Persistent intestinal dysbiosis after SARS-CoV-2 infection in Brazilian patients

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Research Article

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Persistent intestinal dysbiosis after SARS-CoV-2 infection in Brazilian patients

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Abstract

The massive secretion of inflammatory cytokines is associated with the COVID-19 severity and poor prognosis, as well as, in long COVID, the pathophysiology seems to be related to immune deregulation. The patient's immune status can influence the response to SARS-CoV-2, and this immunity is affected by the intestinal microbiota condition (eubiotic or dysbiotic). This study aimed to evaluate the intestinal microbiota of patients infected with SARS-CoV-2 with different clinical manifestations and post-COVID-19 periods, and correlate with the use of antibiotics during the acute disease. According to the beta diversity, we observed significant differences between microbial communities in stool samples from post-COV patients when compared with healthy controls. Additionally, we detected four different clusters when we grouped patients into asymptomatic, mild, moderate, and severe disease. Patients who took antibiotics during the COVID-19 course showed decreased richness of the gut microbiota, even months after the disease resolution. We detected some genera possibly associated with the persistent post-COVID dysbiosis, including increased *Prevotella, Dialister, Haemophillus, Barnesiella, Desulfovibrio, Bilophila, Alistipes, Parabacteroides* and *Bacteroides*, suggesting the impact of the disease in the gut microbiota. Besides that, we found some genera associated with antibiotic-induced dysbiosis in post-COVID-19 patients, including decreased *Akkermansia* and *Bifidobacterium* species. Therefore, we hypothesized that persistent dysbiosis and indiscriminate use of antibiotics during the COVID-19 pandemic may be associated with long COVID syndromes, suggesting the involvement of the gut-lung axis. These data suggest that intestinal microbiota modulation may represent a therapeutic approach for long COVID.

Keywords: COVID-19, gut-lung axis, gut microbiota, intestinal dysbiosis, antibiotics, long COVID

I. Introduction

Coronavirus Disease 2019 (COVID-19), an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), rapidly spreads worldwide and was declared a Global Pandemic on 11 March, 2020 (1,2). According to the World Health Organization (WHO), SARS-CoV-2 has already infected more than 217 million people worldwide, with 4,517,240 deaths (3). In Brazil, there are 20,751,108 confirmed cases and 579,643 deaths (4). SARS-CoV-2 infects humans via droplets or aerosols through speaks, sneezes or coughs, so physical distancing, masks use and ventilated areas reduce the virus transmission (5,6).

COVID-19 comprises a wide spectrum of clinical manifestations, ranging from asymptomatic to critical ill patients characterized by respiratory and/or multi-system organ failure (MSOF) (7). Despite the predominance of respiratory tract involvement, the gastrointestinal tract can also be affected, presenting as nauseas, vomiting, abdominal pain and diarrhea (8). In addition, several patients are experiencing long-term sequelae and symptoms (long COVID) after resolution of acute disease, including myalgic encephalomyelitis/chronic fatigue syndrome (9). The influence of the immune system on both acute and long COVID-19 has been demonstrated in several studies (8,10–12). In acute disease, massive cytokine production, also known as cytokine storm, has been associated with the progression to severe cases and with the development of acute respiratory distress syndrome (ARDS), MSOF and coagulation dysfunction (10,11). Cytokine storm in COVID-19 is characterized by overproduction of TNF-alpha, IL-1, IL-2, IL-6 and interferons (10,11). In chronic fatigue syndrome, the pathophysiology seems to be related to immune dysregulation, including changes in cytokine profile, immunoglobulin levels and T and B-cell phenotype (12).

The gut microbiota plays an important role on the immune response against viral infections, modulating both innate and adaptive response (13). Growing evidences suggest that factors produced by a healthy gut microbiota is essential in the development and maturation of the immune system, arming the lungs against respiratory viral infections (8,13). Respiratory infections and medications,

including antibiotics, promotes modifications on diversity and composition of the gut microbiota, leading to dysbiosis, which alters the beneficial cross-talk between lung and gut (8,13). This bidirectional relationship, also known as gut-lung axis, has been associated with different outcomes in respiratory tract infections, including influenza and respiratory syncytial virus, emphasizing the importance between both mucosal compartments (8,13).

In COVID-19, older age and comorbidities are known risk factors for predicting severe diseases and both has been associated with dysbiosis, which might explain the development of severe cases on those conditions. To date, there are few studies evaluating the gut-lung axis in SARS-COV-2 infection and only few relationships has already been demonstrated (14). The few available data supports that in SARS-CoV-2 infection, the gut-lung axis and its influence on immune response plays an important role on progression to cytokine storm, MSOF and long-term COVID syndromes (8,12,13). So, we sought to evaluate the intestinal microbiota of Brazilian patients infected with SARS-CoV-2 at different clinical manifestations and post-COVID-19 periods, and correlate with the use of antibiotics during the acute disease.

II. Material and Methods

II.1 Post COVID-19 patients and control subjects

Patients that were diagnosed with COVID-19 (post-COV), with positive test for SARS-CoV-2 RNA, detected by PCR from nasopharyngeal swab, were enrolled for this study from October to December 2020. The Research Ethics Committee from Institute of Biosciences, Humanities and Exact Sciences from Sao Paulo State University approved this study (Process number 4,310,336/2020), and patients signed the informed consent form.

One hundred, forty-nine post-COV patients, 126 females and 23 males, aged 18 to 82 years (mean age ± standard deviation (SD) = 42.5 ± 14.5 years) were enrolled at Hemat Clinical Analysis Laboratory. Post-COV patients were classified in 1) asymptomatic (n =10): Positive SARS-CoV-2

test and no symptoms; 2) Mild (n = 117): symptoms like fever, cough, anosmia, ageusia, diarrhea, without dyspnea; 3) Moderate (n = 10): Clinical evidence of lower respiratory tract disease, non-invasive oxygen support (oxygen saturation < 94%); and Severe (n = 12): invasive oxygen support, admission to the intensive care unit, orotracheal intubation (15,16). Table 1 summarizes the demographic and clinical data from post-COV patients. Table 2 shows the distribution of main symptoms reported by post-COV patients and the use of antibiotics in acute phase of disease.

The most common eight persistent reported symptoms (long COVID) among the mild post-COVID-19 patients include fatigue, anosmia, anxiety, depression, myalgia, alopecia, memory loss, and depression, following by muscle weakness, ageusia, tachycardia, sweating, parosmia, breathlessness, paresthesia, skin rashes, arthralgia, headache, and dizziness. The rarest symptoms include muscle spasms, insomnia, nausea, polydipsia, inappetence, tremors, diarrhea, blurry vision, and changes in bowel function. For moderate and severe patients, the most common post-COVID-19 sequelae were fatigue, sarcopenia, dyspnea, myalgia, cough, paresthesia, post-traumatic stress, memory loss, anosmia, ageusia, alopecia, and edema. The rarest sequelae include plegia, dysphonia, hypothyroidism, hyperinsulinemia, dyslipidemia, systemic arterial hypertension, and some patients still require oxygen support and pulmonary rehabilitation.

Seventy-one healthy controls, 51 females and 20 males, aged 18 to 79 years (mean age ± SD = 46.1 ± 16.6 years), enrolled at the pre-pandemic period were included in this study. Exclusion criteria for control group included use of anti-inflammatories, immunosuppressant drugs, antibiotics, laxatives, probiotics and vaccination in the last 30 days, as well as gastrointestinal surgeries, inflammatory bowel diseases, and chronic diarrheas.

After the informed consent, 8 mL of peripheral blood was collected and stool samples were requested within 3-5 days. Total anti-SARS-CoV-2 antibodies and C-reactive protein (CRP) evaluations in serum samples were performed by electrochemiluminescence and immunoturbidimetric assay, respectively, at Hemat Clinical Analysis Laboratory.

Table 1: Demographic and clinical data from post-COVID-19 patients.

Patients	Gender	Age (years)	BMI (Kg/m^2)	Total SARS-CoV-2 antibodies	CRP (mg/dL)	Days post-COVID mean
Asymptomatic (N=10)	10F/2M	36.5 ± 13.8	26.5 ± 4.7	29.2 ± 49.1	0.42 ± 0.27	72
Mild (N=117)	78F/39M	40.4 ± 14.1	27.9 ± 5.0	68.6 ± 58.8	0.56 ± 0.92	84
Moderate (N=10)	7F/3M	49.6 ± 13.4	35.2 ± 5.3	101.1 ± 57.5	1.56 ± 1.68	81
Severe (N=12)	5F/7M	43.7 ± 15.7	31.4 ± 4.7	87.3 ± 43. 4	3.22 ± 4.35	105

Table 2: Main symptoms and use of antibiotics in post-COVID-19 patients.

Patients	Diarrhea	Fever	Dyspnoea	Anosmia	Ageusia	Antibiotics
Asymptomatic (N=10)	0	0	0	0	0	4
Mild (N=117)	37	54	0	75	59	90
Moderate (N=10)	4	7	10	4	6	9
Severe (N=12)	8	10	12	5	6	10

II.2 DNA extraction from stool samples and 16S sequencing

DNA was obtained from 200 mg from stool samples from post-COV patients and controls by using QIAamp Fast DNA Stool Mini Kit (Qiagen, CA, USA), according to manufacturer instructions. For bacterial 16S sequencing, DNA was quantified by Quantus fluorometer and adjusted to 5 ng/mL using Tris buffer (10 mM, pH 8.5). V3 and V4 regions of the bacterial 16S were amplified by using bacterial DNA, V3/V4 primers and the 2X KAPA HiFi HotStart Ready Mix (Kapa Biosystems, MA, USA). PCR purification was performed using AMPure XP Beads Kit (BD Biosciences, CA, USA). DNA libraries were constructed according to the Illumina protocols and the sequencing was conducted by an Illumina MiSeq platform system. The sequences generated by 16S sequencing were deposited at the NCBI repository (BioProject ID: PRJNA758913).

II.3 Statistical analysis

All statistical analyses were performed using the R software package (v. 4.0.3) (R Core Team, 2021). Microbiome data wrangling was carried out using the tidyverse (v. 1.3.0) and phyloseq (v.

