Germany). ZymoBIOMICS® DNA Microprep Kit (Zymo Research, Irvine, CA) was used for DNA extraction.

Targeted Library Preparation

Bacterial 16S ribosomal RNA gene-targeted sequencing was performed using the *Quick*-16S™ NGS Library Prep Kit (Zymo Research, Irvine, CA), using the bacterial 16S primers to amplify the V3-V4 region of the 16S rRNA gene. These primers have been custom-designed by Zymo Research to provide the best coverage of the 16S gene while maintaining high sensitivity.

The sequencing library was prepared using real-time PCR. The final PCR products were quantified with qPCR fluorescence readings and pooled together based on equal molarity. The final pooled library was cleaned with the Select-a-Size DNA Clean & Concentrator™ (Zymo Research, Irvine, CA), then quantified with TapeStation®(Agilent Technologies, Santa Clara, CA) and Qubit® (Thermo Fisher Scientific, Waltham, WA).

Control Samples

The ZymoBIOMICS® Microbial Community Standard (Zymo Research, Irvine, CA) was used as a positive control for each DNA extraction. The ZymoBIOMICS® Microbial Community DNA Standard (Zymo Research, Irvine, CA) was used as a positive control for each targeted library preparation. Negative controls (i.e., blank extraction control and blank library preparation control) were included to assess the level of bioburden carried by the wet lab process. The final library was sequenced on Illumina® MiSeq™ with a v3 reagent kit (600 cycles). The sequencing was performed with a 10% PhiX spike-in.

Bioinformatics Analysis

Unique amplicon sequence variants were inferred from raw reads using the DADA2 pipeline[16]. Chimeric sequences were also removed with the DADA2 pipeline. Taxonomy assignment was performed using Uclust from Qiime v.1.9.1 with the Zymo Research Database, a 16S database that is internally designed and curated, as a reference. Composition visualization, alpha-diversity, and beta-diversity analyses were performed with

Qiime v.1.9.1[17]. The taxonomy with significant abundance among different groups was identified by LEfSe[18] using default settings. Other analyses, such as heatmaps, Taxa2ASV Deomposer, and PCoA plots, were performed using internal scripts by Zymo Research Europe.

Statistical Analysis

The data collected from the surveys and the clinical records were entered into a spreadsheet and analyzed using IBM SPSS Statistics for Windows, version 26 (IBM Corp, Armonk, NY, USA). Descriptive statistics obtained included the mean and standard deviation for each continuous variable measured. Moreover, frequencies in terms of numbers and percentages were obtained for discrete variables. Chi-square was used to identify the relationship between discrete variables and IBD risk. On the other hand, the Independent T-test was used to assess the relationship between continuous variables and IBD risk. Variables measured on an ordinal scale were analyzed using the Mann-Whitney test to investigate their relationship with IBD risk. Binary logistic regression was employed to identify independent predictors of diagnostic delay. A significance level was set at 0.05.

Ethical Approval and Consent of Participation

Ethical approval was obtained from the Academic Research Council of the School of Medicine at the University of Jordan; IRB approval number (205/2021), following the ethical principles of the Helsinki Declaration. Written consent was obtained from all respondents.

RESULTS

Sociodemographic Summary

This study aimed to investigate the microbial community structure in human gut mucosal biopsies from healthy individuals and those diagnosed with IBD, incorporating sociodemographic variables to contextualize findings. Participants represented a diverse group of respondents (n=20), both healthy controls (n=10) and cases of IBD patients (n=10). The cohort's mean age was 33.45 ± 11.20 years, indicative of a mid-adult population, with a gender distribution of 11 males and 9 females. The Body Mass Index (BMI), a measure of body fat based on height and weight, averaged at 24.97 ± 5.04 kg/m², suggesting a generally healthy weight range among the participants. However, there is variability indicating both underweight and potentially overweight individuals within the cohort. Cases and controls were age, gender, and BMI-matched (P>0.05) (Table 1). This distribution of age and BMI provides a baseline for examining how these factors may influence or correlate with the microbial community structure within the gut mucosa of both healthy and IBD-affected individuals.

Endoscopic Finding

Clinical diagnosis and Ilealcolonospoy confirmed the status for all 20 participants (n=20); the healthy controls (n=10) and the cases of IBD patients (n=10), of which 4 were diagnosed with Crohn's disease, and 6 were with ulcerative colitis (UC) (Table 2).

Table 1. Comparison between the cases and controls.

