Characteristics of faecal microbiota in Korean patients with *Clostridioides difficile*-associated diarrhoea

Yong Duk Jeon

Department of Medicine

The Graduate School, Yonsei University

Characteristics of faecal microbiota in Korean patients with *Clostridioides difficile*-associated diarrhoea

Yong Duk Jeon

Department of Medicine

The Graduate School, Yonsei University

Characteristics of faecal microbiota in Korean patients with *Clostridioides difficile*-associated diarrhoea

Directed by Professor Jun Yong Choi

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Yong Duk Jeon

December 2020

This certifies that the Doctoral Dissertation
of Yong Duk Jeon is approved.

Thesis Supervisor: Jun Yong Choi

Thesis Committee Member #1: Dongeun Yong

Thesis Committee Member #2: Young Goo Song

Thesis Committee Member #3: Hyo Youl Kim

Thesis Committee Member #4: Sang Sun Yoon

The Graduate School
Yonsei University

December 2020

ACKNOWLEDGEMENTS

I dedicate this dissertation to Professor Jun Yong Choi, who has been supportive and patient. Without his guidance, it would not have been possible to complete my PhD. I am grateful to be a student under his guidance in clinical training during which I learned what it takes to be an infectious disease physician.

I would like to express my appreciation to the members of my dissertation committee, Professor Dongeun Yong, Young Goo Song, Hyo Youl Kim and Sang Sun Yoon, who have enriched my dissertation with valuable theoretical and methodological guidance.

Finally, I would like to thank my parents, Jeon Keun Ho and Lee Oak Hwa, and my wife, Yerheen Ha, for their devoted love and support.

Yong Duk Jeon

<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Characteristics of faecal microbiota in Korean patients with *Clostridioides difficile*-associated diarrhoea

Yong Duk Jeon

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Jun Yong Choi)

Background: The intestinal microbiota plays an important role in the pathogenesis of *Clostridioides difficile*-associated diarrhoea, and regional and racial characteristics influence the microbiome composition and diversity. We investigated the intestinal microbiome characteristics of patients with *C. difficile* colitis (CD+) compared to those of patients with colitis not due to *C. difficile* (CD-), patients with vancomycin-resistant enterococci (VRE) colonization, and healthy controls, in Korea.

Methods: We collected stool samples from 24, 18, 11 and 13 subjects within CD+, CD-, VRE and healthy control groups, respectively. Based on clinical severity, the CD+ group was divided into two subgroups: mild and severe. The microbial communities were evaluated by 454-pyrosequencing of bacterial 16s rRNA.

Results: The species richness and microbial diversity were significantly lower in

the CD+ group compared to those in healthy controls, but not compared to those in CD- and VRE groups. Phylum-level analysis showed that the proportion of Actinobacteria in the CD+ group was significantly lower than in the healthy control, but was unchanged compared to that in CD- and VRE groups. At the genus level, compared to the healthy group, the CD+ group showed significantly lower proportions of several major gut-associated genera including *Blautia, Bifidobacterium, Faecalibacterium, Anaerostipes* et al. However, the proportions of *Enterococcus, Hungatella, Eubacterium_g1, Clostridium_g6 and Clostridium_g4* were significantly higher in the CD+ group than those in the healthy control. Compared to those in the CD- group, the proportions of *Hungatella* and *Clostridium_g4* in the CD+ group were significantly higher. Compared to those in the VRE group, subjects in the CD+ group showed significantly higher proportions of *Anaerostipes* and *Hungatella*. At the species level, the CD+ group had a significantly higher proportion of *C. difficile* than CD- group and healthy control. Compared to that in the VRE group, *C. difficile* was higher in the CD+ group, but not significantly. In comparison of mild and severe *C. difficile* infection, the severe CD+ group had a significantly higher proportion of *Clostridium_g4* (0.2 % in mild CD+ group; 3.9 % in severe CD+ group, $p = 0.044$). The mean relative abundance of *C. difficile* in severe CD+ group showed the higher tendency ($p = 0.065$) compared to that in mild CD+ group.

Conclusion: We could identify the intestinal microbiome characteristics of Koreans with *C. difficile* colitis. It might help to develop microbiome based diagnostic and treatment modalities.

Key words: faecal microbiota, intestinal microbiota, *Clostridioides difficile* infection, next generation sequencing

Characteristics of faecal microbiota in Korean patients with *Clostridioides difficile*-associated diarrhoea

Yong Duk Jeon

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Jun Yong Choi)

I. INTRODUCTION

The human gut microbiota plays an important role in nutrition and physiology, and is closely related to health and disease [1, 2]. The gut microbial composition differs depending on age, geography, lifestyle, and health status [2-4]. The methods of analysing human gut microbiota have rapidly advanced as new technologies have been developed; in particular, next generation sequencing (NGS) plays a key role in improving our perspective on gut microbiota [5]. The characteristics of the gut microbiota in health and disease have been extensively studied, and their importance in intestinal diseases such as inflammatory bowel disease, as well as various systemic diseases such as diabetes, obesity, and cardiovascular disease, has been established [6-8].

Clostridioides difficile infection (CDI) is a major focus of research in the study of gut microbiota[9]. Despite the recent major increase in the incidence and severity of CDI, treatment with antibiotics such as metronidazole and oral vancomycin have remained the most effective strategy [10]. However, in recurrent CDI, antibiotic treatment is associated with a high recurrence rate [11]. Recently, van Nood *et al.* reported the first randomized controlled trial of faecal

microbiota transplantation (FMT) in patients with recurrent CDI. FMT showed much better treatment outcomes than antibiotic treatment [12]. This indicated that CDI is associated with a collapse of the healthy gut microbiota, and that faecal material from a healthy donor can restore the gut microbiota of patients [13]. Currently, an important focus of research is the investigation of the nature of microbial changes in CDI patients, and is connected with the development of new treatment strategies that are more effective and convenient [14].

In South Korea, the incidence of CDI is also increasing; FMT has been introduced to treat recurrent and refractory CDI [15-17]. However, there is a lack of research studying the gut microbiota of healthy people and patients with CDI in Korea. The characteristics of the gut microbiota differ in different countries; therefore, it is necessary to investigate the characteristics of the gut microbiome in Korean subjects. Several studies showed the characteristics of the gut microbiome in healthy Korean populations have significant differences from other countries [18, 19].

Vancomycin-resistant enterococci (VRE) and *C. difficile* are both major nosocomial pathogens and thus have similar risk factors including antibiotic exposure and hospitalization [20]. Previous studies showed the prevalence of VRE in CDI subjects is 10-20% [21, 22]. To better identify the specific characteristics of the gut microbiota of CDI, it is necessary to compare CDI group with other groups having antibiotics exposures and hospitalization such as subjects with VRE colonization.

The purpose of this study is to investigate the characteristics of gut microbiota in patients with CDI compared to not only healthy controls but also patients with colitis other than CDI and patients with VRE colonization. We anticipated that gut microbiota in patients with CDI and patients with VRE colonization would have lower bacterial richness and diversity because both have similar risk factors including antibiotic exposure and hospitalization. Comparing CDI and VRE groups, we intended to gain more fine-grained understanding of the

characteristics of gut microbiota in patients with CDI, which goes beyond examining bacterial richness and diversity. We expect that the results of this investigation may lead to the establishment of novel diagnostic and treatment strategies in patients with CDI in South Korea.

II. MATERIALS AND METHODS

1. Study population and sample collection

This study was conducted at a 2,500 beds tertiary hospital of South Korea. Stool samples were collected from hospitalized patients with CDI (CD+), hospitalized patients with loose stool negative for *C. difficile* (CD-), hospitalized patients with VRE colonisation, and healthy controls between December 2014 and March 2015. CD+ and VRE groups included only patients who were first diagnosed as CDI or VRE colonization. CDI was diagnosed in subjects with diarrhoea (passage of three or more unformed or loose stools per day for more than 2 days) and who satisfied at least one of the following criteria: a toxin-producing *C. difficile* strain was successfully cultured, or a toxin gene of *C. difficile* was detected by PCR [23, 24]. It was confirmed that subjects in the CD+ and CD- groups did not have VRE colonisation within their stool samples. Likewise, subjects in the VRE group were confirmed to be without CDI. Subjects with both VRE and CDI were excluded. The CD+ group was divided into two subgroups in terms of clinical severity: mild and severe. Patients with ≥ 2 points were considered to have severe CDI [24], where one point each was given for age >60 years, temperature >38.3°C, albumin level <2.5 mg/dL, or peripheral WBC count 115,000 cells/mm^3 within 48 h of enrollment. Healthy controls were recruited from individuals visiting the Severance Hospital for routine health check-ups who had no malignancy or gastrointestinal disease and no history of antibiotics or chemotherapy for 3 months prior to investigation. Healthy controls were confirmed to be without CDI or VRE colonisation. All of the participants were over 20 years old. The Institutional Review Board (IRB) of the Severance Hospital approved the study (IRB #4-2014-0792). The participants for this study provided informed signed consent. All methods in this study were performed in accordance with the Declaration of Helsinki.

