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Dysbiosis of gut microbiota in patients with severe COVID-19

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Abstract

Aim: Altered gut microbiota has been proposed as one of the causes of exacerbation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2/COVID-19) from the perspective of the gut–lung axis. We aimed to evaluate gut microbiota in mechanically ventilated patients with COVID-19 prior to using antibiotics.

Methods: We retrospectively selected for enrollment COVID-19 patients who required mechanical ventilation on admission but who had not used antibiotics before admission to observe the influence of SARS-Cov-2 on gut microbiota. Fecal samples were collected serially on admission and were evaluated by 16S rRNA gene deep sequencing.

Results: The phylum of Bacteroidetes decreased, and those of Firmicutes and Actinobacteria increased in COVID-19 patients compared with those in healthy controls ($p < 0.001$). The main commensals of *Bacteroides*, *Faecalibacterium*, and *Blautia* at the genus level were significantly decreased in the COVID-19 patients, and opportunistic bacteria including *Corynebacterium*, *Anaerococcus*, *Finegoldia Peptoniphilus*, *Actinomyces*, and *Enterococcus* were increased ($p < 0.001$). α-Diversity and β-diversity in COVID-19 patients significantly changed compared with those in the healthy controls.

Conclusion: The commensal gut microbiota were altered, and opportunistic bacteria increased in patients with severe COVID-19 who required mechanical ventilation on admission.

KEYWORDS
COVID-19, dysbiosis, gut, ICU, microbiota

BACKGROUND

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2/COVID-19) pandemic is an emergency situation throughout the world. COVID-19 has been reported to cause inflammation and cytokine storm and lead to multiple organ dysfunction syndrome.[1] The gut has been a target organ following critical illnesses.[2] Cross talk between the intestinal epithelium, immune system, and commensal bacteria is central to initiating the systemic inflammatory response. The gut–lung axis has been proposed as one of the causes of the exacerbation of disease severity.[3]

The human gut microbiota is estimated to contain 10^{14} microbes comprising over 1000 different bacterial species that reside in the host's colon.[4] These bacteria have a close connection with human metabolism and homeostasis of the immune system. Dysbiosis, defined as an imbalance in the microbial communities living in or on the body,[5] leads to various diseases such as obesity, metabolic syndrome, cardiovascular diseases, cancer, and autoimmune diseases. In the acute phase of critical care, gut microbiota are altered

following injury and dysbiosis develops thereafter. The decreased number of gut microbiota or dysbiosis was associated with infectious complications and mortality in critical illness.[6,7] It is thus important to know the composition of the gut microbiota because SARS-CoV-2 could cause intestinal inflammation and deteriorate the gut microbiota, which could further exacerbate COVID-19.

There are many reports on the gut microbiota in COVID-19, but there are few reports on the effects of COVID-19 on the gut microbiota free of the influence of antibiotics. Because antibiotics change the microbiota, we aimed to evaluate the gut microbiota in mechanically ventilated patients with COVID-19 prior to the use of antibiotics.

METHODS

Patients

Patients who were intubated and required mechanical ventilation on admission and were diagnosed as having COVID-19 in the Department of Traumatology and Acute Critical Medicine and Intensive Care Unit, Osaka University Hospital, during the period November 2020 to May 2021 were eligible for enrollment in this study. We retrospectively selected the COVID-19 patients who had not used antibiotics before admission to observe the influence of SARS-CoV-2 on the gut microbiota prior to using antibiotics. Healthy adult subjects were also included as a healthy control (HC) group.

The emergency medical system in our country has three designated levels according to the perceived acuity of the patients. Our designated tertiary hospital deals with patients who need to be managed in the operating room or the intensive care unit (ICU).[8] During the study period, only patients with severe COVID-19 who required a ventilator were transferred to our hospital.