Variables	Cases, n = 10 (%)	Controls, n = 10 (%)	Test value	P- value
Age, mean ± SD	33.00 ± 10.822	33.90 ± 12.133	- 0.175 A	0.863
Sex			0.833 B	0.65
Male	7 (70.0)	5 (50.0)		
Female	3 (30.0)	5 (50.0)		
Marital status			0.000 B	0.999
Single	5 (50.0)	5 (50.0)		
Married	5 (50.0)	5 (50.0)		
Area of residence			0.833 B	0.65
Urban	7 (70.0)	5 (50.0)		
Countryside	3 (30.0)	5 (50.0)		
Vitamin D usage			0.952 B	0.628
Yes	4 (40.0)	2 (20.0)		
Feeding during infancy			2.484 B	0.211
Breast fed	10 (100.0)	7 (77.8)		
Formula fed	0 (0.0)	2 (22.2)		
Times of washing hands daily			3.81 B	0.141
Less than 10 times	5 (50.0)	1 (10.0)		
More than 10 times	5 (50.0)	9 (90.0)		
Smoking status			2.949 B	0.2

Smoker	6 (60.0)	5 (50.0)		
Non-smoker	2 (20.0)	5 (50.0)		
Passive smoker	2 (20.0)	0 (0.0)		
Routine exercise			5.00 B	0.087
Performed	0 (0.0)	4 (40.0)		
Not performed	10 (100.0)	6 (60.0)		
Recent travel outside Jordan during the past 3 months			0.392 B	0.999
Yes	2 (20.0)	1 (10.0)		
Family history of DM			0.202 B	0.999
Yes	6 (60.0)	5 (50.0)		
Family history of CVD			0.800 C	0.371
Yes	4 (40.0)	6 (60.0)		
Female patients a history of giving birth to a baby weighing more than 4 kg (macrosomia)			1.600 B	0.464
Yes	0 (0.0)	2 (40.0)		
Drug allergies			0.000 B	0.999
Yes	1 (10.0)	1 (10.0)		
Diet type			0.933 B	0.836
Vegetables dominant	1 (10.0)	2 (20.0)		
Meats dominant	2 (20.0)	3 (30.0)		
Mix	6 (60.0)	4 (40.0)		
High fat (Fast food)	1 (10.0)	1 (10.0)		
Daily caffeine intake			1.945 B	0.275
1-3 times	9 (90.0)	5 (62.5)		
More than 3 times	1 (10.0)	3 (37.5)		
Herbal products consumption			0.000 B	0.999
Yes	4 (40.0)	4 (40.0)		
Liver disorders			3.529 B	0.211
Presented	0 (0.0)	3 (30.0)		
Not presented	10 (100.0)	7 (70.0)		
Pain killers' consumption			0.833 B	0.65
Yes	5 (50.0)	7 (70.0)		
Pelvic radiation exposure			0.800 C	0.371
Yes	4 (40.0)	6 (60.0)		
BMI (Kg/m2), mean ± SD	23.84 ± 4.61	26.11 ± 5.44	- 1.009 A	0.327
FBG (mg/dl), mean ± SD	86.23 ± 14.79	91.65 ± 7.85	- 0.817 A	0.429
Vitamin D, mean ± SD	14.15 ± 11.24	15.83 ± 10.43	- 0.172 A	0.874
Creatinine, mean ± SD	0.64 ± 0.16	0.73 ± 0.16	- 1.089 A	0.295
Urea, mean ± SD	21.62 ± 7.51	26.58 ± 5.10	- 1.408 A	0.183
CRP, mean ± SD	11.32 ± 12.32	3.05 ± 2.20	1.302 A	0.217
ESR, mean ± SD	19.33 ± 7.43	8.00 ± 2.83	2.050 A	0.071
Ferritin, mean ± SD	19.70 ± 22.03	46.57 ± 52.83	- 0.907 A	0.386
ALT, mean ± SD	29.24 ± 20.32	33.14 ± 27.72	- 0.312 A	0.760
AST, mean ± SD	23.26 ± 8.90	23.38 ± 8.35	- 0.028 A	0.978
Hemoglobin, mean ± SD	13.65 ± 2.37	14.40 ± 2.69	- 0.662 A	0.516
Hematocrit, mean ± SD	41.69 ± 5.71	44.34 ± 6.68	- 0.954 A	0.353