1.34.0) R packages (17,18). Libraries with less than 500 reads were removed. The beta diversity analysis included Principal Coordinate Analysis using Bray-Curtis dissimilarity and taxa proportions as input. PERMANOVA models were computed using the adonis function from the vegan (v. 2.5.7) R package (19). The alpha diversity analysis included richness and Shannon index assessment using non-parametric tests (Kruskal-Wallis or Wilcoxon rank-sum) as appropriate. Differential abundance analysis was performed using the corncob (v. 0.2.0) R package (20). Taxa present in less than 10% of samples were filtered out prior to this analysis. Confidence intervals for the taxa prevalence were computed using the exact method from the binom (v. 1.1.1) R package (Dorai-Raj, 2014). P values were adjusted for the control of false-discovery rate (FDR) at 5% using the Benjamini–Hochberg step-up procedure (21).

III. Results

III.1 Detection of intestinal dysbiosis in patients after SARS-CoV-2 infection

In order to evaluate the gut microbiota composition in patients after SARS-CoV-2 infection, we sequenced the V3/V4 regions from bacterial 16S and determined the alpha and beta diversities by using the annotated operational taxonomic units (OTUs). According to the alpha diversity analysis, we observed significant differences ($P = 0.0251$) in richness and evenness when we evaluated fecal samples from post-COV patients and healthy controls (Figure 1A-B). Likewise, we also detected significant differences ($P = 0.0015$) between microbial communities found in post-COV patients and controls (Figure 1C-D). When we grouped patients into asymptomatic, mild, moderate, and severe disease, we did not observe significant differences ($P = 0.0527$) in richness and evenness of the intestinal microbiota (Figure 2A-B). However, we detected significant differences ($P = 0.0015$) in beta diversity among these clinical manifestation groups, with four different clusters in Principal Coordinate Analysis (PCoA) using Bray-Curtis dissimilarity and taxa proportions (Figure 2C-D).

Alpha and beta diversity analyzes were also performed by allocating groups by age and body mass index (BMI), but no significant differences were detected in any of these analyzes (Supplementary Figures 1A-D and 2A-D). Furthermore, there are no differences (P = 0.1403) between microbial communities in female or male gut microbiota after SARS-CoV-2 infection (Supplementary Figure 3A-B).

III.2 Patients who took antibiotics presented decreased richness of the intestinal microbiota

To assess the impact of antibiotic use on patients who had COVID-19, we divided the groups and performed alpha and beta diversity analyses. Differences in richness (P = 0.0327) and evenness (P = 0.0251) were observed in post-COV patients (no antibiotics) and healthy controls (Figure 3A-B). Besides that, patients who took antibiotics showed decreased richness (P = 0.044) of the gut microbiota, even months after the disease. Differences in beta diversity were not observed (P = 0.0682) between patients who took or not antibiotics and controls (Figure 3C-D).

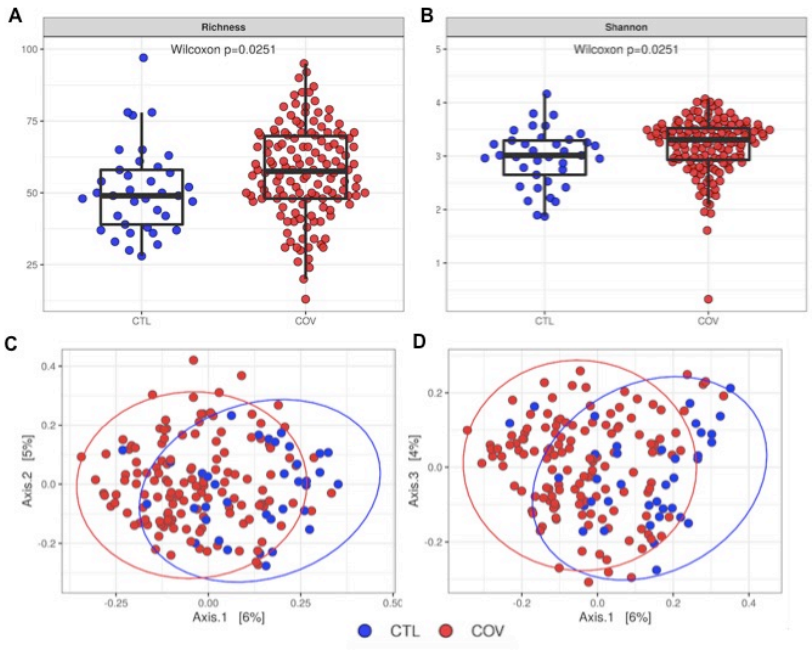


Figure 1: Alpha and beta diversity analysis of the intestinal microbiota from post-COVID-19 patients (COV) and healthy controls (CTL).

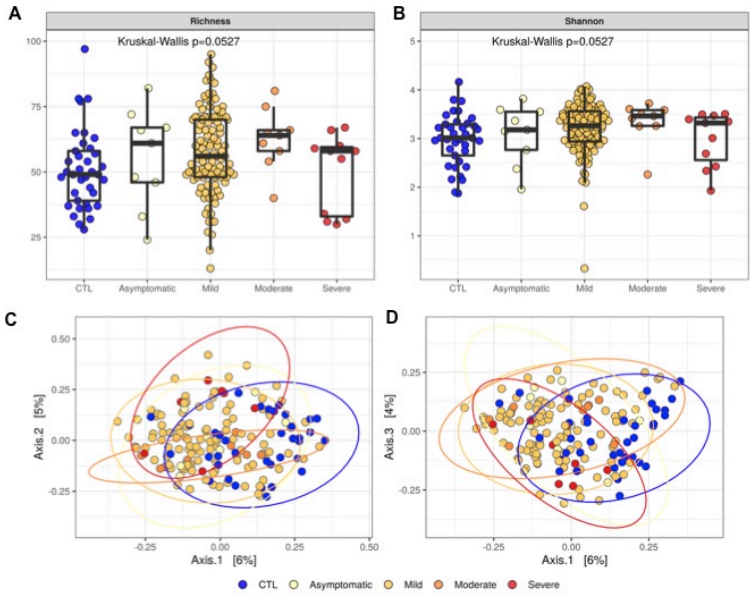
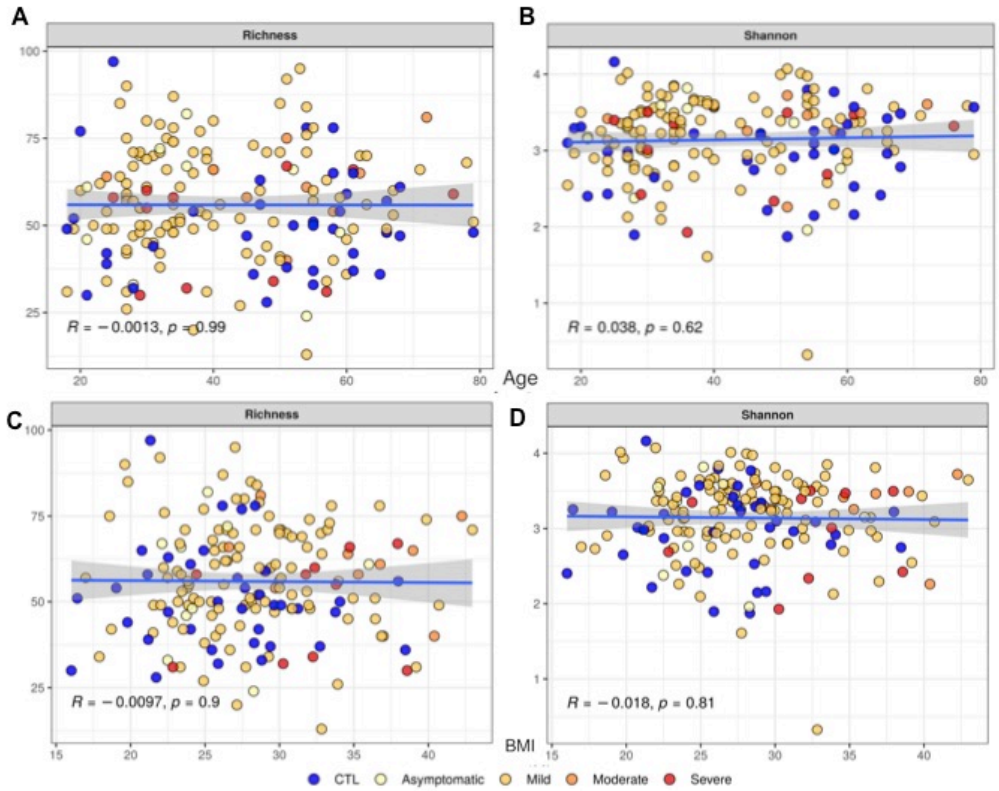
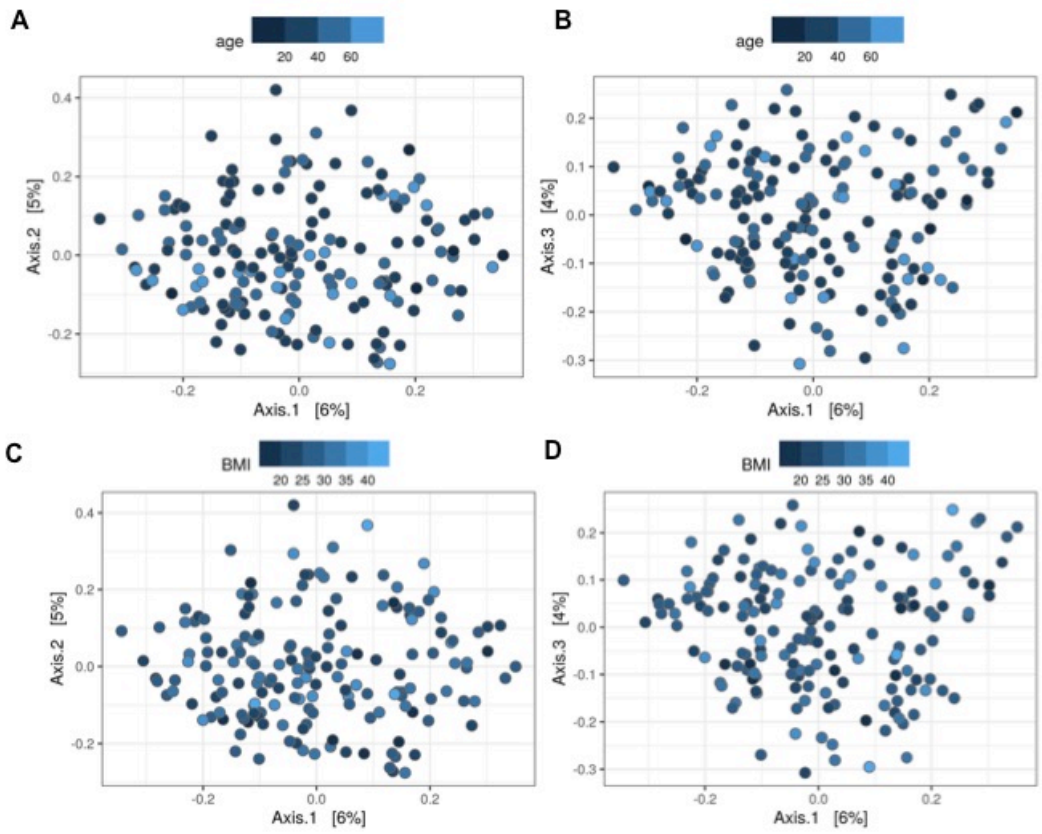