DNA extraction and 16S rRNA sequencing

Fecal samples were collected from the patients on admission. All samples were collected by inserting a sterile cotton-tipped swab 1–2 cm beyond the anus and rotating the swab for several seconds. Swabs were placed in sterile centrifuge tubes and immediately stored in a freezer at −78°C until use. Fecal samples in the HC group were collected from feces on an arbitrary day. DNA was extracted from all fecal samples using a DNeasy PowerSoil Pro Kit (QIAGEN). To monitor changes in gut microbiota of the study population, 16S rRNA gene (V1-V2 region) deep sequencing was performed on a MiSeq system (Illumina). The paired-end sequences obtained were merged, filtered, and denoised using DADA2. Taxonomic assignment was performed using the QIIME2 feature-classifier plugin with the Greengenes 13_8 database. The QIIME2 pipeline, version 2020.2, was used as the bioinformatics environment for the processing of all relevant raw sequencing data.[9] The differential bacterial taxonomy between groups was identified by linear discriminant analysis effect size (LEfSe).[10]

For microbial community comparisons, we performed unique fraction (UniFrac) distance analysis of our 16S rRNA gene sequencing data. Both unweighted and weighted UniFrac distances were calculated to evaluate the diversity of microbial components and the structure of the microbiotas, respectively.[11] Although "Unweighted UniFrac" is a qualitative method, "Weighted UniFrac" is a quantitative measure that takes the number of RNA sequence reads into consideration. Subsequently, the analyzed data were processed using UniFrac principal coordinate analysis and presented in three-dimensional coordinates.

Statistical analysis

This study is an observational prospective cohort study. Sample size was computed based on feasibility. With 30 patients and 30 controls, the analysis was thought to have 85% power at a two-sided significance level of 5% to detect 1.5 standard deviation difference in the means of the outcome variables in which the standard deviation was for the corresponding outcome variable. A significance level of a two-sided $p < 0.05$ was used for statistical inferences. All statistical analyses were performed using JMP 15 (SAS Institute Inc., Cary, NC, USA). Comparisons of the α-diversity, UniFrac distance, and bacterial richness between the groups were performed by the Kruskal–Wallis test, using the Mann–Whitney *U* test with Bonferroni adjustment as a post hoc test, and data are presented using GRAPHPAD PRISM, version 6.04 (Graph-Pad Software, La Jolla, CA, USA).

RESULTS

Of the 109 ventilated patients assessed, 79 patients were excluded because antibiotics were used before admission or feces were not collected. Subjects included 30 patients (80% male) whose patient characteristics are listed in Table 1. All of the patients were intubated on admission, and six patients had been intubated at their previous hospital. Dexamethasone was administered in 60% of the patients, and remdesivir was used in 40% of them in the previous hospitals. The main comorbidities were hypertension, diabetes mellitus, and hyperlipidemia. Among the complications, diarrhea occurred in 11 (36.7%) patients. The median number [interquartile range] of ventilator-free days was 16.5 [0–21.5] days. Mortality within 28 days was 6.7% (2 of 30 patients).

In the analysis of the gut microbiota, the phylum of Bacteroidetes decreased, and those of Firmicutes and Actinobacteria increased in the COVID-19 patients

TABLE 1 Baseline patient clinical and demographic characteristics.

N	30
Age	65.5 [56.5, 73.8]
Male	80.0 (24)
Respiratory condition	
P/F ratio	276.5 [212.8, 312.3]
ECMO	6.7 (2)
Days from onset to admission (day)	7.0 [5.0–11.5]
Days from diagnosis to admission (day)	3.5 [1.8, 8.8]
Days from admission to intubation (day)	0 [0, 0]
Concomitant drugs	
Dexamethasone	60.0 (18)
Remdesivir	26.7 (8)
Favipiravir	23.3 (7)
Methylprednisolone	10.0 (3)
Comorbidities	
Hypertension	40.0 (12)
Diabetes mellitus	26.7 (8)
Hyperlipidemia	23.3 (7)
Hyperuricemia	16.7 (5)
Chronic kidney disease	10.0 (3)
Cardiovascular disease	6.7 (2)
COPD	3.3 (1)

Note: Values are expressed as median [interquartile range] or percentage (frequency).