WBC, mean ± SD	7.34 ±1.58	7.78 ± 2.16	- 0.631 A	0.536
MCV, mean ± SD	84.59 ± 7.23	82.37 ± 8.16	0.644 A	0.528
MCH, mean ± SD	27.69 ± 3.68	26.69 ± 4.05	0.578 A	0.571
RBC, mean ± SD	4.93 ± 0.48	5.41 ± 0.65	- 1.865 A	0.079
MCHC, mean ± SD	32.62 ± 1.99	32.22 ± 2.22	0.424 A	0.677
RDW, mean ± SD	16.80 ± 1.48	16.35 ± 2.06	0.560 A	0.582
Neutrophils, mean ± SD	63.32 ± 7.52	58.35 ± 9.40	1.306 A	0.208
Eosinophils, mean ± SD	1.72 ± 1.12	1.88 ± 1.14	- 0.315 A	0.757
Basophils, mean ± SD	0.58 ± 0.19	0.61 ± 0.31	- 0.242 A	0.812
Lymphocytes %, mean ± SD	28.09 ± 7.47	32.77 ± 8.38	- 1.318 A	0.204
NLR, mean ± SD	2.44 ± 0.78	1.93 ± 0.67	1.566 A	0.135
Monocytes, mean ± SD	5.84 ± 1.58	5.51 ± 1.10	0.536 A	0.599
Platelets count, mean ± SD	303.60 ± 93.6	284.10 ± 70.56	0.526 A	0.605
MPV, mean ± SD	9.14 ± 0.93	8.65 ± 0.69	1.322 A	0.204

A: Independent Samples t-test, B: Fisher's Exact Test, C: Chi-square test.
ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), BMI (Body Mass Index), CRP (C-reactive protein), CVD (Cardiovascular diseases), DM (Diabetes Mellitus), ESR (Erythrocyte sedimentation rate), FBG (Fasting blood glucose), HbA1c (Hemoglobin A1c), Kg (Kilogram), MCH (Mean corpuscular hemoglobin), MCHC (Mean corpuscular hemoglobin concentration), MCV (Mean corpuscular volume), m (Meter), MPV (Mean platelet volume), NLR (Neutrophil-to-lymphocyte ratio), RBC (Red blood cell count), RDW (Red cell distribution width), SD (Standard Deviation), and WBC (White blood cell count).

Table 2. Characteristics of IBD patients (n = 10)

Variables	**Frequency (%)**
Age of onset (years), mean ± SD	24.40 ± 4.40
Performed surgery since diagnosis	
Yes	0 (0.0)
No	10 (100.0)
Medication currently taking	
5-ASA	6 (60.0)
Azathioprine	2 (20.0)
Sulfasalazine	1 (10.0)
None	1 (10.0)
Stool pattern among UC patients	
Normal number of daily stools	4 (80.0)
Five or more stools than usual	1 (20.0)
IBD Type	
Crohn's	4 (40.0)
UC	6 (60.0)
Endoscopic finding	
TI narrowing, normal colon (SES-CD 3)	1 (10.0)
Remission	2 (20.0)
Mild	2 (20.0)
Moderate	3 (30.0)
Severe	2 (20.0)

SD (Standard deviation), **5- ASA** (5-aminosalicylic acid)

Decrease in the diversity of intestinal flora in IBD patients

Using 16S rRNA taxonomic profiling, we examined alterations in community structure for the IBD patients and control (normal) individuals. The majority of rarefaction curves for the samples reached saturation (Figure 1), demonstrating adequate sequencing depth for this study. The volume of sequencing data was both reasonable and sufficiently extensive to capture diversity information for the majority of individuals. Amplicon sequence variants (ASVs) served as a metric for diversity. Chao1 was used to assess community richness by studying the total number of species in healthy and IBD patients, while the Simpson Diversity Index, the Shannon Diversity Index, and PD Whole Tree, were used to evaluate community diversity using alpha diversity (Figure 1). The diversity of intestinal flora in IBD patients was notably reduced compared to that observed in the healthy control subjects (Figure 2). Further, Chao1 and observed species analyses revealed a significant reduction in the diversity of intestinal flora among IBD patients.

Principal coordinates analysis (PCoA), a beta diversity analysis, was employed to calculate the Bray–Curtis distance. Every point displayed on the beta diversity plot symbolizes the complete microbial composition profile. Samples exhibiting similar microbial composition profiles are clustered closer together, whereas those with differing profiles are situated farther apart. This analysis unveiled distinct dissimilarities in the flora structure between the healthy and IBD groups (Figure 3).