2. Microbiological tests

Stool specimens were cultured to confirm the presence of toxigenic *C. difficile*. Before inoculation, each specimen was pre-treated using the alcohol-shock method, mixed with an equivalent volume of alcohol, and incubated for 30 min. Alcohol-shocked specimens were inoculated into *C. difficile* selective agar (BD Co., Franklin Lakes, NJ, USA) and incubated at 35 ℃ for 48 h under anaerobic conditions. Colonies suspected of being *C. difficile* were identified using a VITEK 2 automated microbiology system (bioMerieux, Marcy l'Etoile, France). After isolating *C. difficile*, PCR was performed to determine the presence of *C. difficile* toxin genes (tcdA, tcdB, cdtA and cdtB) as previously described [25, 26]. The presence of the *C. difficile* toxin B gene in patient samples was examined by real-time PCR using the GeneXpert DX system (Cepheid, Sunnyvale, CA, USA). The identification of enterococcal growth was performed using the VITEK 2 automated system (bioMerieux), and antibiotic susceptibility was determined using a VITEK 2 antibiotic susceptibility test card (bioMerieux).

3. DNA extraction

DNA extraction was performed using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Stool samples stored at -70℃ since the day of sample collection were used for DNA extraction. First, 500 μg (wet weight) stool sample was collected, 10 ml PBS was added, and the solution was vortexed vigorously until the stool sample was thoroughly homogenized. Then, the diluted sample was filtered through a cell strainer (Falcon, Corning Inc, Corning, NY, USA) and centrifuged at 1,300 rpm for 10 min. The sample was resuspended in AL buffer (Qiagen) and disrupted the bacterial cell walls by bead-beating according to the manufacturer's recommendations. The quantity and quality of DNA was confirmed by measuring the absorbance at 260 and 280 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technology, Rockland, DE, USA).

4. PCR amplification and pyrosequencing

PCR amplification was performed using primers targeting the V1 to V3 regions of the bacterial 16S rRNA gene. For bacterial amplification, barcoded primers of 9F (5′-CCTATCCCCTGTGTGCCTTGGCAGTC-TCAG-AC-AGAGTTTGATCMTGGCTCAG-3′; underlined sequence indicates the target region primer) and 541R (5′-CCATCTCATCCCTGCGTGTCTCCGAC-TCAG-X-AC-ATTACCGCGGCTGCTGG-3′; 'X' indicates the unique barcode for each subject). Amplification was carried out under the following conditions: initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final elongation at 72°C for 5 min. The PCR product was confirmed by 2% agarose gel electrophoresis and visualized under a Gel Doc system (BioRad Laboratories, Hercules, CA, USA). The amplified products were purified with the QIAquick PCR purification kit (Qiagen). Equal concentrations of purified products were pooled, and short fragments (non-target products) were removed with the Ampure beads kit (Agencourt Bioscience, Beverley, MA, USA). The quality and size of the products was assessed on a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) using a DNA 7500 chip. Mixed amplicons were analysed by emulsion PCR, and then deposited on Picotiter plates. Sequencing was carried out using the GS Junior Sequencing system (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions.

5. Pyrosequencing data analysis

Basic analysis was conducted as described previously [27-29]. The obtained reads from the different samples were sorted based on the unique barcodes of each PCR product. The sequences of the barcode, linker, and primers were removed from the original sequencing reads. Any reads containing two or more ambiguous nucleotides, low-quality reads (average score < 25), or reads shorter than 300 bp, were discarded. Potential chimeric sequences were detected using the

Bellerophon method, which compares the BLASTN search results between forward half and reverse half sequences [30]. After removing chimeric sequences, the taxonomic classification of each read was assigned against the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net) [31], which contains the 16S rRNA gene sequences of type strains with valid published names and representative species level phylotypes of either cultured or uncultured entries in the GenBank database with complete hierarchical taxonomic classification from the phylum to the species level. The richness and diversity of samples were determined by Chao1 estimation and Shannon diversity index at the 3% distance. Random subsampling was conducted to equalize the read size of samples to compare different read sizes among samples. The overall phylogenetic distance between communities was estimated using Fast UniFrac [32] and visualized using PCoA. To compare operational taxonomic units (OTUs) between samples, shared OTUs were obtained by XOR analysis in the CLcommunity software (Chunlab Inc., Seoul, Korea).

6. Statistical Analysis

Statistical tests were performed using SPSS v. 19 (SPSS Inc., Chicago, IL, USA) and CLcommunity (ChunLab Inc.). Data are shown as the mean ± standard deviation (SD). The statistical significance of continuous variables was assessed using Student's *t*-test or the Mann-Whitney *U* test between two groups. One-way analysis of variance (ANOVA) and the Kruskal-Wallis test were used to evaluate the differences between more than two groups. A value of $p < 0.05$ was considered statistically significant.

III. RESULTS

1. Characteristics of study subject

We collected stool samples from hospitalized patients with CDI (CD+), hospitalized patients with loose stool negative for *C. difficile* (CD-), hospitalized patients with VRE colonization, and healthy controls. A total of sixty-six stool samples were collected from twenty-four patients with CD+, eighteen patients with CD-, eleven patients with VRE colonization, and thirteen healthy controls. The baseline characteristics of each group are shown in Table 1. The mean age was 59.6 years and there were 35 male patients (53.0%). The Charlson comorbidity index was the lowest in the healthy controls (CD+: 4.2; CD-: 2.1; VRE: 4.1; Healthy: 0.5). The rate of hospitalization in the 3 months prior to the investigation was 79.2% in CD+, 44.4% in CD-, 90.9% in VRE, and 0% in healthy controls. The mean white blood cell count was highest in the CD+ group (CD+: 11232.6 (/μL); CD-: 7680.6; VRE: 7147.3; Healthy: 5701.5) and the mean albumin was the lowest in the CD+ group (CD+: 3.0 (g/dL); CD-: 3.1; VRE: 3.6; Healthy: 4.2). The clinical symptoms of the patients are also shown in Table 1.

Table 1. Demographic and clinical characteristics of subjects

Characteristics	CD+ (n=24)	CD- (n=18)	VRE (n=11)	Healthy (n=13)
Age	64.8 ± 15.7	62.1 ± 20.2	56.3 ± 23.6	49.2 ± 11.5
Gender (% males)	45.8	55.6	45.5	69.2
BMI (kg/m^2)	21.2 ± 3.1	21.1 ± 3.2	20.5 ± 2.8	24.7 ± 3.1
Underlying disease (%)				
Cancer	41.7	38.9	36.4	0
Cardiovascular disease	16.7	22.2	27.3	15.4
Diabetes mellitus	20.8	5.6	9.1	15.4

Cerebrovascular disease	41.7	16.7	45.5	0
Chronic lung disease	12.5	11.1	0	7.7
Chronic liver disease	4.2	16.7	9.1	0
Chronic kidney disease	16.7	5.6	45.5	0
Charlson comorbidity index	4.2 ± 2.8	2.1 ± 1.6	4.1 ± 1.6	0.5 ± 0.9
Hospitalization in past 3 months (%)	79.2	44.4	90.9	0
Laboratory findings				
White blood cell (/μL)	11232.6 ± 7479.7	7680.6 ± 5353.2	7147.3 ± 2469.7	5701.5 ± 1455.0
Haemoglobin (g/dL)	10.7 ± 2.2	10.5 ± 1.8	10.8 ± 1.7	15.1 ± 1.6
Platelet (/μL)	239.0k ±132.1k	243.6k ± 181.2k	205.7k ± 81.0k	223.0k ±31.0k
Albumin (g/dL)	3.0 ± 0.9	3.1 ± 0.5	3.6 ± 0.9	4.2 ± 0.4
CRP (mg/L)	74.7 ± 73.4	31.8 ± 32.4	20.8 ± 16.4	
Clinical symptom				
Diarrhoea	95.8	88.9	18.2	
Fever	33.3	22.2	9.1	
Vomiting	4.2	0	0	
Abdominal pain	12.5	22.2	0	
Haematochezia	0	5.6	0	

CD: *Clostridioides difficile*; VRE: vancomycin-resistant enterococci, BMI: body mass index, CRP: C-reactive protein.