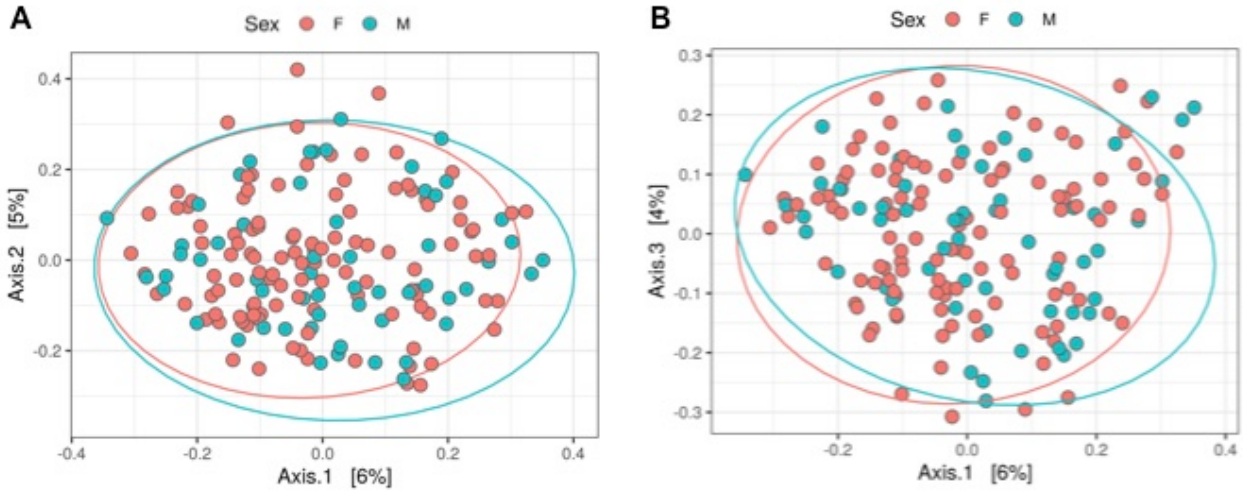
Figure 2: Alpha and beta diversity analysis of the gut microbiota from asymptomatic, mild, moderate, and severe post-COVID-19 patients and healthy controls (CTL).



Supplementary figure 1: Alpha diversity analysis of the gut microbiota from asymptomatic, mild, moderate, and severe post-COVID-19 patients, according to age and body mass index (BMI)



Supplementary figure 2: Beta diversity analysis of the gut microbiota from post-COVID-19 patients, according to age and body mass index (BMI).



Supplementary figure 3: Beta diversity analysis of the gut microbiota from post-COVID-19 patients, according to gender. F: female; M: male.

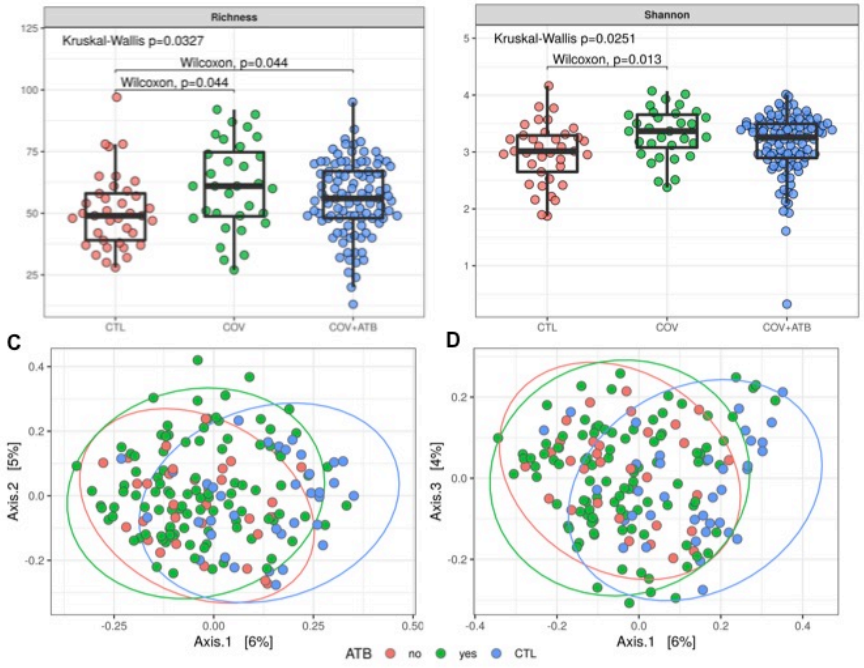


Figure 3: Alpha and beta diversity analysis of the gut microbiota from post-COVID-19 patients that took (COV+ATB) or not antibiotics (COV), and healthy controls (CTL).

III.3 Differential genera relative abundance in overall patients after SARS-CoV-2 infection

For a purpose to investigate the presence of specific genera in our cohort, including all post-COV patients, we evaluated the differential relative abundance in patients' samples, compared to controls. The relative abundance of *Acidaminococcus, Parasutterella, Anaerotignum, Barnesiella, Flavonifractor, Bilophila, Butyricimonas, Dynosmobacter, Alistipes, Bacteroides* and *Parabacteroides* genera were significantly increased in the feces of post-COV patients when compared to controls. On the other hand, *Collinsella, Coprococcus, Akkermansia, Bifidobacterium, Streptococcus*, and *Dorea* genera were significantly reduced in patients' samples, compared to control group (Figure 4). Supplementary Figures 4 and 5 show the relative abundances of these genera in post-COV patients and control individuals.

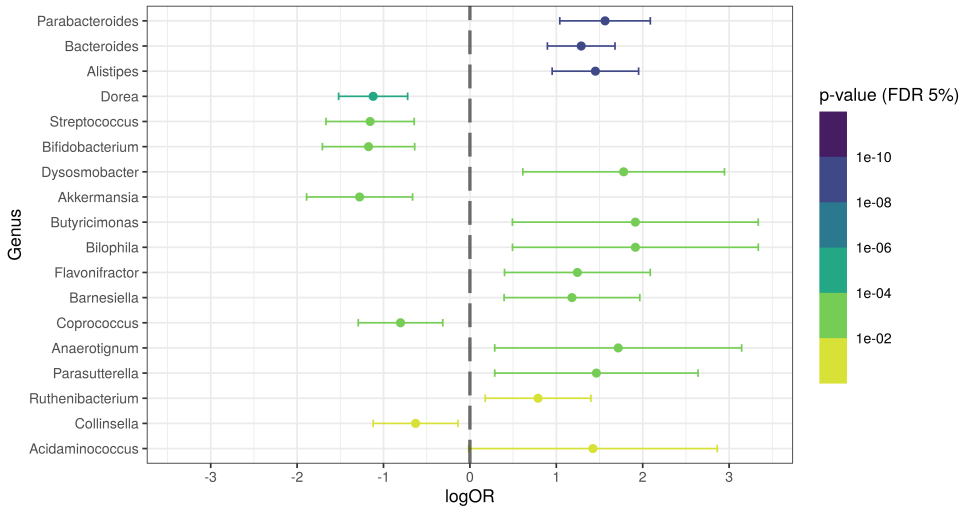
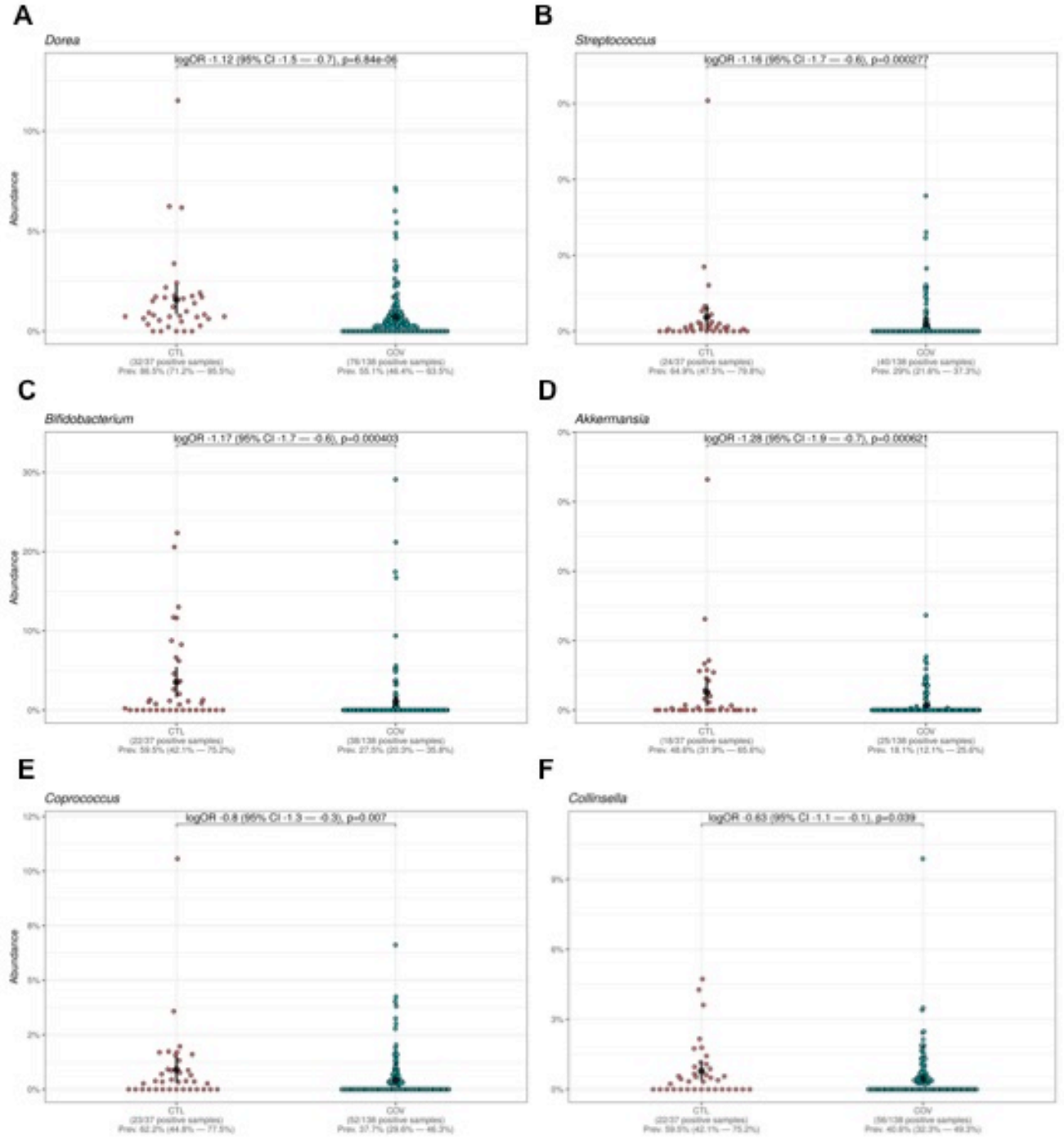