Differences in the taxonomy of the gut microbiota among individuals diagnosed with IBD

Analysis of bacterial abundance determined the microbiota composition of stool samples at the phyla and genus level. Proteobacteria, Bacteroidota, Actinobacteriota, Firmicutes were the dominant phyla among both healthy and

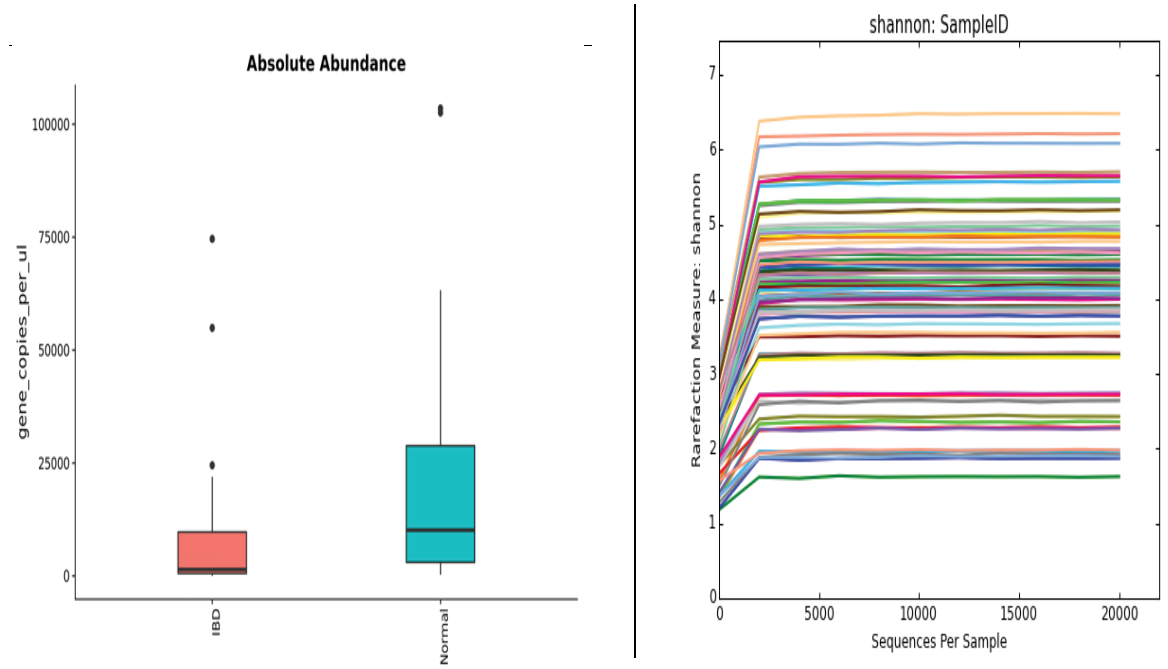

Figure 1. Shannon-Wiener curve saturation. The figure shows that the Shannon-Wiener curve is saturated, suggesting that the amount of sequencing data collected was adequate. This implies that the data comprehensively represents species richness.

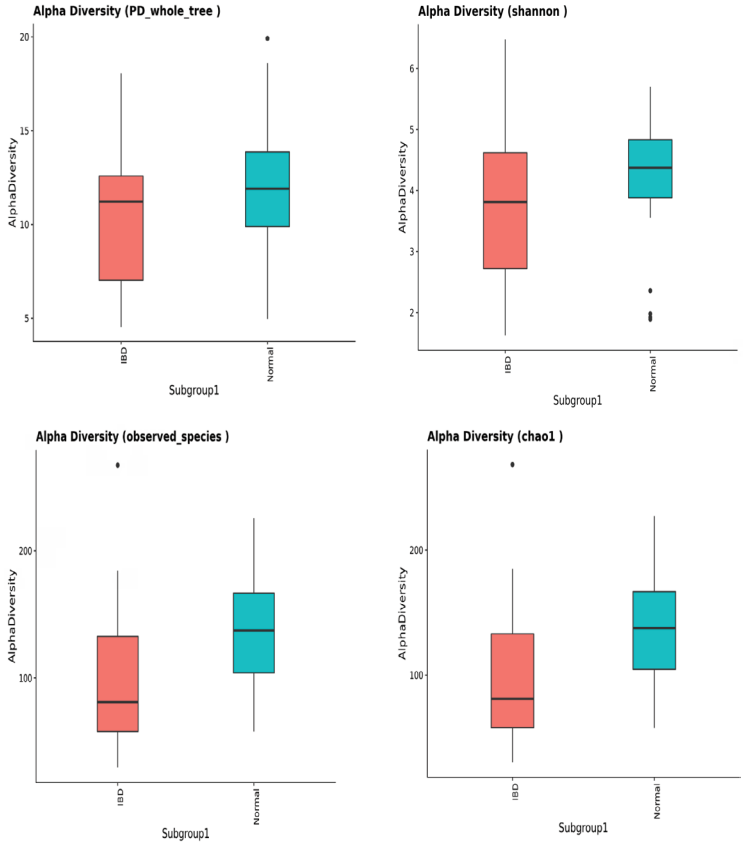

Figure 2. Alpha diversity analysis. Alpha diversity analysis was conducted on the intestinal flora of both healthy individuals and those with IBD (A–D). The results from Chao 1, observed species, PD Whole Tree, and the Shannon Diversity Index indicated a notable reduction in the diversity of intestinal flora among IBD patients in remission.