Abbreviations: COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation.

TABLE 2 Gut microbiota at the phylum level.

	Healthy controls	COVID-19	*p* Value
Bacteroidetes	37.2 [34.4, 43.5]	18.2 [12.3, 27.7]	<0.001
Firmicutes	52.6 [43.1, 56.1]	62.9 [49.3, 72.2]	<0.001
Proteobacteria	3.7 [2.6, 6]	2.3 [1, 5.8]	0.107
Actinobacteria	3.4 [2.4, 7.5]	9.5 [5.6, 16.2]	<0.001
Fusobacteria	0 [0, 0.4]	0 [0, 0.3]	0.476

compared with those in the HCs (Table 2). At genus level, the dominant commensals of *Bacteroides*, *Faecalibacterium*, and *Blautia* were dramatically decreased in the COVID-19 patients compared with the HCs (Table 3; Figure 1). *Sutterella*, *Ruminococcus*, *Coprococcus*, *Lachnospira*, *Roseburia*, and *Anaerostipes* were also significantly decreased compared with the HCs in the LEfSe analysis and log2-transformed change in expression (Figures 2 and 3). In contrast, the genera of *Corynebacterium*, *Anaerococcus*, *Finegoldia*, *Peptoniphilus*, *Actinomyces*, and *Enterococcus* were the main genera showing a significant increase in the COVID-19 patients versus the HCs.

Regarding bacterial diversity, α-diversity in the COVID-19 patients decreased significantly compared with that in the HCs by the Simpson index ($p = 0.004$) and the Shannon index ($p = 0.002$) (Figure 4A). β-Diversity in the COVID-19 patients significantly increased in pairwise PERMANOVA comparisons ($p < 0.001$). The weighted and weighted UniFrac distance analysis showed significant differences in the gut microbiota composition of the COVID-19 patients compared with those in the HC group ($p < 0.001$) (Figure 4B). In the three-dimensional principal coordinate analysis based on weighted UniFrac distances, the plots in COVID-19 group were more scattered than those in the HC group (Figure 4C).

DISCUSSION

There are previous reports on the gut microbiota in COVID-19,[12] but there are few reports of the effects of severe COVID-19 on the gut microbiota in patients not influenced by antibiotics. Because antibiotics change microbiota such as *Bifidobacterium* and *Roseburia*,[13] we aimed to evaluate gut microbiota prior to using antibiotics. This study showed that the gut microbiota in patients with severe COVID-19 who required intubation was altered on admission prior to using antibiotics. The gut microbiota in severe insults can change within 6 h after injury, and the changes can persist for more than 6 weeks in critically ill patients.[14] These changes can result from multiple factors including broad-spectrum antibiotics use and disease severity.[7] We showed that even without any of the COVID-19 patients receiving antibiotics, the gut microbiota had already been disrupted, possibly due to SARS-COV-2 infection.

Normal gut microbiota have important roles in metabolism, nutrition, and protection against pathogens. Disruption of the gut microbiota, or "dysbiosis", can lead to many diseases such as infection, inflammatory bowel disease, metabolic syndrome, and cancer.[15] In critically ill patients, the gut microbiota is altered significantly especially with regard to the number of obligate anaerobes,[16] which are the dominant bacteria and are associated with infectious complications and mortality. In the present study, bacterial diversity had decreased, and dysbiosis had already progressed at admission prior to using antibiotics. The phylum of Bacteroidetes in the COVID-19 group, especially the genera of *Bacteroides*, *Faecalibacterium*, and *Blautia*, was decreased significantly compared with the HC group.[17] *Bacteroides* species provide protection from pathogens and supply nutrients to other microbial residents.[18] *Faecalibacterium* has an anti-inflammatory effect on intestinal disorders.[19] *Blautia* has a series of potential probiotic properties, which are reported to be associated with mortality from graft-versus-host disease.[20] Ojima et al.[7] reported in their investigation of gut microbiota in the ICU that dysbiosis developed within the first week from

TABLE 3 Gut microbiota at the genus level.