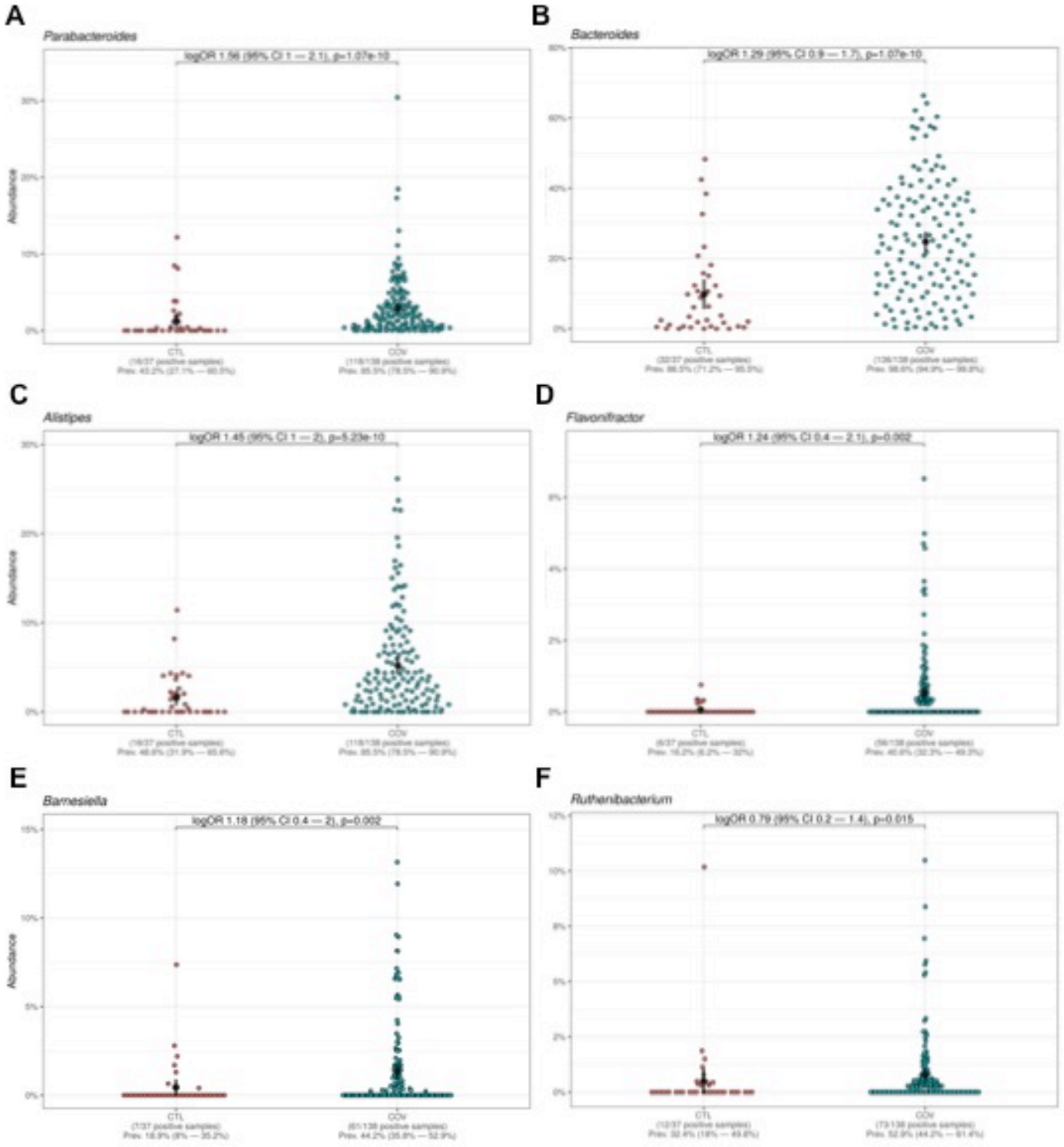
Figure 4: Differential genera relative abundance analysis of the intestinal microbiota in post-COVID-19 patients.

III.4 Specific gut microbiota signature in post-COVID-19 patients

To identify possible signatures of the gut microbiota in post-COVD patients, we compared patients without antibiotic use to the pre-covid control group (CTL *versus* COV), and we observed some genera possibly associated with the persistent post-COVID dysbiosis. The relative abundance of *Prevotella, Dialister, Haemophillus, Barnesiella, Desulfovibrio, Bilophila, Alistipes, Parabacteroides* and *Bacteroides* genera were significantly increased in the feces of the post-COVID patients when compared to the controls. Interestingly, *Streptococcus* genus was significantly decreased in the patients' samples, compared to the control group (Figure 5).



Supplementary figure 4: Relative abundance of *Dorea, Streptococcus, Bifidobacterium, Akkermansia, Coprococcus* and *Collinsella* genera in the gut microbiota from post-COVID-19 patients (COV) and healthy controls (CTL).



Supplementary figure 5: Relative abundance of *Parabacteroides, Bacteroides, Allistipes, Flavonifractor, Barnesiella* and *Ruthenibacterium* genera in the gut microbiota from post-COVID-19 patients (COV), and healthy controls (CTL).

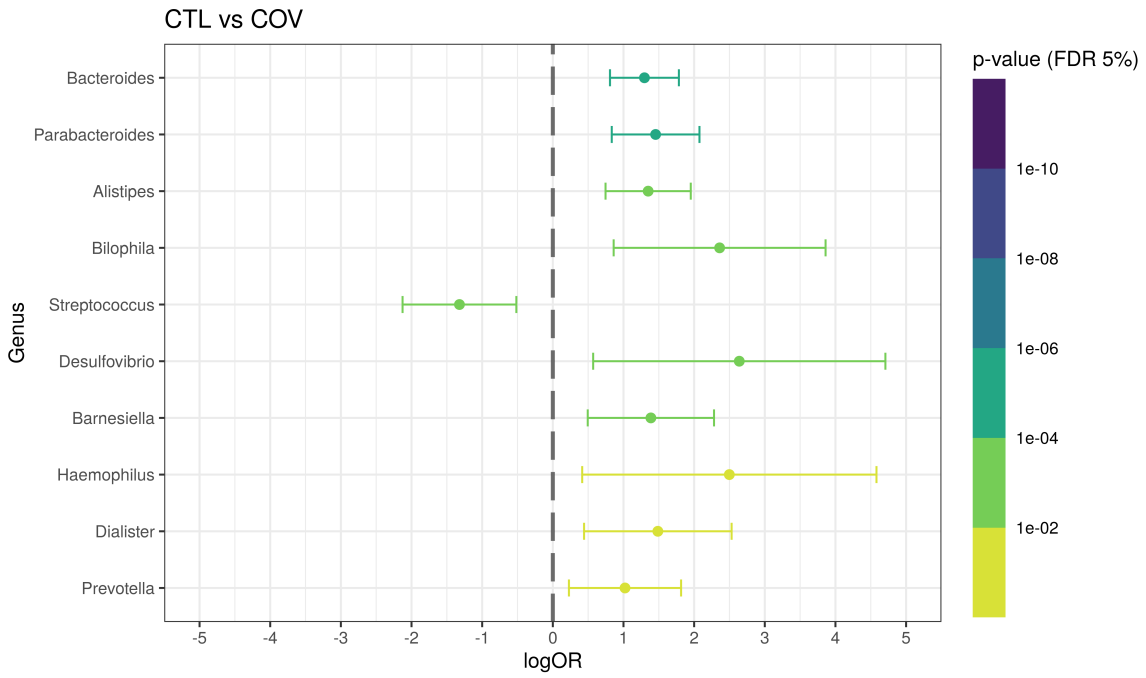


Figure 5: Differential genera relative abundance analysis of the intestinal microbiota in post-COVID-19 patients, without antibiotic use.