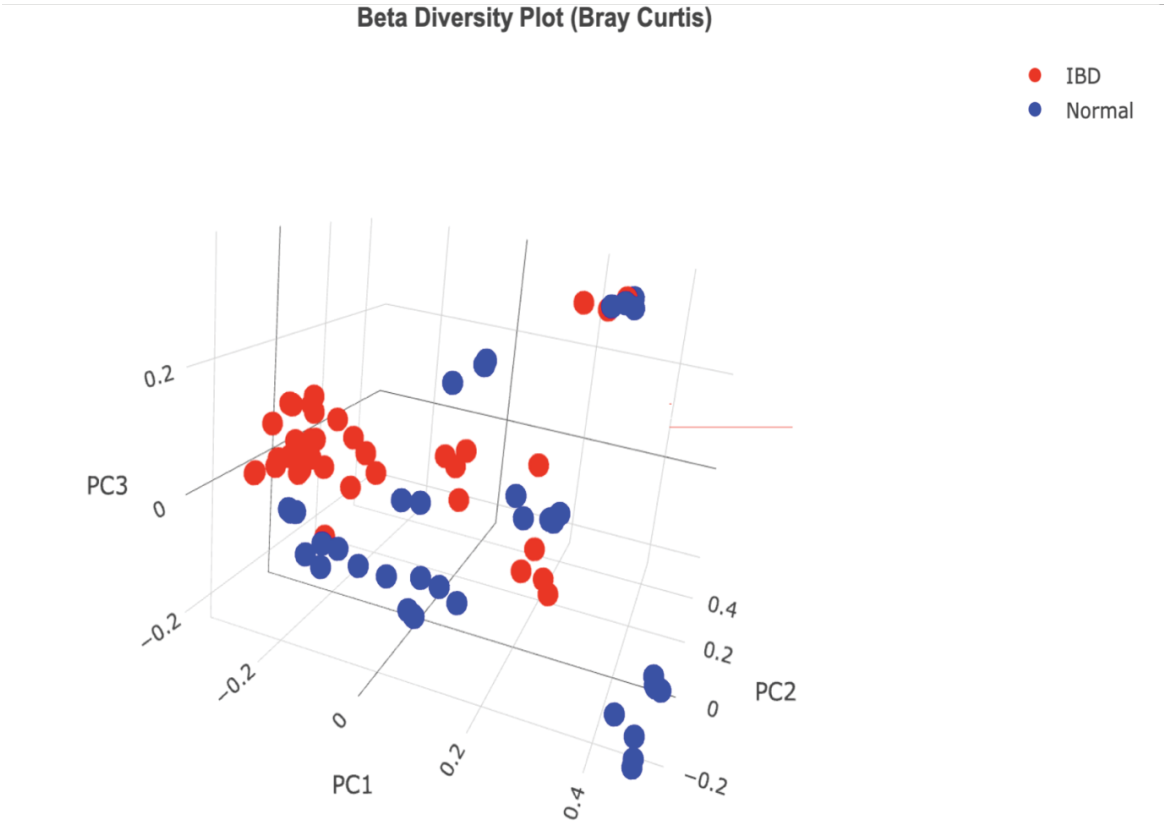

Figure 3. Principal coordinates analysis (PCoA). Principal coordinates analysis (PCoA) shows findings from beta diversity analysis. PCoA was conducted based on Bray–Curtis distance. Two colors of points were used to represent distinct sample groupings, and the horizontal and vertical axis scales indicate relative distances.

diseased individuals, each with different relative abundances. In particular, Proteobacteria and Firmicutes showed a higher occurrence than Bacteroidota and Actinobacteria. Patients with active IBD had significantly higher levels of Proteobacteria than healthy controls (Figure 4).