The proportion of patients who had used antibiotics in the 3 months prior to this study was 95.2% in CD+, 83.3% in CD-, and 100 % in VRE (Table 2). The total duration of antibiotic use was the longest in VRE patients (CD+: 26.2 (days); CD-: 20.9; VRE: 45.2). The duration of treatment with each antibiotic class is also shown in Table 2.

Table 2. History of antibiotic use in subjects within 3 months before the start of experiments

	CD+ (n=24)	CD- (n=18)	VRE (n=11)	P-value
Proportion of subjects with any antibiotic use (%)	95.2	83.3	100.0	
Duration of antibiotic use (days, mean ± SD)				
Any antibiotics	26.2 ± 22.9	20.9 ± 20.3	45.2 ± 30.5	0.032
Cephalosporin	11.2 ± 11.3	4.6 ± 8.1	11.0 ± 14.1	0.144
Penicillin	5.9 ± 10.9	7.1 ± 10.3	4.8 ± 5.3	0.829
Fluoroquinolone	5.4 ± 9.4	6.7 ± 13.8	11.0 ± 26.7	0.639
Aminoglycoside	0.7 ± 2.0	0	3.1 ± 10.3	0.254
Macrolide	0.3 ± 0.9	0	0	0.248
Carbapenem	4.0 ± 9.3	2.4 ± 5.4	5.8 ± 9.2	0.541
Glycopeptide	2.7 ± 5.6	3.7 ± 7.2	9.4 ± 13.1	0.093
Others	10.2 ±14.0	7.3 ± 16.2	27.0 ± 34.3	

CD: *Clostridioides difficile*; VRE: vancomycin-resistant enterococci.

2. Species richness and diversity of intestinal microbiota

The alpha diversity of the bacteria in the analysed samples is shown in Table 3 and Fig. 1. The species richness, as estimated by the Chao1 index, was significantly lower in the CD+ group compared to that in the healthy control ($p < 0.001$), but did not differ significantly between the CD+ group and any other group. Microbial diversity, as calculated by the Shannon index, was significantly lower in the CD+ group compared to that in the healthy control group ($p = 0.001$), but did not differ significantly between the CD+ group and any other group.

Principal coordinate analysis (PCoA) was used to evaluate the beta diversity within each group (Fig. 2). Healthy controls were clustered separately from the

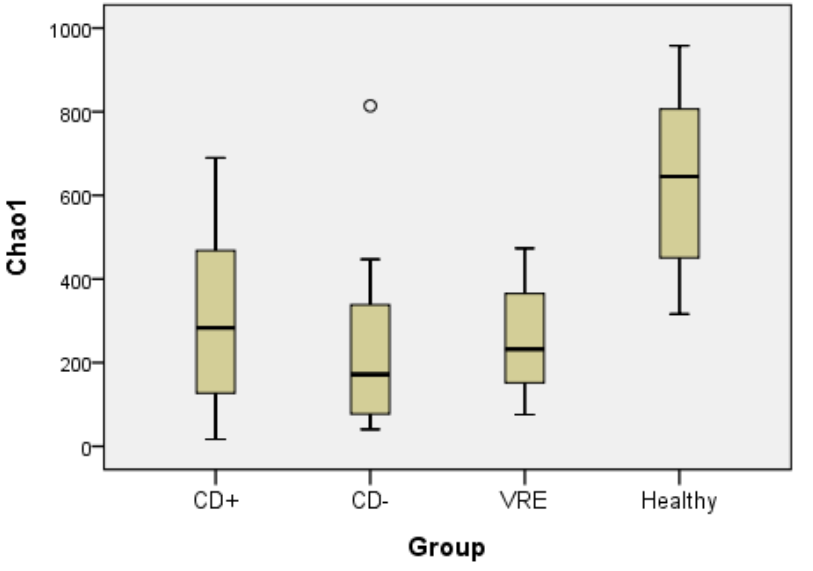
CD+, CD-, and VRE group, while the CD+, CD-, and VRE groups could not be separated.

Table 3. Alpha diversity of analysed bacterial samples

	CD+ (n=24)	CD- (n=18)	VRE (n=11)	Healthy (n=13)	P-value			
Alpha diversity (mean ± SD)					Overall	CD+ vs healthy	CD+ vs CD-	CD+ vs VRE
Chao1 index	285.3 ± 190.2	224.0 ± 189.9	251.3 ± 135.5	642.9 ± 219.6	<0.001	<0.001	0.307	0.597
Shannon index	3.5 ± 0.8	3.2 ± 1.2	3.2 ± 0.6	4.5 ± 0.6	0.001	0.001	0.224	0.225

CD, *Clostridioides difficile*; VRE, vancomycin-resistant enterococci.

A. Chao1



B. Shannon

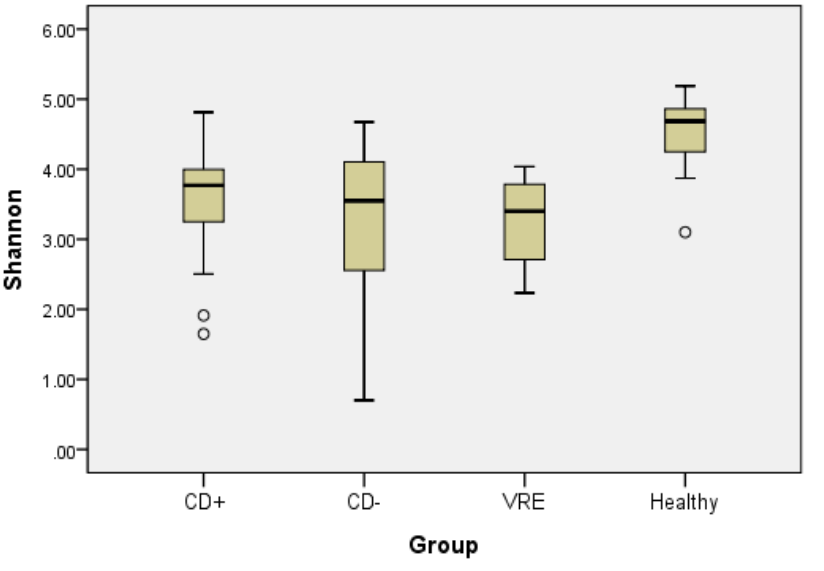


Figure 1. Alpha diversity of analysed bacterial samples. (A) Chao1 index to determine species richness. (B) Shannon index to determine microbial diversity. CD: *Clostridioides difficile*; VRE: vancomycin-resistant enterococci.

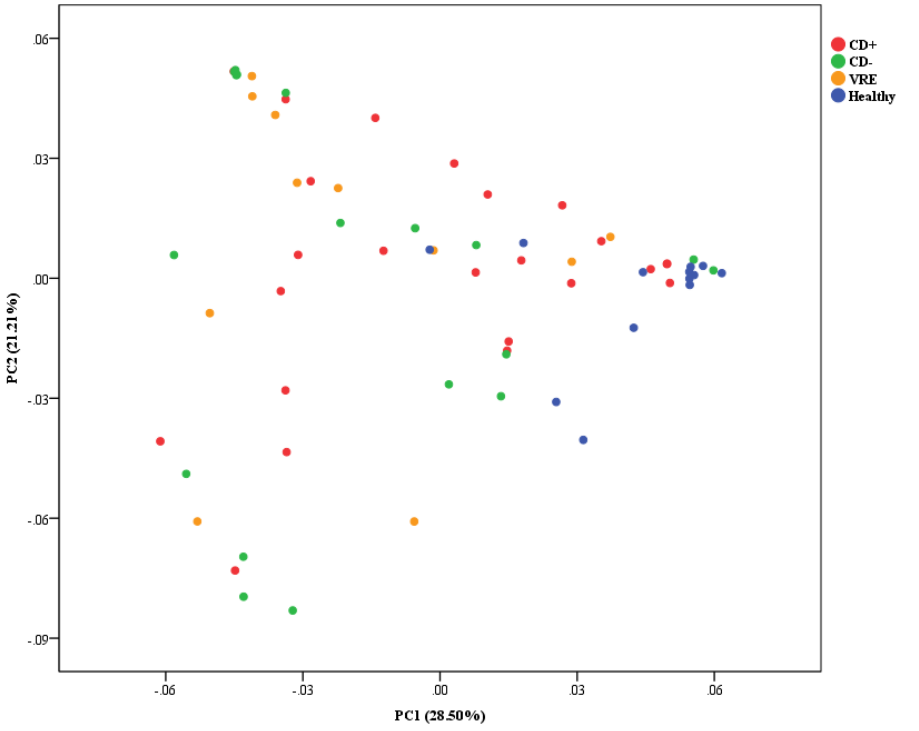


Figure 2. Beta diversity of each group of bacteria. Principal coordinate analysis (PCoA) was used to evaluate the beta diversity of each group of bacteria. CD: *Clostridioides difficile*; VRE: vancomycin-resistant enterococci.