III.5 Antibiotic induced decrease in *Akkermansia* and *Bifidobacterium* in post-COVID-19

After, to demonstrate the effect of antibiotic use on the post-COV patients, we evaluated the differential relative abundance comparing patients who took antibiotics during the disease, compared to control group (CTL *versus* COV+ATB). We detected some genera possibly associated with antibiotic-induced dysbiosis. The relative abundance of *Acidaminococcus, Ruthenibacterium, Barnesiella, Parasutterella, Bilophila, Anaerotignum, Flavonifractor, Butyricimonas, Dysosmobacter, Bacteroides, Alistipes,* and *Parabacteroides* genera were significantly increased in the feces of the post-COV+ATB patients when compared to the controls. In addition, *Clostridium*, *Collinsella, Coprococcus, Akkermansia, Streptococcus*, *Bifidobacterium,* and *Dorea* genera were significantly decreased in the post-COV+ATB patients (Figure 6). Supplementary figure 6 shows some genera up regulated after COVID-19 infection and Supplementary figure 7 stand out the main genera down regulated by use of antibiotics by COVID-19 patients.

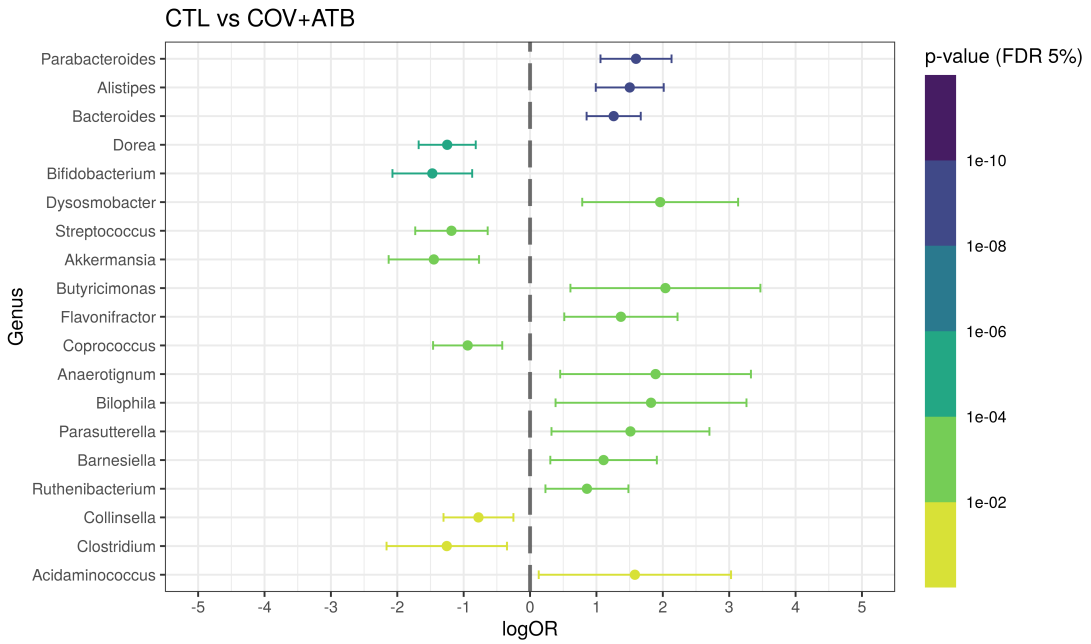
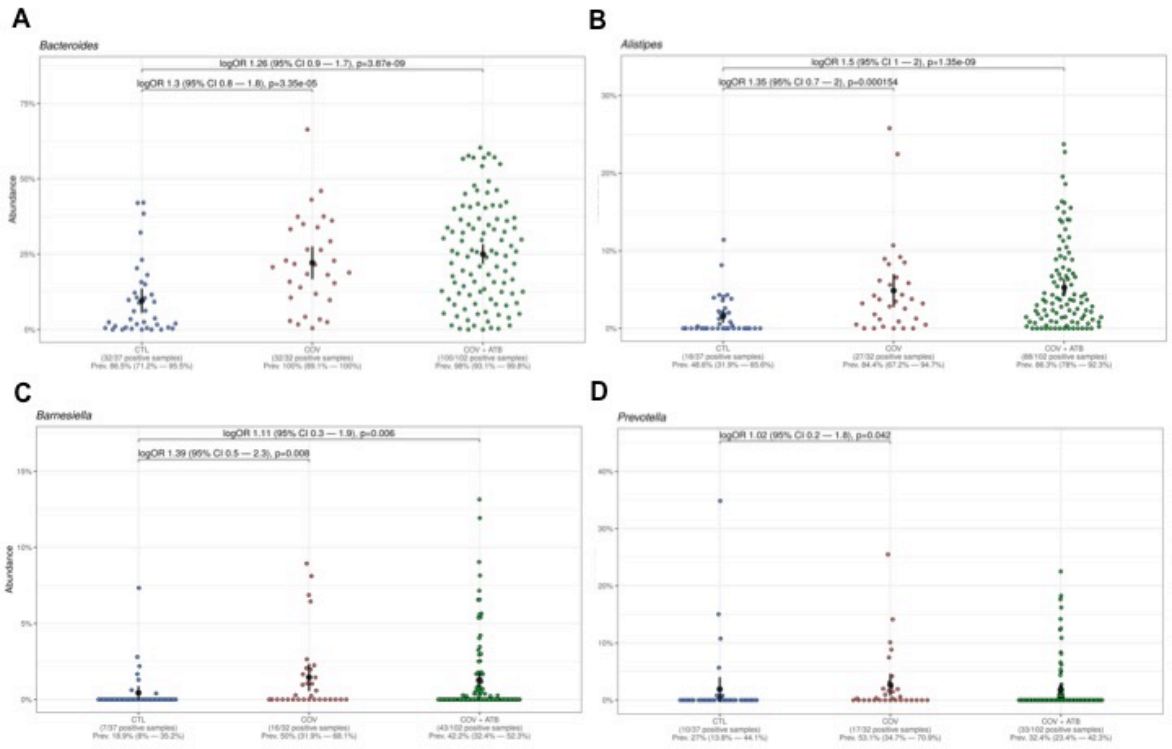
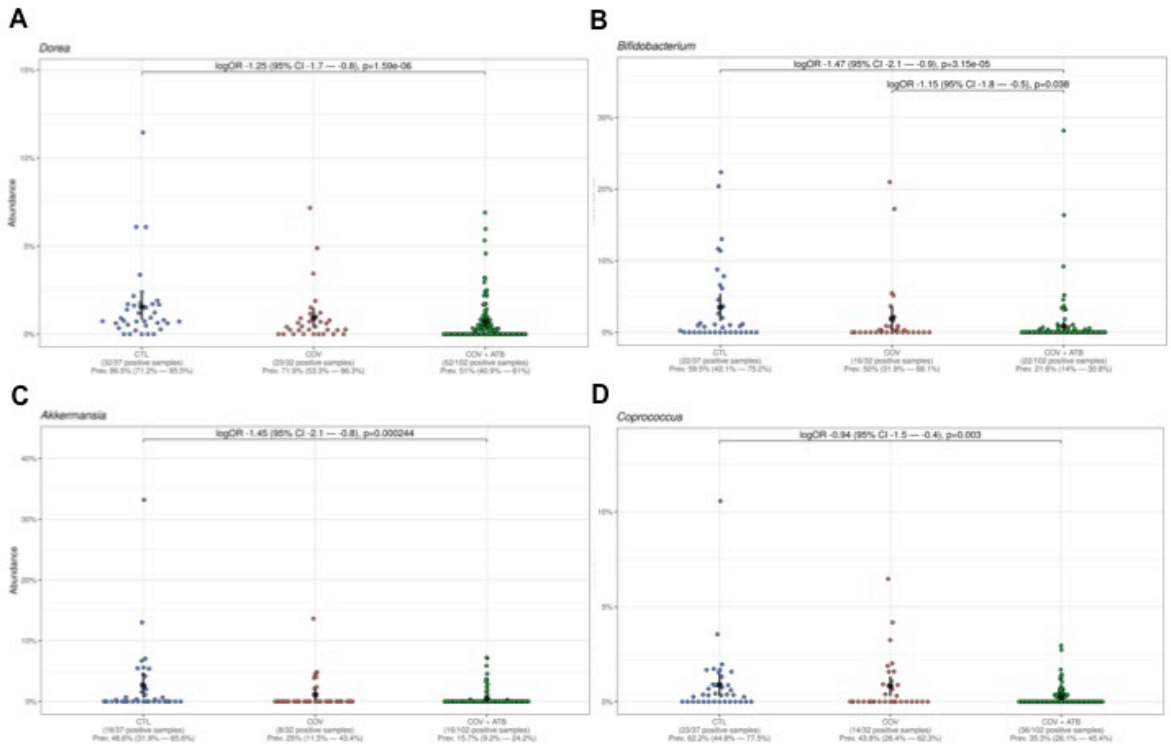


Figure 6: Differential genera relative abundance analysis of the intestinal microbiota in post-COVID-19 patients that took antibiotics.



Supplementary figure 6: Relative abundance of *Bacteroides, Alistipes, Barnesiella* and *Prevotella* genera in the gut microbiota from post-COVID-19 patients that took (COV+ATB) or not antibiotics (COV), and healthy controls (CTL).



Supplementary figure 7: Relative abundance of *Dorea, Bifidobacterium, Akkermansia* and *Coprococcus* genera in the gut microbiota from post-COVID-19 patients that took (COV+ATB) or not antibiotics (COV), and healthy controls (CTL).

IV. Discussion

The gut microbiota is important for several physiological process, including the modulation of both innate and adaptive immune response to maintain a systemic immune homeostasis (22). The role of gut microbiota in several autoimmune conditions have already been demonstrated and for the past few decades, an increasing body of evidence points towards the microbiota as a major player in infectious diseases (8,13,22–24).

There are few studies evaluating the gut-lung axis in SARS-COV-2 infection and only few relationships has already been demonstrated (14). The few available data supports that in SARS-CoV-2 infection, the gut-lung axis and its influence on immune response plays an important role on progression to cytokine storm, MSOF and long-term COVID syndromes (8,12,13). However, most of the studies included just a small sample of patients, which could affect the analysis and outcomes. To our knowledge, our study is the first analysis to evaluate the intestinal microbiota in post-COVID-19

patients. We observed that a specific gut microbiota signature and intestinal dysbiosis in post-COVID-19 patients persisted even after several months of the disease. In addition, antibiotic treatment was associated with decreased richness of the intestinal microbiota and decreased beneficial microbes, such as *Bifidobacterium* and *Akkermansia* species.