The Linear Discriminant Analysis (LDA) coupled with effect size measurements (LEfSe) identified several bacterial taxa that differ significantly between the gut microbiomes of patients with IBD and healthy (normal) individuals. Figure 5 shows an overrepresentation of the taxa *Escherichia coli,* and *Pseudomonas* within the *Proteobacteria* phylum in IBD patients, as indicated by the highest LDA scores, and an overrepresentation of the taxa *Clostridium perfringens* and *Lachnoclostridium* from the *Firmicutes phylum.* The other three different genera*, Erysipelotrichaceae, Ruminococcaceae, and Lachnospiraceae,* decreased in the IBD group compared to healthy controls. For the normal group, several taxa within the *Firmicutes* and *Bacteroidetes* phyla were more prominent in healthy controls, including *Faecalibacterium prausnitzii*, *Bifidobacterium*, *Blautia*, *Coprococcus*, and *Bacteroides dorei* which are known for their beneficial roles in maintaining gut health and often associated with anti-inflammatory properties and gut barrier's integrity.

DISCUSSION

Our current study illustrated the normal gut microbiome signature among a group of normal Jordanian individuals. When compared to IBD patients, IBD patients show diminished microbial diversity compared to healthy individuals. IBD is known to be linked to gut microbiome changes once compared to healthy individuals[19,20], among others, have demonstrated differences in the quantity and diversity of microbial species within the gut of individuals with IBD and healthy individuals. This reduction in diversity can influence the presence of different bacterial species among different patients. For instance, certain species, such as *Faecalibacterium prausnitzii*, are frequently found in lower quantities among IBD patients, while other potentially detrimental species might show an increase, such as *E. coli*.

Changes in the equilibrium between the prominent gut phyla, Firmicutes, and Bacteroidetes, are commonly observed among individuals with IBD. A decline in Firmicutes and an elevation in Bacteroidetes has been demonstrated previously[21]. Similar to our findings, it has been shown that individuals with IBD exhibited reduced species diversity in their gut microbiota compared to healthy volunteers[22–25]. Other studies indicated a possible increase of Proteobacteria in the gut of IBD patients. Elevated levels of Proteobacteria, particularly