3. Relative abundance of intestinal microbes

A comparison of the mean relative abundance of each major phylum is shown in Table 4 and Fig. 3. Phylum Firmicutes was predominant in all groups. In the healthy group, Actinobacteria was the second-most abundant. The abundance of Actinobacteria was significantly lower in the CD+ group (p = 0.001) compared to that in healthy control, but there was no difference in its abundance between the CD+ group and any other group. Proteobacteria was the second-most predominant phylum in the CD+, CD-, and VRE groups. The proportion of

Proteobacteria was slightly higher in the CD+, CD-, and VRE groups than in healthy controls, but not significantly (12.8% in CD+; 23.6% in CD-; 17.5% in VRE; 3.5% in healthy control). Compared to that in the VRE group, the proportion of Bacteroidetes was higher in the CD+ and CD- groups, but not significantly (7.1% in CD+; 5.9% in CD-; 2.7% in VRE; 4.3% in healthy control, p=0.673). Verrucomicrobia was detected at a proportion of 2.5% in the CD+ group, but was below the detection threshold in the CD-, VRE, and healthy groups.

Table 4. Relative abundance within each group at the phylum level

	CD+ (n=24)	CD- (n=18)	VRE (n=11)	Healthy (n=13)	P-value			
					Overall	CD+ vs healthy	CD+ vs CD-	CD+ vs VRE
Firmicutes	73.6 ± 24.4	67.1 ± 33.5	77.2 ± 27.2	78.6 ± 19.8	0.972	0.899	0.889	0.594
Proteobacteria	12.8 ± 23.0	23.6 ± 33.3	17.5 ± 27.3	3.5 ± 7.4	0.849	0.435	0.959	0.644
Bacteroidetes	7.1 ± 11.0	5.9 ± 11.7	2.7 ± 4.6	4.3 ± 8.0	0.673	0.749	0.482	0.693
Actinobacteria	2.8 ± 5.0	3.1 ± 7.8	2.4 ± 6.0	13.6 ± 14.7	0.001	0.001	0.095	0.155
Synergistetes	0.8 ± 2.8	0.2 ± 0.9	0	0.0 ± 0.0	0.323	0.637	0.457	0.227
Verrucomicrobia	2.5 ± 8.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.402	0.163	0.357	0.263
Fusobacteria	0.4 ± 1.8	0.1 ± 0.3	0.1 ± 0.3	0.0 ± 0.0	0.958	0.595	0.857	0.797

CD, *Clostridioides difficile*; VRE, vancomycin-resistant enterococci.

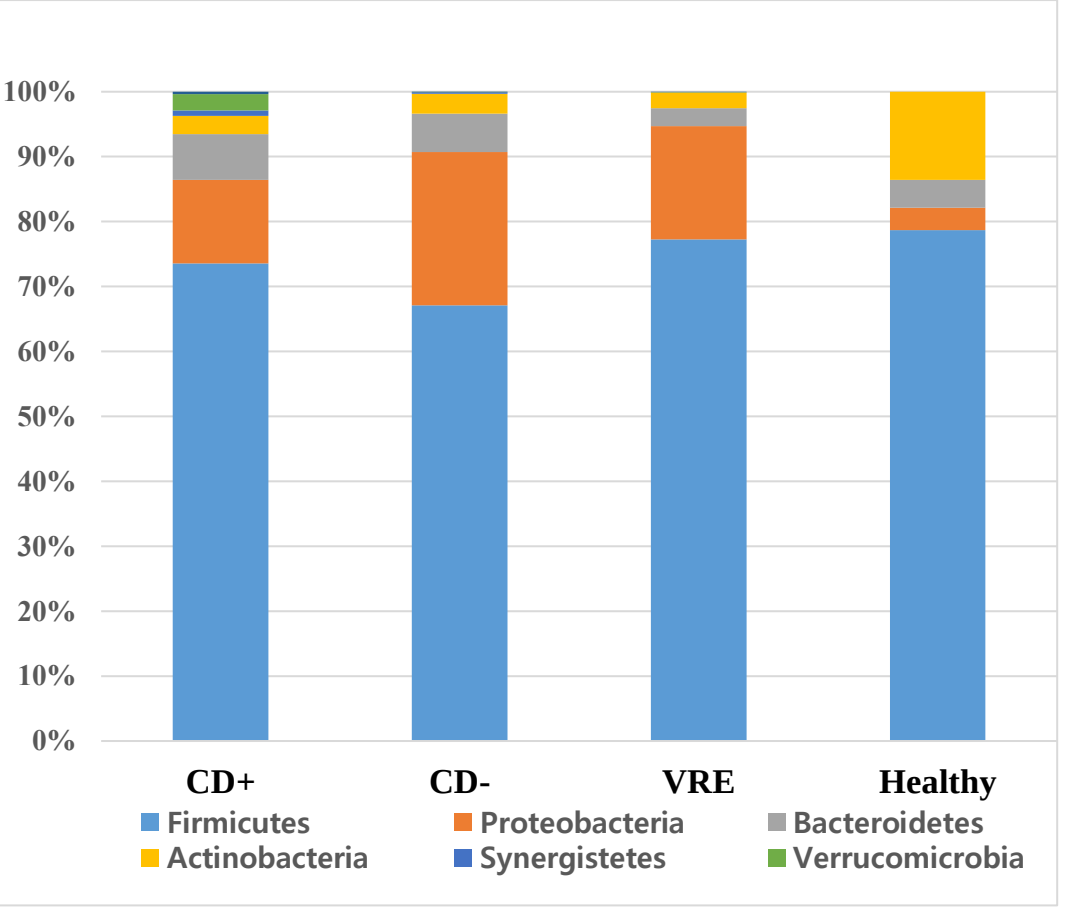


Figure 3. Relative abundance within each group at the phylum level. CD: *Clostridioides difficile*; VRE: vancomycin-resistant enterococci.

At the genus level, genera with a mean relative abundance of >1.0% (based on healthy controls or CD+ group) were analysed, as described in Table 5. Compared to those in the healthy group, the CD+ group showed significantly lower proportions of *Blautia*, *Bifidobacterium*, *Faecalibacterium*, *Anaerostipes*, *Dorea*, *Collinsella*, *Eubacterium*_g5, *Fusicatenibacter*, *Prevotella*, *Roseburia*, *Subdoligranulum*, GQ871709_g, *Catenibacterium*, *Clostridium*, *Ruminococcus*_g2, *Dialister*, and *Ruminococcus*_g5. However, the proportions of *Enterococcus*, *Hungatella*, *Eubacterium_g1*, *Clostridium_g6 and*

Clostridium_g4 in the CD+ group were significantly higher than those in the healthy control. Compared to those in the CD- group, the proportions of *Hungatella* and *Clostridium_g4* in the CD+ group were significantly higher. Compared to those in the VRE group, subjects in the CD+ group showed significantly higher proportions of *Anaerostipes* and *Hungatella*.