In others respiratory viral infections, the influence of gut-lung axis has already been evaluated. In influenza murine models, an intact intestinal microbiota is necessary for adaptive T-cells respond to infection and studies have demonstrated that colonization by *Bifidobacterium* genus is strongly associated with survival, suggesting that this bacteria mediates an anti-influenza effect via several metabolic molecules (13,24). Another study have demonstrated that after exposure to influenza, lungs-derived T cells migrate to intestine and cause intestinal dysbiosis, resulting in an aberrant immune response and gastroenteritis (25). In addition, transfer of fecal microbiota from mice that have survived influenza infection to antibiotic treated mice conferred resistance to influenza (23).

To date, few research studies evaluated the role of the gut-lung axis in COVID-19 and several hypothesis were postulated to explain the impact of intestinal dysbiosis (8,14,25–28). The disease severity and mortality, which is associated to cytokine storm, are higher in patients over 65 years old, particularly those with comorbidities, including diabetes, cardiovascular, metabolic and renal disorders (8,25). Intestinal dysbiosis in these same conditions has been extensively evaluated and decreased abundance of *Bacteroides* species has been reported (8,14,25). Interestingly, *Bacteroides* species have been shown to downregulate angiotensin-converting enzyme 2 (ACE2) expression in murine models (14). In addition, immunological aging is associated with a subclinical inflammatory state characterized by Th1 immune response, while in children, a Th2 immune response that is associated with less pro-inflammatory cytokines, seems to be more common (25). These findings together suggest that individual microbiome may affect the immune response to SARS-COV-2 infection (14). Although our results demonstrated no significant difference in gut microbiota when

patients were allocated by age, it is important to note that the mean age of our sample was 42 and just few cases were older than 65, making it difficult to draw any conclusion.

Our results demonstrated a specific microbiota signature in post-COVID-19 patients when compared to control group, characterized by increased abundance of *Acidaminococcus, Parasutterella, Anaerotignum, Barnesiella, Flavonifractor, Bilophila, Butyricimonas, Dynosmobacter, Alistipes, Bacteroides, Parabacteroides,* and decreased *Collinsella, Coprococcus, Akkermansia, Bifidobacterium, Streptococcus*, and *Dorea* genera. Furthermore, our findings of decreased richness and evenness in post-COVID-19 patients, even after several months, suggest that intestinal dysbiosis might play a role in post-COVID-19 syndromes. Previous reports have already demonstrated the influence of the immune system in post-COVID-19 syndromes, and the crosstalk between gut microbiome and immune regulation has been extensive evaluated (8,12,22). Two previous studies demonstrated similar findings during acute COVID-19 (14,26). In a pilot study of 15 acute COVID-19 patients, persistent alterations in fecal microbiome were demonstrated during hospitalization, even after clearance of SARS-COV-2 infection on nasopharyngeal swab (14). Moreover, Gu and colleagues demonstrated significant decrease in gut microbiota diversity and abundance in H1N1 and COVID-19 patients (26).

The further depletion of gut microbiome on those who used antibiotics in our study corroborate previous reports suggesting that antibiotics is associated with pervasive and long-term effects on the intestinal microbiota (24). In our attempt to understand the impact of antibiotics on intestinal dysbiosis, we compared post-COV+ATB with the control group and detected increased abundance of *Acidaminococcus, Ruthenibacterium, Barnesiella, Parasutterella, Bilophila, Anaerotignum, Flavonifractor, Butyricimonas, Dysosmobacter, Bacteroides, Alistipes,* and *Parabacteroides* genera. In addition, *Clostridium*, *Collinsella, Coprococcus, Akkermansia, Streptococcus*, *Bifidobacterium,* and *Dorea* genera were significantly decreased. These findings are also supported by a study published by Zuo and colleagues, with COVID-19 patients during hospitalization, demonstrating that

in antibiotic-treated patients, there were a further depletion of beneficial bacterial species, including *Faecalibacterium prausnitzii, Lachnospiraceae bacterium 5_1_63FAA, Eubacterium rectale, Ruminococcus obeum*, and *Dorea formicigenerans* (14). Therefore, we hypothesize that persistent dysbiosis and indiscriminate use of antibiotics during the COVID-19 pandemic may be associated with long COVID-19 syndromes.

Although our results demonstrated no significant differences in richness and evenness of the gut microbiota when patients were allocated by disease severity, it is important to note that our samples were collected several weeks after acute disease and included mostly mild cases. Actually, two previous studies have evaluated the gut microbiota during COVID-19 hospitalization and interesting results were found (14,26). First, they demonstrated a relative abundance of opportunistic pathogens, including *Streptococcus, Rothia, Veillonella, Erysipelatoclostridium, Bacteroides nordii* and *Actinomyces* (14,26). Interestingly, *Rothia* was already associated with susceptibility to secondary bacterial lung infection in patients with avian H7N9 virus infection (26). Second, the presence of *C. hathewayi*, which is associated to upregulation of angiotensin-converting enzyme 2 (ACE2) in murine gut, were positively associated with COVID-19 severity (14). Lastly, *Alistipes onderdonkii* and *Faecalibacterium prausnitzii* were top bacterial species to show protective effect, probably due to its effects on maintaining gut immune homeostasis and anti-inflammatory properties, respectively (14).

The main strength of our study is the large sample size, which improves our power to assess gut microbiota relationships. Nevertheless, this study had some limitations. First, intestinal dysbiosis could be affected by several factors not addressed in this present study, including dietary and lifestyle (24). To minimize this limitation, we did not recruit patients who used prebiotics or probiotics and performed analysis allocating patients by BMI. Second, the absence of samples during acute COVID-19 leads to uncertain regarding temporal microbiome changes. Notwithstanding the limitations, our study is the first to demonstrated the persistent intestinal dysbiosis in post-COVID-19 patients and

suggest the role of the gut-lung axis in post-COVID-19 syndromes. These data stand out this possible novel association and suggest that gut microbiota may represent a therapeutic approach for long COVID-19.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

The sequences generated by 16S sequencing were deposited at the NCBI repository (BioProject ID: PRJNA758913), and can be accessed through https://www.ncbi.nlm.nih.gov/bioproject/758913.

Author Contributions

ASFJ: post-COV patients' enrollment, clinical data registration, samples collection, and manuscript writing; TFB, LVVS, AZL, GVVS, ASD: Controls and post-COV patients' enrollment, samples collection, DNA extraction and quantification; GFC, LFO: library construction, 16S sequencing and bioinformatics analysis; EG and ALBP: Financial support, manuscript revision; GLVO: experimental design, data interpretation, manuscript writing and revision. All authors read and approved the final manuscript.

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Figures

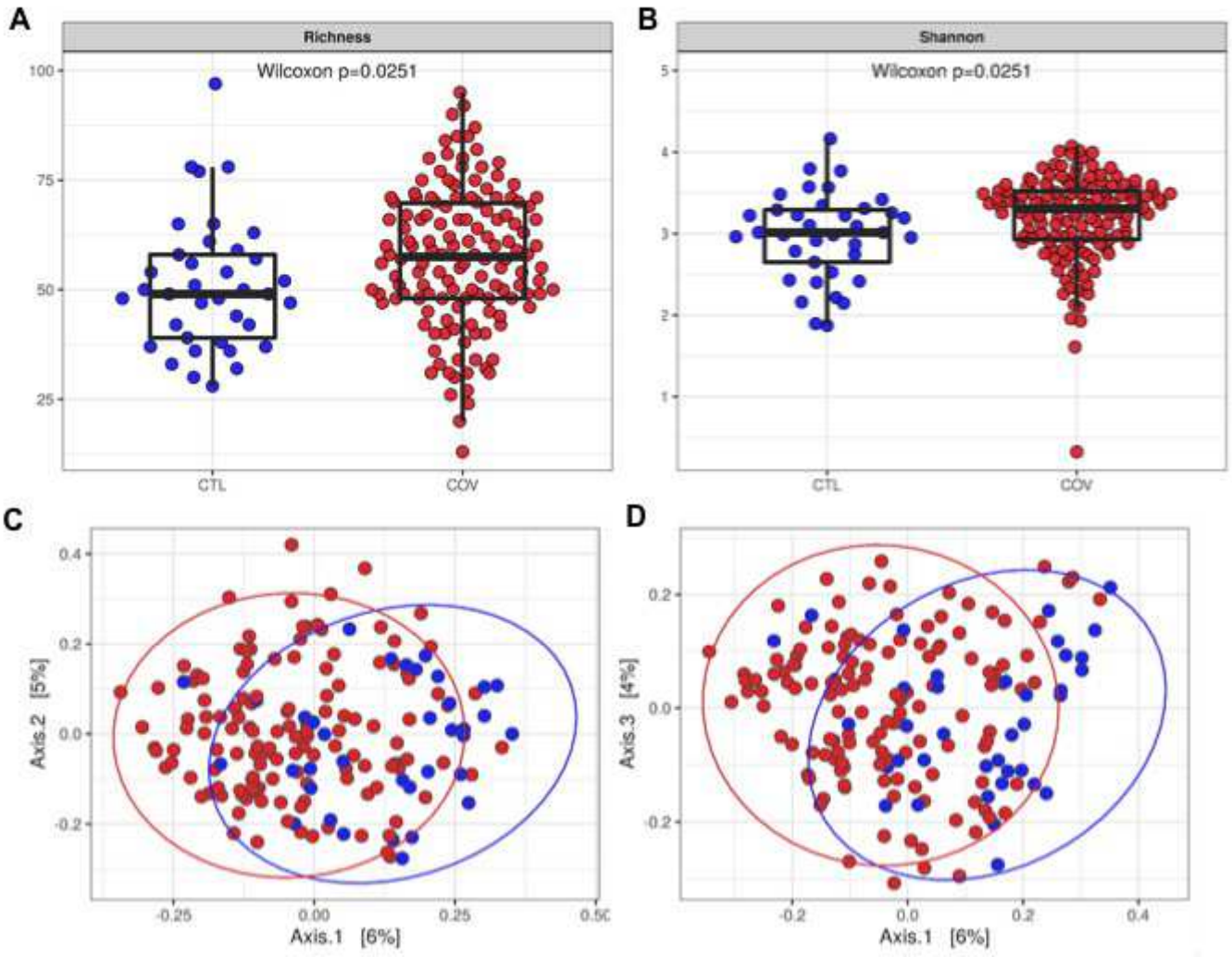

Figure 1

Alpha and beta diversity analysis of the intestinal microbiota from post-COVID-19 patients (COV) and healthy controls (CTL).