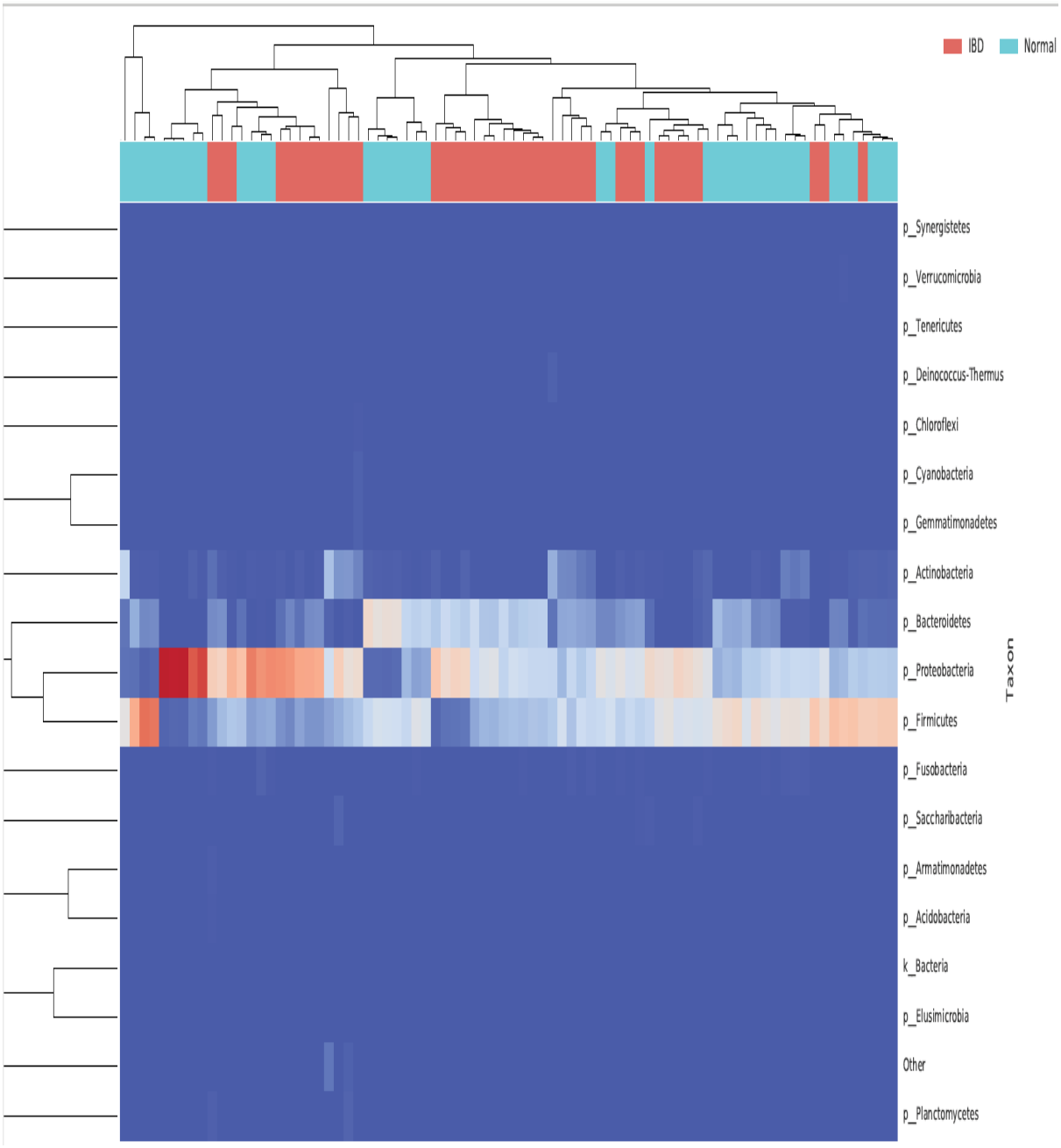

Figure 4. Heatmap of the microbiological compositions at the phylum level.
This heatmap shows the average relative abundances of all phyla identified in IBD patients and healthy control counterparts.