Table 5. Relative abundance within each group at genus level

	CD+ (n=24)	CD- (n=18)	VRE (n=11)	Healthy (n=13)	*P*-value			
					Overall	CD+ vs healthy	CD+ vs CD-	CD+ vs VRE
Blautia	6.9 ± 13.1	2.5 ± 6.6	7.0 ± 14.1	19.1 ± 14.1	<0.001	0.001	0.065	0.390
Bifidobacterium	2.1 ± 5.0	0.8 ± 1.6	2.0 ± 6.1	9.4 ± 11.7	0.001	0.001	0.372	0.369
Lactobacillus	3.6 ± 8.7	2.1 ± 3.8	14.4 ± 25.3	7.2 ± 12.5	0.269	0.084	0.202	0.351
Faecalibacterium	1.3 ± 5.0	1.1 ± 4.4	0.0 ± 0.0	7.0 ± 6.7	<0.001	<0.001	0.483	0.743
Anaerostipes	0.7 ± 1.4	0.2 ± 0.5	0.3 ± 0.8	6.1 ± 9.5	<0.001	0.002	0.389	0.048
Dorea	2.6 ± 6.4	3.0 ± 7.9	0.2 ± 0.5	4.3 ± 2.9	<0.001	<0.001	0.594	0.167
Collinsella	0.1 ± 0.4	0.4 ± 1.1	0.0 ± 0.0	3.6 ± 3.9	0.003	0.003	0.411	0.330
Streptococcus	5.0 ± 12.3	2.5 ± 5.6	6.7 ± 9.9	3.3 ± 8.9	0.617	0.190	0.573	0.406
Escherichia	5.6 ± 13.9	11.0 ± 21.8	7.8 ± 16.8	2.9 ± 7.5	0.830	0.618	0.410	0.665
*Eubacterium*_g5	0.2 ± 0.7	0.2 ± 0.7	1.5 ± 4.9	2.9 ± 3.1	<0.001	<0.001	0.723	0.661
Fusicatenibacter	0.7 ± 3.1	0.1 ± 0.2	0.0 ± 0.0	2.4 ± 3.0	<0.001	<0.001	0.434	0.741
Prevotella	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	2.3 ± 5.7	0.004	0.001	0.343	0.884
Roseburia	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.2	2.2 ± 2.9	<0.001	<0.001	0.802	0.614

*Ruminococcus*_g6	2.4 ± 4.8	1.7 ± 6.0	4.9 ± 15.0	2.0 ± 3.7	0.176	0.885	0.092	0.157
Subdoligranulum	0.2 ± 0.6	0.2 ± 0.6	0.0 ± 0.0	2.0 ± 2.0	<0.001	<0.001	0.442	>0.999
GQ871709_g	0.1 ± 0.2	0.1 ± 0.3	0.0 ± 0.0	2.0 ± 1.8	<0.001	<0.001	0.630	0.157
Catenibacterium	0.0 ± 0.0	0.1 ± 0.4	0.0 ± 0.0	2.0 ± 3.6	0.001	0.001	0.248	>0.999
Clostridium	0.2 ± 0.4	0.2 ± 0.6	0.4 ± 1.1	1.8 ± 2.7	0.047	0.035	0.784	0.344
*Ruminococcus*_g2	0.1 ± 0.6	0.0 ± 0.1	0.1 ± 0.2	1.7 ± 2.9	<0.001	<0.001	0.469	0.797
Dialister	0.0 ± 0.1	0.1 ± 0.2	0.0 ± 0.0	1.6 ± 3.2	0.003	0.002	0.181	0.331
*Eubacterium*_g2	0.7 ± 3.5	0.8 ± 3.2	0.0 ± 0.0	1.1 ± 3.0	0.308	0.218	0.416	0.331
*Ruminococcus*_g5	0.2 ± 0.5	0.2 ± 0.4	0.0 ± 0.0	1.0 ± 1.2	<0.001	<0.001	0.440	0.392
Bacteroides	6.2 ± 10.5	3.0 ± 5.5	2.6 ± 4.4	1.0 ± 1.4	0.527	0.747	0.195	0.404
Enterococcus	23.9 ± 27.0	34.6 ± 40.4	31.8 ± 33.1	0.1 ± 0.2	<0.001	<0.001	0.889	0.456
Hungatella	5.6 ± 14.6	0.3 ± 0.7	0.2 ± 0.3	0.2 ± 0.3	0.005	0.017	0.003	0.011
Eubacterium_g1	4.4 ± 9.4	6.1 ± 22.3	0.6 ± 1.4	0.0 ± 0.1	0.056	0.005	0.242	0.265
Klebsiella	2.9 ± 13.1	6.8 ± 19.9	7.8 ± 24.9	0.1 ± 0.3	0.478	0.151	0.761	0.739
Clostridium_g6	2.7 ± 6.2	1.2 ± 3.1	3.1 ± 5.5	0.1 ± 0.2	0.138	0.033	0.106	0.971
Akkermansia	2.5 ± 8.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.402	0.163	0.357	0.263
Enterobacter	2.4 ± 7.4	3.0 ± 9.2	0.2 ± 0.3	0.0 ± 0.0	0.176	0.093	0.956	0.406
Clostridium_g4	1.9 ± 7.0	0.0 ± 0.1	0.5 ± 1.1	0.0 ± 0.0	<0.001	<0.001	<0.001	0.074
Anaerofilum	1.2 ± 3.2	0.2 ± 0.5	0.1 ± 0.2	0.0 ± 0.0	0.141	0.132	0.074	0.072
Raoultella	1.1 ± 5.0	0.2 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.515	0.71	0.221	0.267

CD, *Clostridioides difficile*; VRE, vancomycin-resistant enterococci.

Interestingly, the proportion of *Clostridium_g4*, including *Clostridioides difficile*,

was 1.89 % in the CD+ group, 0.03 % in the CD- group, 0.45 % in the VRE group, and 0.002 % in the healthy control group (Table 5). At the species level, the CD+ group showed a significantly higher proportion of *C. difficile* than the CD- and healthy groups; however, this difference was not significant when compared to the VRE group (Table 6).

Table 6. Relative abundance of *Clostridioides difficile* within each group

	CD+ (n=24)	CD- (n=18)	VRE (n=11)	Healthy (n=13)	P-value			
					Overall	CD+ vs healthy	CD+ vs CD-	CD+ vs VRE
Relative abundance	1.9 ± 7.0	0.0 ± 0.1	0.4 ± 1.1	0	<0.001	<0.001	<0.001	0.064

CD, *Clostridioides difficile*; VRE, vancomycin-resistant enterococci.

4. Comparison between mild and severe *C. difficile* infection

CD+ group was devided into two subgroups in terms of clinical severity: mild and severe CD+ groups. A comparison of the alpha diversity of the gut microbiota between the two subgroups is shown in Table 7. The Chao1 index and Shannon index were not significantly different between two groups. The beta diversity within each group was evaluated using PCoA (Fig. 3). The mild and severe CD+ groups were not separated. A comparison of the mean relative abundance of each major phylum is shown in Table 7. *Firmicutes* was higher in the mild CD+ group compared to the severe CD+ group, but not significantly (82.2 % in mild CD+ group; 63.4% in severe CD+ group, p=0.060). Compared to the mild CD+ group, the proportions of *Proteobacteria*, *Bacteroidetes* and *Verrucomicrobia* were higher in the severe CD+ group, but not significantly. *Actinobacteria* was insignificantly higher in the mild CD+ group. At the genus level, genera with a mean relative abundance of >1.0% (based on CD+ group) were analysed, as described in Table 8. Compared to the mild CD+ group, the severe CD+ group

showed a significantly higher proportion of *Clostridium_g4* (0.2 % in mild CD+ group; 3.9 % in severe CDI group, p = 0.044). Other genera had no significant differences between the two groups. At the sepcies level, the mean relative abundance of *C. difficile* was higher in the severe CD+ group, but the p value was 0.065 (Table 9).

Table 7. Comparison of alpha diversity and relative abundance at the phylum level between mild and severe CD+ groups

	Mild CD+ (n=13)	Severe CD+ (n=11)	P-value
Alpha diversity (mean ± SD)			
Chao1 index	247.0 ± 182.3	330.6 ± 197.8	0.311
Shannon index	3.5 ± 0.8	3.6 ± 0.8	0.931
Relative abundance at phylum level (%, mean ± SD)			
Fiumicutes	82.2 ± 16.3	63.4 ± 28.9	0.060
Proteobacteria	6.8 ± 12.4	20.0 ± 30.4	0.258
Bacteroidetes	5.2 ± 9.4	9.3 ± 12.7	0.703
Actinobacteria	3.8 ± 6.3	1.7 ± 2.7	0.469
Synergistetes	0.6 ± 2.3	1.1 ± 3.5	0.450
Verrucomicrobia	0.7 ± 2.4	4.6 ± 11.4	0.253
Fusobacteria	0.7 ± 2.5	0.0 ± 0.1	0.512