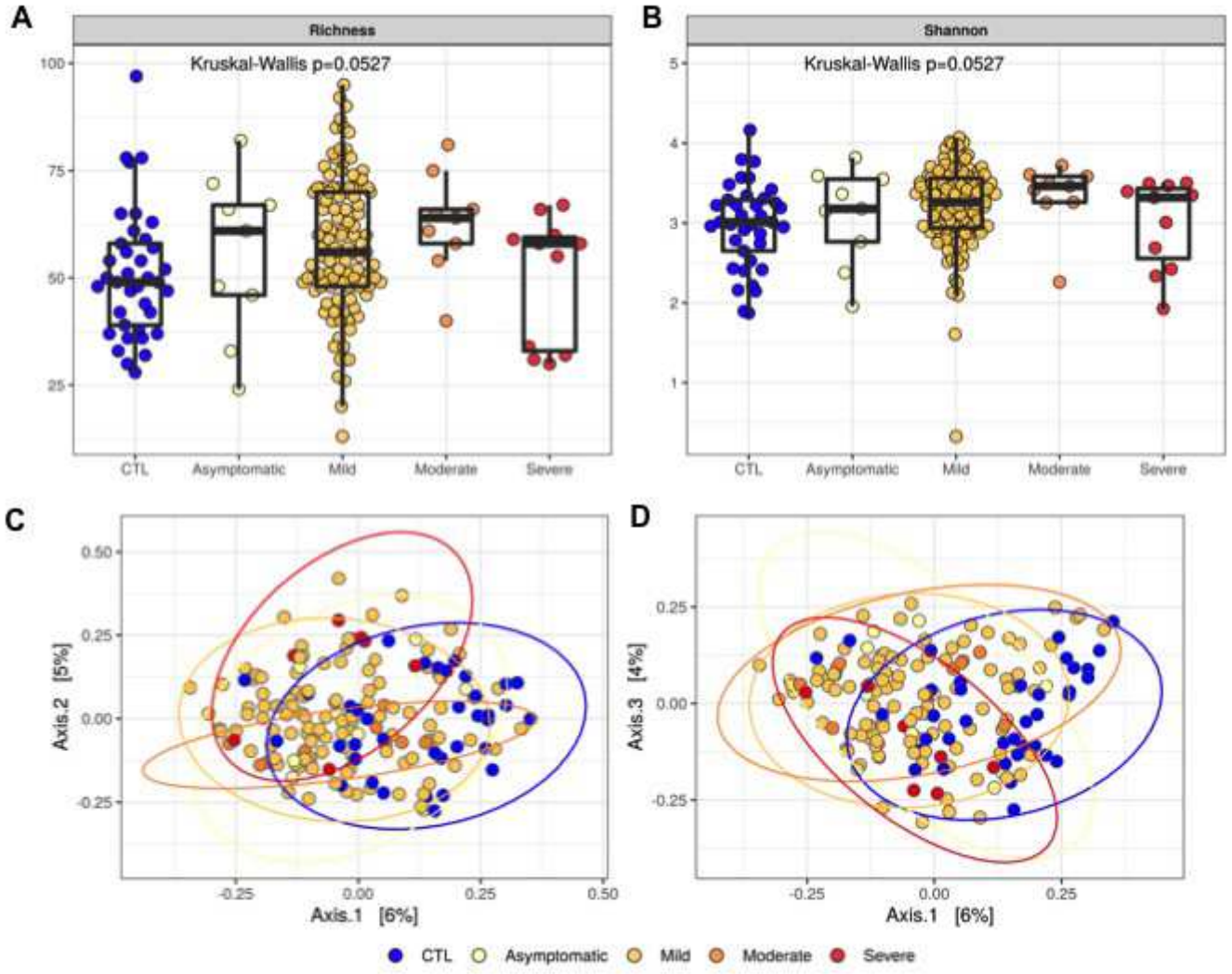

Figure 2

Alpha and beta diversity analysis of the gut microbiota from asymptomatic, mild, moderate, and severe post COVID-19 patients and healthy controls (CTL).

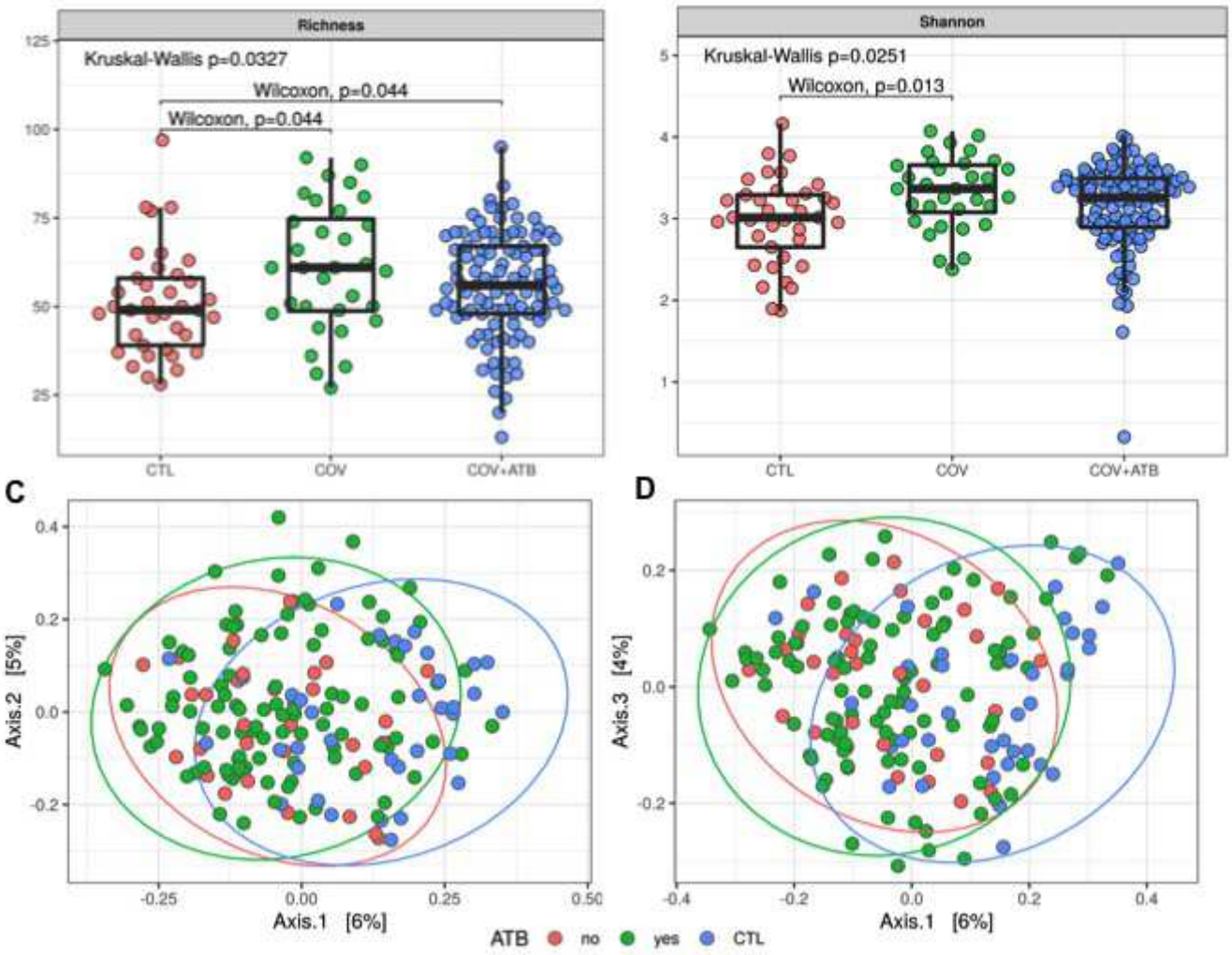

Figure 3

Alpha and beta diversity analysis of the gut microbiota from post-COVID-19 patients that took (COV+ATB) or not antibiotics (COV), and healthy controls (CTL).