	Healthy controls	COVID-19	*p* Value
Bacteroides	22.6 [9.6, 30.8]	6.0 [1, 13.6]	<0.001
Faecalibacterium	6.9 [2.9, 9.1]	0.1 [0, 0.3]	<0.001
Blautia	4.3 [2.9, 5.1]	1.8 [0.1, 4.2]	0.001
Unclassified *Lachnospiraceae*	3.9 [2.7, 5.0]	0.7 [0, 1.8]	<0.001
Ruminococcus	3.7 [1.7, 6.1]	0.5 [0.2, 3.4]	0.002
Coprococcus	3.0 [1.8, 3.8]	0.1 [0, 1.0]	<0.001
Parabacteroides	2.4 [1.7, 3.2]	0.9 [0, 3.3]	0.091
Sutterella	2.4 [1.1, 3.6]	0.1 [0, 0.7]	<0.001
Prevotella	1.6 [0, 18.3]	0.8 [0, 7.9]	0.781
Collinsella	1.4 [0, 2.0]	0.1 [0, 0.7]	0.013
Clostridium	1.3 [0.5, 1.9]	0.3 [0, 1.4]	0.004

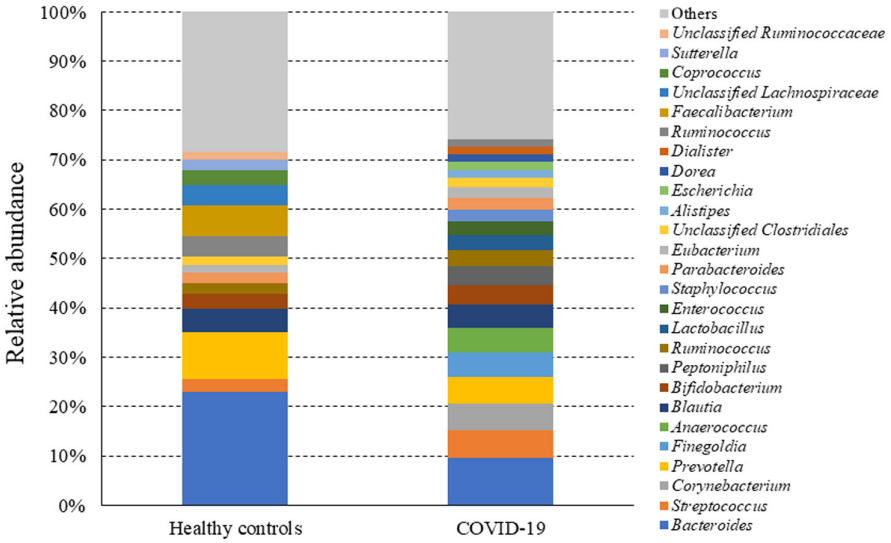

FIGURE 1 Gut microbiota composition at the genus level.

admission, and anti-inflammatory-related bacteria, such as *Blautia*, *Clostridium*, and *Faecalibacterium* decreased, whereas *Enterococcus* increased, and the Bacteroidetes/Firmicutes ratio was associated with mortality. In the gut microbiota of hospitalized COVID-19 patients, a decrease in normal gut microbiota bacteria such as *Eubacterium*, *Faecalibacterium*, *Roseburia*, and *Lachnospiraceae* was observed.[21] In this study, Actinobacteria increased significantly in the COVID-19 group. The administered drugs, especially dexamethasone might have effect to increase Actinobacteria.[22] Those results indicate the same tendency shown by the present results,[23] but our results showed that these changes could already occur prior to using antibiotics.

COVID-19 has been reported to have about 15% of gastrointestinal symptoms. In the present study, 36.7% of the patients had diarrhea. Angiotensin-converting enzyme (ACE) 2, a receptor for SARS-CoV-2, is expressed not only in the lungs but also in the small intestine and could induce enteritis and diarrhea with gut dysbiosis.[24] In a clinical study, it has been reported that fecal viral loads were detected[25] and digestive histology revealed gastrointestinal infection in patients with COVID-19.[26] Severely ill patients who require mechanical ventilation may have more intestinal inflammation, which could alter the gut microbiota and cause diarrhea.