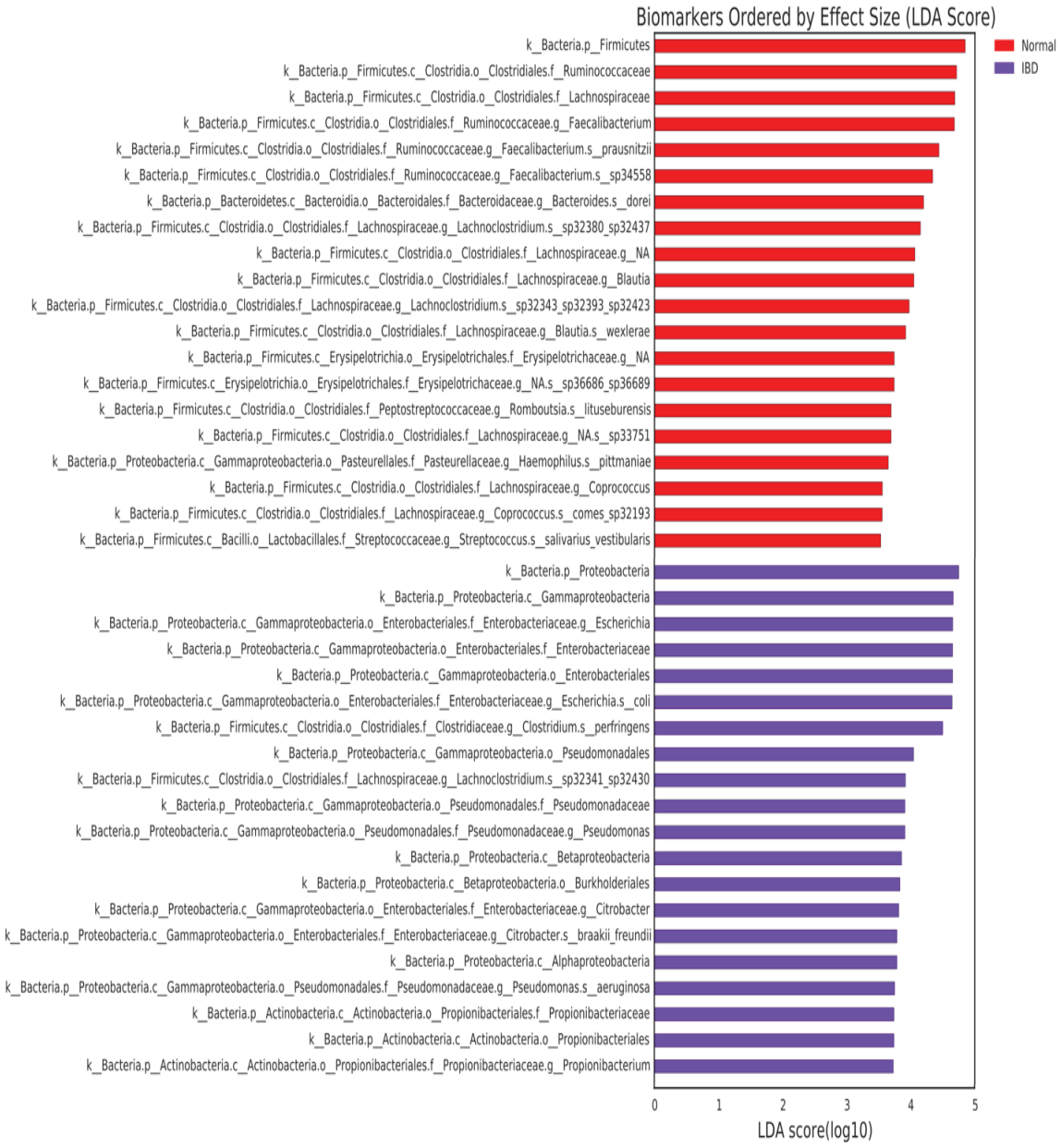

Figure 5. LEfSe analysis
LEfSe bar plots of the bacterial communities represent the significantly differential taxa between IBD patients (violet) and normal (red), based on effect size (LDA score) logarithmically scaled to reflect the magnitude of difference.

Enterobacteriaceae, have been linked to gut inflammation.

Moreover, a reduction in beneficial butyrate-producing bacteria like *Roseburia* spp. and *Faecalibacterium prausnitzii* has been noted in individuals with IBD[26], which goes along with the observed data in our study. *Bifidobacterium*, *Blautia*, *Coprococcus*, and *Bacteroides dorei* were beneficial bacteria that showed an increase in healthy individuals and a significant decrease in IBD patients, which indicates their importance in maintaining healthy status. These observations support the findings of other studies reporting that *Blautia*, *Coprococcus*, and *Bacteroides dorei* as potential probiotics[27–29]. These differences in species abundance and microbial composition between IBD patients and healthy individuals are inconsistent across all studies and vary based on the subtype of IBD, disease severity, environmental factors, and individual variations[24,26,30]. This may indicate a potential lack of uniformity in microbiome methodologies or could stem from the diversity observed in the microbiome linked to the disease. Understanding these differences can provide insights into potential therapeutic strategies like fecal microbiota transplantation, prebiotics, probiotics, or dietary interventions to modulate the gut microbiome and potentially alleviate symptoms in IBD patients.

The human gut microbiome holds significant importance in well-being, impacting diverse human functions like immune response, metabolism, and behavior. Its composition and diversity can be shaped by multiple elements, including diet and environmental factors[31]. Numerous studies indicate that establishing a healthy gut microbiota relies on various dietary elements, among which breastfeeding plays a significant role.

The altered gut microbiota associated with IBD is believed to interact with both the immune system and the intestinal barrier, potentially playing a role in persistent inflammation and the distinct symptoms of the condition. *Escherichia coli* (*E. coli*) comprises diverse bacteria, some considered commensal and others pathogenic. In the context of IBD, the role of *E. coli* is intricate and not fully comprehended. Adherent-Invasive *E. coli* (AIEC) has been specifically implicated in the pathogenesis of Crohn's disease[32,33]. AIEC strains possess the capability to adhere to and invade intestinal epithelial cells, leading to chronic inflammation. These strains are found in higher abundance in the gut of certain individuals with CD than healthy ones[33]. Our present study revealed an enrichment of *E. coli* bacteria in IBD patients compared to controls.

Similarly, other studies have reported variations in the prevalence and characteristics of *E. coli* strains in IBD patients compared to controls[34]. Most *E. coli* strains in healthy individuals were non-pathogenic and constituted a normal part of the gut microbiota. These strains coexist with other beneficial bacteria in a balanced ecosystem within the gut. Consequently, understanding the role of *E. coli* in IBD is complex due to the diverse nature of *E. coli* strains and their interactions with the host immune system. While certain studies have linked specific *E. coli* strains to inflammation and disease progression in IBD, further research is necessary to elucidate the precise mechanisms through which *E. coli* may contribute to developing or worsening IBD in susceptible individuals. It is essential to recognize that the presence of *E. coli* alone might not be adequate to cause IBD, given that the disease is multifactorial and influenced by various genetic, environmental, and microbial factors.

This study represents the first report on human gut microbiota from the Middle Eastern population[35]. And represents the foundation for future microbiota-based therapies and the development of probiotics that are Middle-Eastern specific.

CONCLUSION

The metagenomic analysis of both groups (IBD and Controls) shall give enough data to conclude the linkage between the microbial community structure in both normal and IBD samples. IBD is marked by an imbalance in gut microbial composition, featuring a reduction in beneficial/commensal bacteria such as Firmicutes, Actinobacteria, and Bacteroides, and an increased presence of pathogenic/colitogenic Proteobacteria. Our study highlights the importance of *Roseburia* spp., *Faecalibacterium prausnitzii*, *Blautia*, *Coprococcus*, and *Bacteroides dorei* as potential probiotics and important biomarkers for IBD diagnosis for Middle Eastern populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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