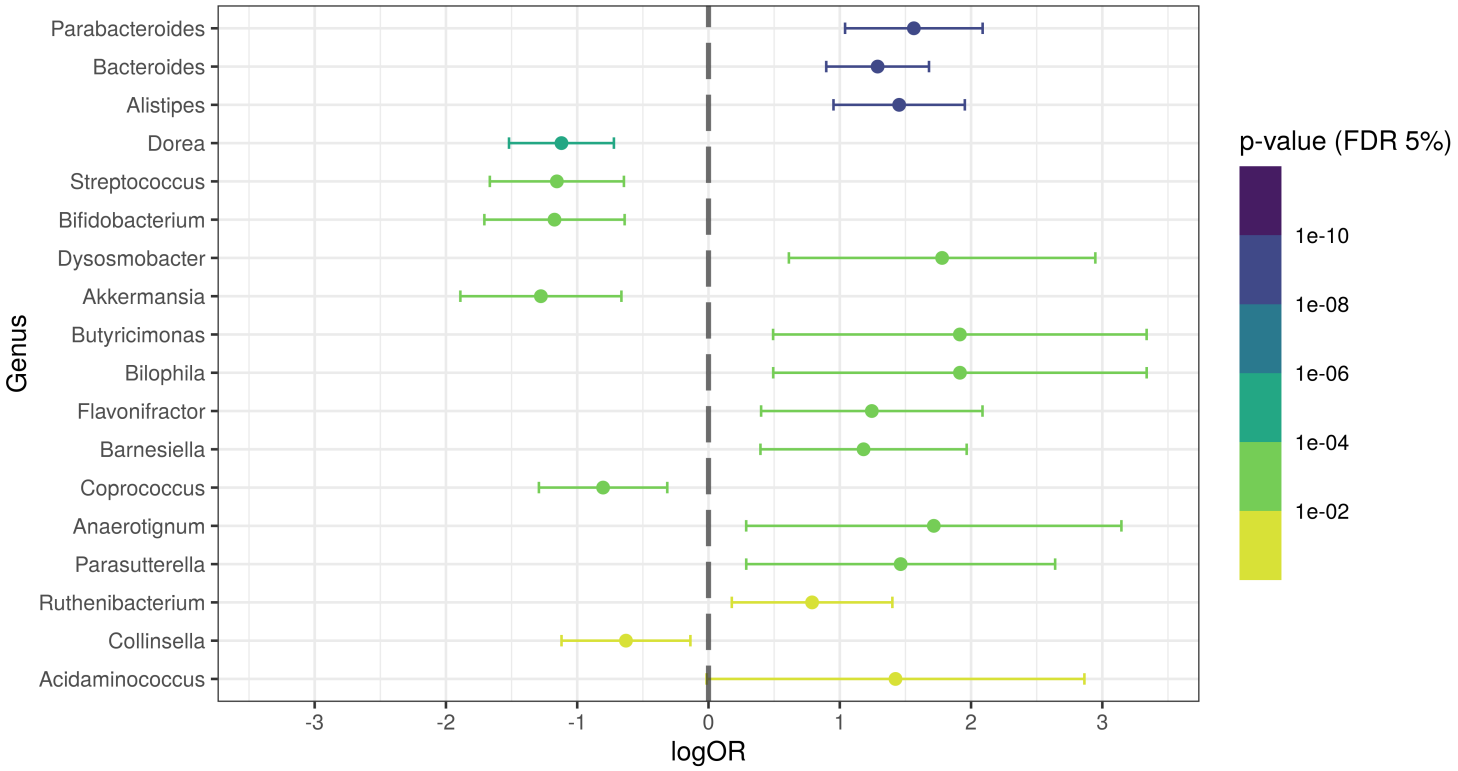

Figure 4

Differential genera relative abundance analysis of the intestinal microbiota in post-COVID-19 patients.

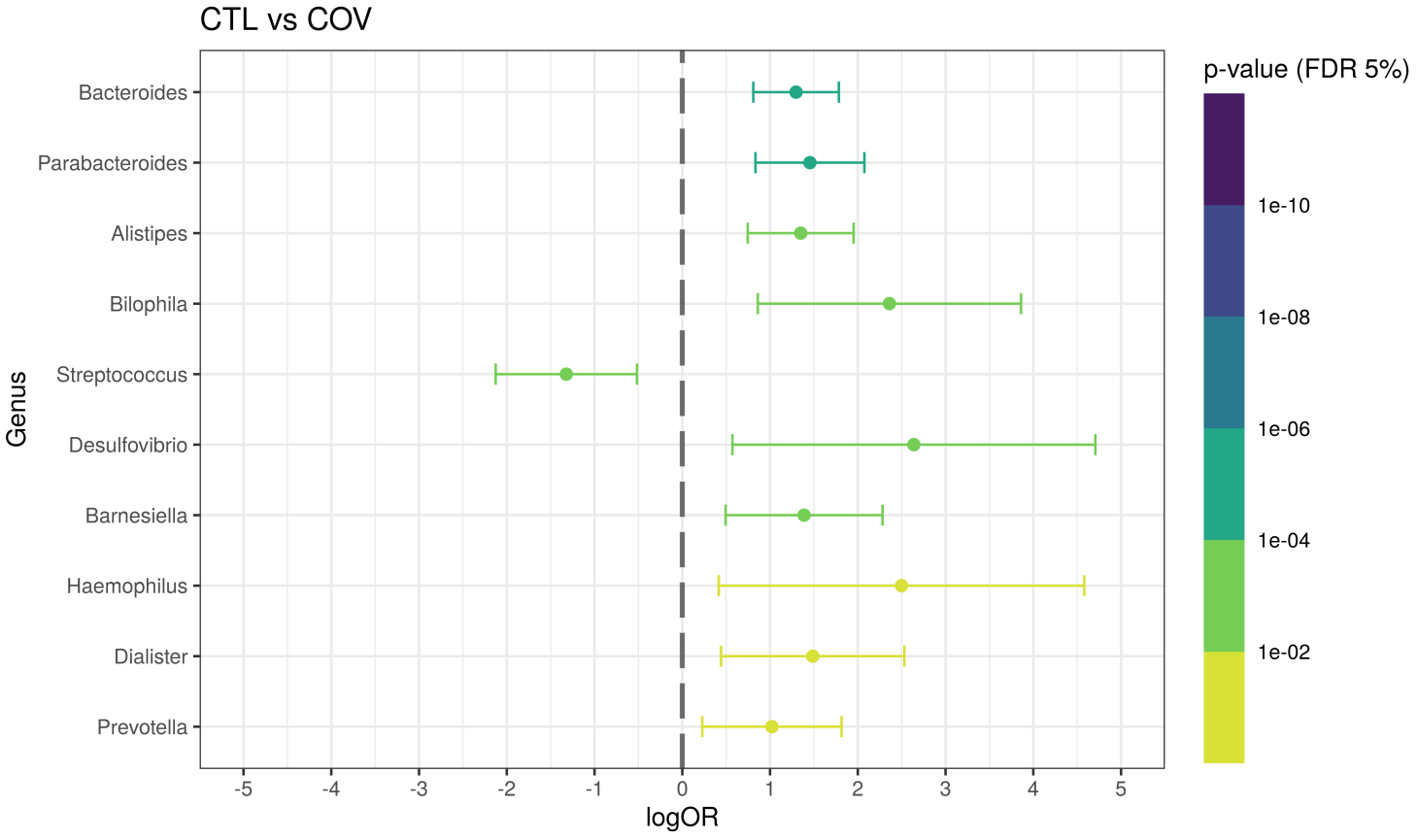

Figure 5

Differential genera relative abundance analysis of the intestinal microbiota in post-COVID-19 patients, without antibiotic use.

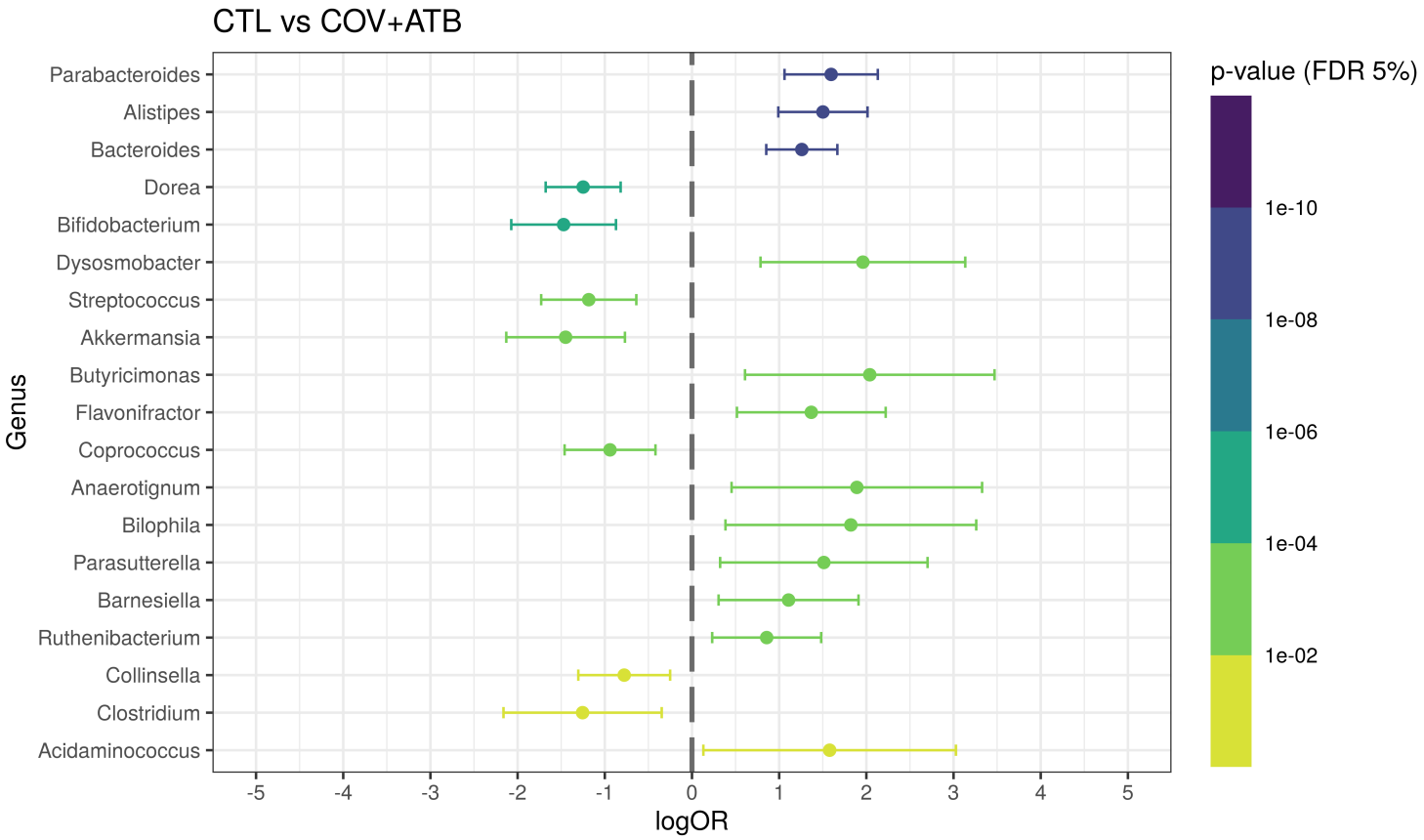

Figure 6

Differential genera relative abundance analysis of the intestinal microbiota in post-COVID-19 patients that took antibiotics.

Supplementary Files

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