The gut–lung axis perspective is important because intestinal dysbiosis is associated with increased pneumonia and mortality in critically ill patients.[3,6] Moreira-Rosario reported[27] that α-diversity index decreased with COVID-19 severity defined by the WHO Clinical Progression Scale.[28] In another report, Mazzarelli et al.[29] reported that α-diversity of COVID-19 patients in the ICU was significantly lower than that of those in the ward and controls. In this research, alpha diversity of COVID-19 significantly decreased, which were the same tendency of the previous research. One of the mechanisms could be the role of progressive intestinal dysbiosis in producing

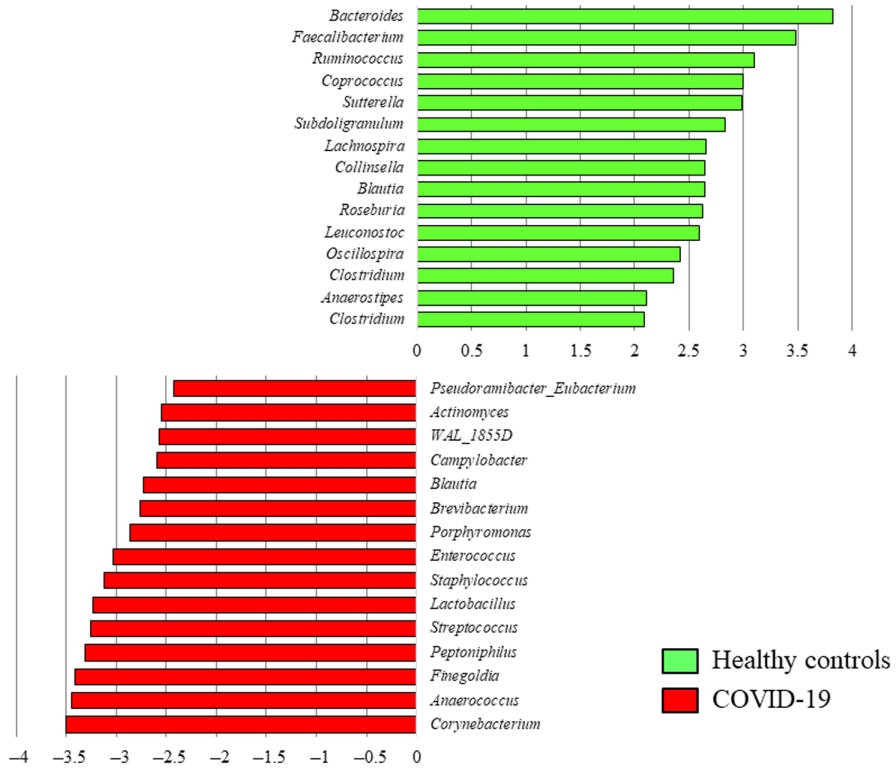

FIGURE 2 Linear discriminant analysis effect size in healthy controls and the COVID-19 group.