CD: *Clostridioides difficile*

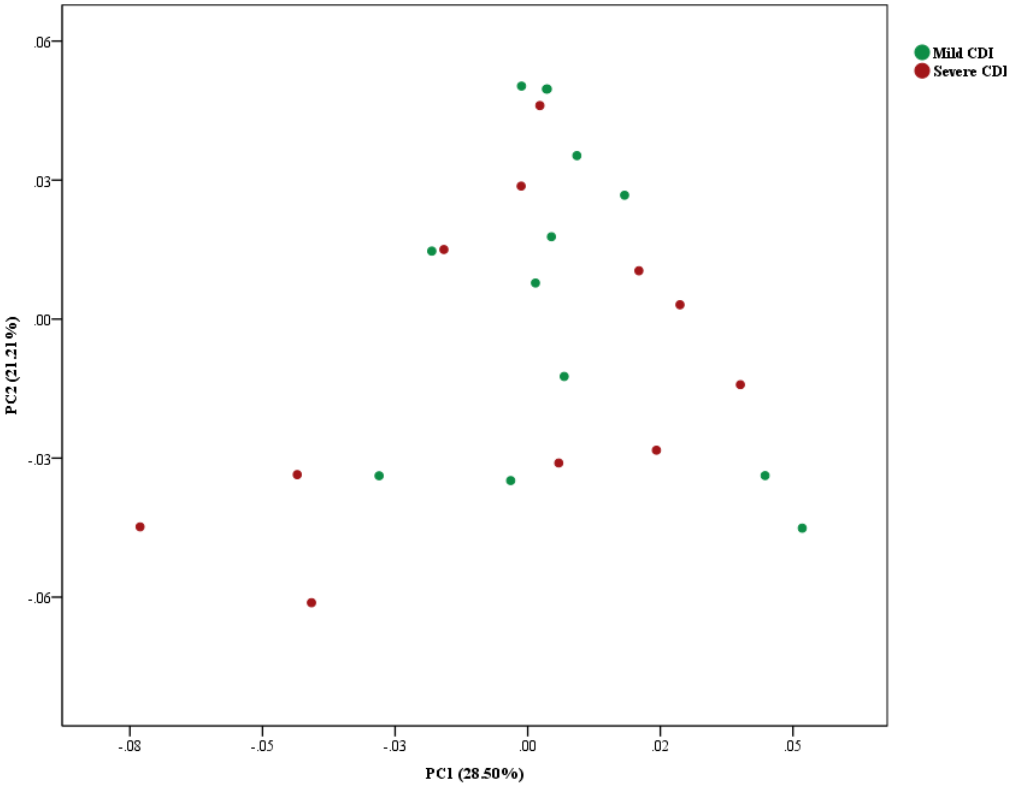


Figure 4. Beta diversity of mild and severe CD+ groups. Principal coordinate analysis (PCoA) was used to evaluate the beta diversity of mild and severe CD+ groups. CDI: *Clostridioides difficile* infection

Table 8. Comparison of relative abundance at genus level between mild and severe CD+ groups

	Mild CD+ (n=13)	Severe CD+ (n=11)	P-value
Relative abundance at genus level (%, mean ± SD)			
Enterococcus	24.0 ± 30.7	23.7 ± 23.2	0.931
Blautia	8.8 ± 16.5	4.7 ± 7.5	0.541
Bacteroides	5.6 ± 9.1	6.9 ± 12.3	0.637
Hungatella	8.5 ± 19.6	2.2 ± 2.9	0.353
Escherichia	3.3 ± 6.9	8.2 ± 19.3	0.747
Streptococcus	5.2 ± 14.5	4.8 ± 10.0	0.976
Eubacterium_g1	4.3 ± 8.3	4.5 ± 11.0	0.953
Lactobacillus	3.8 ± 11.0	3.3 ± 5.4	0.726
Klebsiella	0.4 ± 0.8	5.9 ± 19.4	0.789
Clostridium_g6	3.2 ± 6.8	2.0 ± 5.6	0.175
Dorea	3.6 ± 8.4	1.3 ± 2.7	0.588
Akkermansia	0.7 ± 2.4	4.6 ± 11.4	0.253
Ruminococcus_g6	4.1 ± 6.0	0.4 ± 0.7	0.107
Enterobacter	2.7 ± 9.5	2.0 ± 4.4	0.975
Bifidobacterium	3.1 ± 6.4	0.9 ± 2.3	0.605
Clostridium_g4	0.2 ± 0.3	3.9 ± 10.3	0.044
Faecalibacterium	2.2 ± 6.7	0.3 ± 0.9	0.351
Anaerofilum	1.0 ± 1.9	1.5 ± 4.4	0.976
Raoultella	0.1 ± 0.2	2.2 ± 7.3	0.228

CD: *Clostridioides difficile*

Table 9. Comparison of relative abundance of *Clostridioides difficile* between mild and severe CD+ groups

	Mild CD+ (n=13)	Severe CD+ (n=11)	P-value
Relative abundance	0.2 ± 0.3	3.9 ± 10.2	0.065

CD: *Clostridioides difficile*

IV. Discussion

This study evaluated the characteristics of faecal microbiota in patients with *C. difficile*-associated diarrhoea. It is well known that the disruption of the gut microbiota is a major cause of CDI, and previous studies have consistently shown that a reduction in the diversity and abundance of faecal microbiota is a common phenomenon in CDI patients.[33-35] Exposure to antibiotics is a major cause of gut microbiota disruption and CDI. Antibiotic use also promotes and maintains a high density of enterococci, including VRE.[36, 37] To better characterize changes in the gut microbiota due to CDI, we included VRE-colonised subjects in addition to healthy controls and subjects with CD- loose stool. Although CDI subjects showed a lower bacterial richness and diversity than the healthy controls, the species richness and diversity of intestinal microbiota was not significantly different in the CD+, CD-, and VRE groups. Patients in these groups had similar levels of antibiotic exposure, and antibiotic exposure itself is known to decrease bacterial richness and diversity, regardless of CD toxin positivity or VRE colonisation. We also observed no notable difference in bacterial phyla between the CD+, CD-, and VRE groups, although the proportion of *Actinobacteria* in the CD+ group was significantly lower than in the healthy control group. At the genus level, the CD+ group had a significantly higher proportion of *Hungatella* than the healthy control, CD-, and VRE groups. Moreover, the CD+ group had a significantly higher proportion of *Clostridium_g4* than the healthy control and CD- groups; however, this increase was not significant when compared to the VRE group. This finding is reasonable given that *C. difficile* is included in *Clostridium_g4*

FMT has become an effective treatment for recurrent CDI.[12, 38] Recently, FMT has been attempted to eliminate intestinal colonisation by multi-drug resistant organisms (MDRO) such as extended spectrum b-lactamase (ESBL)-producing and carbapenemase-producing Enterobacteriaceae (CPE), VRE, and methicillin-resistant *Staphylococcus aureus* (MRSA).[39-46] These attempts have shown

considerable successes, and FMT has emerged as a promising therapy for intestinal MDRO decolonisation. The identification of any key microbiome components associated with CDI or VRE would allow researchers to apply this microbiome factor to develop more specific probiotics to treat dysbiosis. Although we could not find any specific phyla to differentiate between CDI and VRE colonisation, the proportion of Verrucomicrobia was slightly higher in the CD+ group, and Synergistetes was detected only in the CD+ group, but not in the VRE group. Furthermore, at the genus level the CD+ group showed a significantly higher proportion of *Anaerostipes* and *Hungatella* than the VRE group.

Consistent with previous studies, decreased diversity and richness were characteristic of the microbiota in CDI patients compared to those in healthy controls.[35] However, the microbial composition at the phylum and genus levels for CDI patients was not fully consistent with previous results. A recent study in South Korea compared the composition of the gut microbiota in patients with toxigenic CDI and healthy controls[35]. They showed that the proportion of Proteobacteria was significantly higher in CDI than in healthy controls at the phylum level, and several genera, such as *Phascolarctobacterium*, *Lachnospira*, *Butyricimonas*, *Catenibacterium*, *Paraprevotella*, *Odoribacter*, and *Anaerostipes*, were not detected in most CDI patients. Decreased Bacteroidetes and increased Proteobacteria in CDI have been observed in previous studies.[34, 47] In our study, the proportion of Proteobacteria was insignificantly higher in CD+ patients than in healthy controls, and the proportion of Actinobacteria was significantly lower in the CD+ group compared to that in healthy controls. The proportion of Bacteroidetes was not significantly different between CD+ group and healthy control. Bacteroidetes was the third-most predominant phylum in our study, but was the second-most predominant phylum in CDI samples in the previous study.

At the species level, the mean relative abundance of *C. difficile* in the CD+ group was significantly higher than that in the CD- group and healthy control.