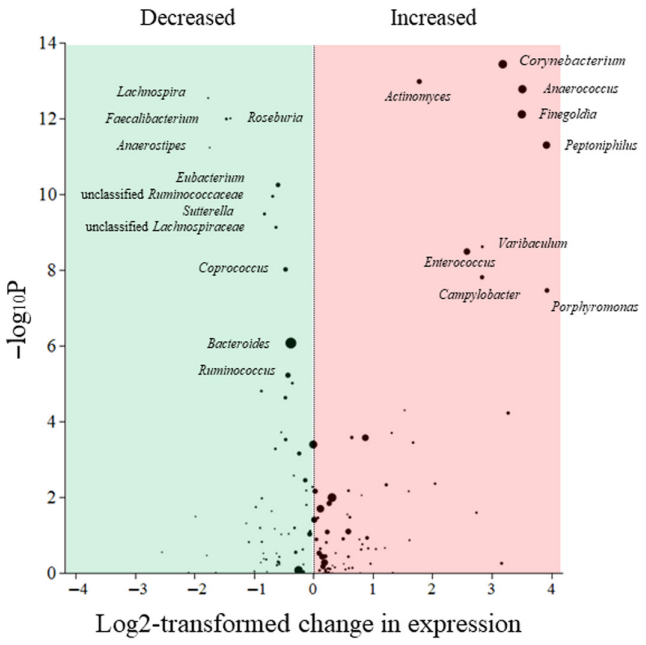

FIGURE 3 Volcano plot of gut microbiota at the genus level in the COVID-19 group compared with the healthy controls. The *x*-axis indicates log-transformed fold change in expression and the *p* values of *y*-axis were analyzed using a Wilcoxon signed-rank test.

immune signals via the microbiota that produce Th17, regulatory T cells, and IFN-γ-producing $CD8^+$ cells. Patients with severe COVID-19 had lower levels of T cells and increased IFN levels over the time course of the disease, which could associate with systemic immunity.[30] Actually, COVID-19 patients have been reported to suffer from fungal disease and severe cytomegalovirus infection.[31] As a partial reason for immune failure might be gut dysbiosis, reconstruction of the gut microbiota might be a potential therapy for COVID-19. In terms of intestinal treatment, decreasing viral loads and preventing inflammation of the intestinal epithelium could be candidate treatment. First, an antiviral oral drug administered for intestinal prophylaxis might be a therapy to reduce intestinal inflammation and subsequent pulmonary complications.[32] Second, the use of probiotics, prebiotics, and synbiotics for modulation of the gut microbiota strengthens innate and adaptive immunity by restoring the microbiota. Foods or supplements containing probiotics have been reported to show efficacy for the production of interleukins, virus titers, interferon, and antibodies. In critically ill patients, probiotics/synbiotics are effective in reducing diarrhea,[33] surgical infectious complications,[34] and ventilator-associated pneumonia.[35] The modulation of the gut–lung axis from the perspective of the gut microbiota could be an important therapeutic target for preventing or attenuating COVID-19 pneumonia.

As a limitation of this study, it did not include gut microbiota of patients with non-severe COVID-19. The comparison of gut microbiota between patients with severe and non-severe disease could be a future target of study to show the association between dysbiosis and severity of COVID-19.

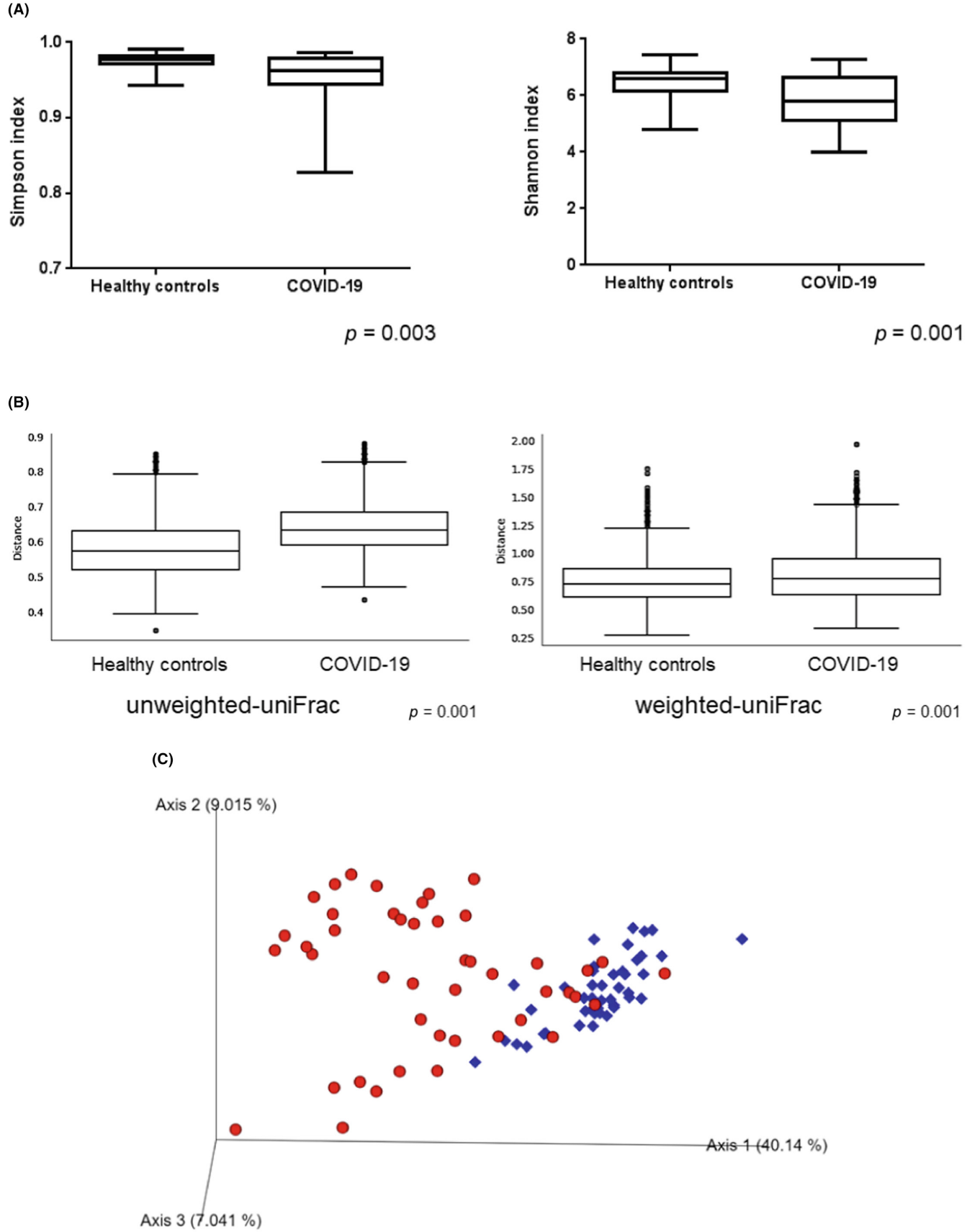

FIGURE 4 (A) α-Diversity of the Simpson index (left) and the Shannon index (right) in healthy controls and the COVID-19 group. (B) β-Diversity of unweighted-unique fraction (uniFrac) (left) and weighted-uniFrac (right) distance analysis in healthy controls and the COVID-19 group. HC, healthy controls. (C) Three-dimensional principal coordinate analysis plots based on weighted uniFrac distances. Blue diamond: Healthy controls, Red circle: COVID-19.

CONCLUSION

In conclusion, the commensal gut microbiota in patients with severe COVID-19 infection was altered, and opportunistic bacteria increased within several days from disease onset.

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CONFLICT OF INTEREST STATEMENT

Dr. Hiroshi Ogura is an Editorial Board member of the AMS Journal and a coauthor of this article. To minimize bias, they were excluded from all editorial decision-making related to the acceptance of this article for publication. Dr. Jun Oda is Editor-in-Chief of the journal and coauthor of this article. They were excluded from the peer-review process and all editorial decisions related to the acceptance and publication of this article. Peer-review was handled independently by Acute Medicine and Surgery editorial office with Dr. Yasuyuki Kuwagata as the Editor to minimize bias.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

APPROVAL OF THE RESEARCH PROTOCOL

Approval of the research protocol: This study was approved by the institutional review board of Osaka University Hospital (approval number: 12035).

INFORMED CONSENT

Informed consent for participation in this study was obtained from each patient or their next of kin.

REGISTRY AND THE REGISTRATION NO. OF THE STUDY/TRIAL

N/A.

ANIMAL STUDIES

N/A.

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