Compared to that in the VRE group, *C. difficile* in the CD+ group was higher, but it was marginally significant (*p* value = 0.064). Another study reported that the average abundance of *C. difficile* was 1.78 % in CDI patients [48] and that *C. difficile* was present in 18 of 211 healthy controls; however, the abundance of *C. difficile* did not differ with clinical severity. In our study, the mean relative abundance of *C. difficile* was 1.9 % in patients with CDI and *C. difficile* was not observed in any of the 13 healthy individuals, but was identified in VRE patients. Moreover, we found that the mean relative abundance of C. difficile was higher in patients with severe CDI; however, this effect was marginally significant (*p* = 0.065).

This study had several limitations. First, the small number of subjects was a major limitation in this study. Second, we could not control the effects of sample storage and interval between sample collection and DNA extraction on the analysis of microbiome. Third, we could not adjust confounding factors such as antibiotic use, hospital days, comorbidities, etc. which may affect the microbiota of subjects. Fourth, we could not compare the characteristics of microbiota according to the type of C. difficile toxin and the genotype of VRE. Furthermore, we could not evaluate paired samples to compare microbiome characteristics before and after CDI. The diet of the subjects may also have affected the characteristics of the microbiome, but we could not collect data to investigate this.

V. CONCLUSION

This research aimed to evaluate the characteristics of faecal microbiota in patients with CDI, compared to not only healthy controls but also patient with colitis other than CDI and patients with VRE colonization. Species richness, diversity and microbial composition were significantly different between CDI patients and healthy persons. But, there was no significant difference in species richness, diversity and relative abundance at the phylum level when CD+ group was compared to CD- and VRE groups that had similar level of antibiotic exposure with CD+ group. Although these findings might be related to the limitations of this study, which were mentioned above, it could also mean that the identification of the key microbiome components is more important than the analysis of overall microbial composition. The identified intestinal microbiome characteristics of Koreans with CDI compared to other patients with antibiotic exposure and healthy control might help to develop microbiome based diagnostic and treatment modalities.

REFERENCES

1. Hooper LV, Midtvedt T, Gordon JI: How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annual review of nutrition* 2002, 22:283-307.
2. Kinross JM, Darzi AW, Nicholson JK: Gut microbiome-host interactions in health and disease. *Genome medicine* 2011, 3(3):14.
3. Mueller S, Saunier K, Hanisch C, Norin E, Alm L, Midtvedt T, Cresci A, Silvi S, Orpianesi C, Verdenelli MC *et al*: Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. *Applied and environmental microbiology* 2006, 72(2):1027-1033.
4. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP *et al*: Human gut microbiome viewed across age and geography. *Nature* 2012, 486(7402):222-227.
5. Dave M, Higgins PD, Middha S, Rioux KP: The human gut microbiome: current knowledge, challenges, and future directions. *Translational research : the journal of laboratory and clinical medicine* 2012, 160(4):246-257.
6. Distrutti E, Monaldi L, Ricci P, Fiorucci S: Gut microbiota role in irritable bowel syndrome: New therapeutic strategies. *World journal of gastroenterology* 2016, 22(7):2219-2241.
7. Patterson E, Ryan PM, Cryan JF, Dinan TG, Ross RP, Fitzgerald GF, Stanton C: Gut microbiota, obesity and diabetes. *Postgraduate medical journal* 2016.
8. Singh V, Yeoh BS, Vijay-Kumar M: Gut microbiome as a novel cardiovascular therapeutic target. *Current opinion in pharmacology* 2016, 27:8-12.

9. Seekatz AM, Young VB: Clostridium difficile and the microbiota. *The Journal of clinical investigation* 2014, 124(10):4182-4189.
10. Kelly CP, LaMont JT: Clostridium difficile--more difficult than ever. *The New England journal of medicine* 2008, 359(18):1932-1940.
11. McFarland LV, Elmer GW, Surawicz CM: Breaking the cycle: treatment strategies for 163 cases of recurrent Clostridium difficile disease. *The American journal of gastroenterology* 2002, 97(7):1769-1775.
12. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG *et al*: Duodenal infusion of donor feces for recurrent Clostridium difficile. *The New England journal of medicine* 2013, 368(5):407-415.
13. Almeida R, Gerbaba T, Petrof EO: Recurrent Clostridium difficile infection and the microbiome. *Journal of gastroenterology* 2016, 51(1):1-10.
14. Rupnik M: Toward a true bacteriotherapy for Clostridium difficile infection. *The New England journal of medicine* 2015, 372(16):1566-1568.
15. Lee JH, Lee S-Y, Kim YS, Park S-W, Park SW, Jo SY, Ryu SH, Lee JH, Moon JS, Whang DH *et al*: The Incidence and Clinical Features of Clostridium difficile Infection; Single Center Study. *Korean J Gastroenterol* 2010, 55(3):175-182.
16. Shin JY, Ko EJ, Lee SH, Shin JB, Kim SI, Kwon KS, Kim HG, Shin YW, Bang BW: Refractory pseudomembranous colitis that was treated successfully with colonoscopic fecal microbial transplantation. *Intest Res* 2016, 14(1):83-88.
17. Moon KR, Sohn KM, Park BM, Kim Y-S, Chun S, Jung H, Song CH: Successful Fecal Transplantation by Enema for Recurrent and Refractory Clostridium difficile Infection. *J Korean Geriatr Soc* 2013, 17(3):152-156.

18. Kook SY, Kim Y, Kang B, Choe YH, Kim YH, Kim S: Characterization of the fecal microbiota differs between age groups in Koreans. *Intestinal Research* 2018, 16(2):246-+.
19. Shin JH, Jung S, Kim SA, Kang MS, Kim MS, Joung H, Hwang GS, Shin DM: Differential Effects of Typical Korean Versus American-Style Diets on Gut Microbial Composition and Metabolic Profile in Healthy Overweight Koreans: A Randomized Crossover Trial. *Nutrients* 2019, 11(10).
20. Safdar N, Maki DG: The commonality of risk factors for nosocomial colonization and infection with antimicrobial-resistant Staphylococcus aureus, enterococcus, gram-negative bacilli, Clostridium difficile, and Candida. *Ann Intern Med* 2002, 136(11):834-844.
21. Ozsoy S, Ilki A: Detection of vancomycin-resistant enterococci (VRE) in stool specimens submitted for Clostridium difficile toxin testing. *Braz J Microbiol* 2017, 48(3):489-492.
22. Ray AJ, Hoyen CK, Das SM, Eckstein EC, Donskey CJ: Undetected vancomycin-resistant Enterococcus stool colonization in a Veterans Affairs Hospital using a Clostridium difficile-focused surveillance strategy. *Infect Control Hosp Epidemiol* 2002, 23(8):474-477.
23. Kyne L, Hamel MB, Polavaram R, Kelly CP: Health care costs and mortality associated with nosocomial diarrhea due to Clostridium difficile. *Clin Infect Dis* 2002, 34(3):346-353.
24. Zar FA, Bakkanagari SR, Moorthi KM, Davis MB: A comparison of vancomycin and metronidazole for the treatment of Clostridium difficile-associated diarrhea, stratified by disease severity. *Clin Infect Dis* 2007, 45(3):302-307.
25. Kato H, Kato N, Watanabe K, Iwai N, Nakamura H, Yamamoto T, Suzuki K, Kim SM, Chong Y, Wasito EB: Identification of toxin A-negative, toxin B-positive Clostridium difficile by PCR. *J Clin*

Microbiol 1998, 36(8):2178-2182.

26. Kwon SS, Gim JL, Kim MS, Kim H, Choi JY, Yong D, Lee K: Clinical and molecular characteristics of community-acquired Clostridium difficile infections in comparison with those of hospital-acquired C. difficile. *Anaerobe* 2017, 48:42-46.
27. Chun J, Kim KY, Lee JH, Choi Y: The analysis of oral microbial communities of wild-type and toll-like receptor 2-deficient mice using a 454 GS FLX Titanium pyrosequencer. *BMC microbiology* 2010, 10:101.
28. Hur M, Kim Y, Song HR, Kim JM, Choi YI, Yi H: Effect of genetically modified poplars on soil microbial communities during the phytoremediation of waste mine tailings. *Applied and environmental microbiology* 2011, 77(21):7611-7619.
29. Kim BS, Kim JN, Yoon SH, Chun J, Cerniglia CE: Impact of enrofloxacin on the human intestinal microbiota revealed by comparative molecular analysis. *Anaerobe* 2012, 18(3):310-320.
30. Huber T, Faulkner G, Hugenholtz P: Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics (Oxford, England)* 2004, 20(14):2317-2319.
31. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H *et al*: Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *International journal of systematic and evolutionary microbiology* 2012, 62(Pt 3):716-721.
32. Hamady M, Lozupone C, Knight R: Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. *The ISME journal* 2010, 4(1):17-27.
33. Milani C, Ticinesi A, Gerritsen J, Nouvenne A, Lugli GA, Mancabelli

L, Turroni F, Duranti S, Mangifesta M, Viappiani A *et al*: Gut microbiota composition and Clostridium difficile infection in hospitalized elderly individuals: a metagenomic study. *Sci Rep* 2016, 6:25945.

34. Zhang LH, Dong DF, Jiang C, Li Z, Wang XF, Peng YB: Insight into alteration of gut microbiota in Clostridium difficile infection and asymptomatic C. difficile colonization. *Anaerobe* 2015, 34:1-7.
35. Han SH, Yi J, Kim JH, Lee S, Moon HW: Composition of gut microbiota in patients with toxigenic Clostridioides (Clostridium) difficile: Comparison between subgroups according to clinical criteria and toxin gene load. *PLoS One* 2019, 14(2):e0212626.
36. Donskey CJ, Chowdhry TK, Hecker MT, Hoyen CK, Hanrahan JA, Hujer AM, Hutton-Thomas RA, Whalen CC, Bonomo RA, Rice LB: Effect of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of colonized patients. *The New England journal of medicine* 2000, 343(26):1925-1932.
37. Ubeda C, Taur Y, Jenq RR, Equinda MJ, Son T, Samstein M, Viale A, Socci ND, van den Brink MRM, Kamboj M *et al*: Vancomycin-resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *Journal of Clinical Investigation* 2010, 120(12):4332-4341.
38. Smits LP, Bouter KE, de Vos WM, Borody TJ, Nieuwdorp M: Therapeutic potential of fecal microbiota transplantation. *Gastroenterology* 2013, 145(5):946-953.
39. Dubberke ER, Mullane KM, Gerding DN, Lee CH, Louie TJ, Guthertz H, Jones C: Clearance of Vancomycin-Resistant Enterococcus Concomitant With Administration of a Microbiota-Based Drug Targeted at Recurrent Clostridium difficile Infection. *Open Forum Infect Dis* 2016, 3(3):ofw133.

40. Sohn KM, Cheon S, Kim YS: Can Fecal Microbiota Transplantation (FMT) Eradicate Fecal Colonization With Vancomycin-Resistant Enterococci (VRE)? *Infect Control Hosp Epidemiol* 2016, 37(12):1519-1521.
41. Bar-Yoseph H, Hussein K, Braun E, Paul M: Natural history and decolonization strategies for ESBL/carbapenem-resistant Enterobacteriaceae carriage: systematic review and meta-analysis. *J Antimicrob Chemother* 2016, 71(10):2729-2739.
42. Lagier JC, Million M, Fournier PE, Brouqui P, Raoult D: Faecal microbiota transplantation for stool decolonization of OXA-48 carbapenemase-producing Klebsiella pneumoniae. *J Hosp Infect* 2015, 90(2):173-174.
43. Singh R, van Nood E, Nieuwdorp M, van Dam B, ten Berge IJ, Geerlings SE, Bemelman FJ: Donor feces infusion for eradication of Extended Spectrum beta-Lactamase producing Escherichia coli in a patient with end stage renal disease. *Clin Microbiol Infect* 2014, 20(11):O977-978.
44. Singh R, de Groot PF, Geerlings SE, Hodiamont CJ, Belzer C, Berge I, de Vos WM, Bemelman FJ, Nieuwdorp M: Fecal microbiota transplantation against intestinal colonization by extended spectrum beta-lactamase producing Enterobacteriaceae: a proof of principle study. *BMC Res Notes* 2018, 11(1):190.
45. Manges AR, Steiner TS, Wright AJ: Fecal microbiota transplantation for the intestinal decolonization of extensively antimicrobial-resistant opportunistic pathogens: a review. *Infect Dis (Lond)* 2016, 48(8):587-592.
46. Stalenhoef JE, Terveer EM, Knetsch CW, Van't Hof PJ, Vlasveld IN, Keller JJ, Visser LG, Kuijper EJ: Fecal Microbiota Transfer for Multidrug-Resistant Gram-Negatives: A Clinical Success Combined

With Microbiological Failure. *Open Forum Infect Dis* 2017, 4(2):ofx047.

47. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, No D, Liu H, Kinnebrew M, Viale A *et al*: Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. *Nature* 2015, 517(7533):205-208.

48. Daquigan N, Seekatz AM, Greathouse KL, Young VB, White JR: High-resolution profiling of the gut microbiome reveals the extent of Clostridium difficile burden. *NPJ biofilms and microbiomes* 2017, 3:35.

국문 요약

한국에서 *Clostridioides difficile* 감염 환자의 대변 미생물총 특성

지도 교수 최준용
연세대학교 대학원 의학과
전용덕

배경: 대장의 미생물총은 *Clostridioides difficile* 감염 환자의 병태생리에 있어서 중요한 역할을 한다. 대장의 미생물총은 지역적 특성 및 인종적 특성에 영향을 받으며, 미생물총의 구성 및 다양성이 다르다. 우리는 한국의 *C. difficile* 환자에서 미생물총의 특성을 밝히기 위하여 *C. difficile* 감염 환자(CD+), *C. difficile*이 아닌 장염환자(CD-), vancomycin-resistant *enterococci* 집락 환자(VRE) 및 정상 성인(healthy control)을 비교하였다.

연구방법: CD+, CD-, VRE 및 정상 성인 그룹 별로 각각 24명, 18명, 11명, 13명의 대상으로부터 대변 검체를 채취하였다. CD+ group은 임상적 중증도를 기준으로 경증 및 중증으로 하위 집단을 나누어 추가 분석을 시행하였다. 미생물총 분석은 세균의 16s rRNA에 대한 454-pyrosequencing을 이용하였다.

결과: CD+ 그룹에서 종의 풍부도(richness) 및 미생물총의 다양성(Diversity)은 정상성인에 비하여 통계학적으로 유의하게 감소되어 있었으나 CD- 및 VRE 그룹과는 차이를 보이지 않았다. 문 단계(phylum level)의 분석에서 CD+ 그룹에서 정상성인에 비해

*Actinobacteria*의 비율이 유의하게 감소되어 있었으나 CD- 및 VRE 그룹과는 차이를 보이지 않았다. 속 단계(genus level)의 분석에서 CD+ 그룹은 *Blautia, Bifidobacterium, Faecalibacterium, Anaerostipes* 등 다수의 장내세균속이 정상 성인에 비해 유의하게 감소되어 있었으나 *Enterococcus, Hungatella, Eubacterium_g1, Clostridium_g6* 그리고 *Clostridium_g4*는 정상성인에 비해 유의하게 증가 되어있는 양상을 보였다. CD- 그룹과 비교하여 CD+ 그룹에서는 *Hungatella* 및 *Clostridium_g4*의 비율이 유의하게 증가되어 있었으며, VRE 그룹과 비교하여서는 *Anaerostipes* 및 *Hungatella*의 비율이 유의하게 증가되어 있었다. *C. difficile* 종은 CD- 그룹 및 정상성인과 비교하여 CD+ 그룹에서 통계학적으로 유의하게 높은 비율을 보였고, VRE 그룹과 비교하여서는 높은 양상을 보였으나 통계학적으로 유의하지는 않았다. 임상적 중증도에 따라 CD+ 그룹을 나누어 비교한 결과에서는, 경증 *C. difficile*환자보다 중증 *C. difficile* 환자에서 *Clostridium_g4* 속이 통계학적으로 유의하게 높은 비율을 보였다(경증: 0.2 %, 중증: 3.9 %, p=0.044). *C. difficile* 종은 중증환자에서 높은 경향을 보였으나 통계학적으로 유의하지는 않았다.

결론: 우리는 한국인 *C. difficile* 감염 환자에서 대변 미생물총의 특성을 분석하였다. 이 분석이 한국인에 있어서 미생물총에 기반한 진단 및 치료에 있어서 도움이 되기를 기대한다.

핵심되는 말: 대변 미생물총, 장내 미생물총, *Clostridioides difficile* 감염 환자, 차세대 염기서열 분석