Linking the gut microbiota to persistent symptoms in survivors of COVID-19 after discharge[§]

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Several follow-up studies have found that COVID-19 (coronavirus disease 2019) patients had persistent symptoms after discharge. Gut microbiota play an important role in human health and immune responses. Therefore, this study investigated the gut microbiota of recovered COVID-19 patients and the correlations between gut microbiota and persistent symptoms after discharge. Stool samples were collected from 15 recovered healthcare workers (HCWs) with COVID-19 at three months after discharge, in addition, stool samples were collected from 14 healthy controls (HCs) to perform 16S rRNA gene sequencing between May and July 2020. Compared with HCs, recovered HCWs had reduced bacterial diversity at three months after discharge, with a significantly higher relative abundance of opportunistic pathogens, and a significantly lower relative abundance of beneficial bacteria. In addition, *Escherichia unclassified* was positively correlated with persistent symptoms at three months after discharge, including fatigue (r = 0.567, p = 0.028), chest tightness after activity (r = 0.687, p = 0.005), and myalgia (r = 0.523, p = 0.045). *Intestinibacter bartlettii* was positively correlated with anorexia (r = 0.629, p = 0.012) and fatigue (r = 0.545, p = 0.036). However, *Faecalibacterium prausnitzii* was negatively correlated with chest tightness after activity (r = -0.591, p = 0.02), and *Intestinimonas butyriciproducens* was negatively correlated with cough (r = -0.635, p = 0.011). In conclusion, the gut microbiota of recovered HCWs with COVID-19 at three months after discharge was different from that of HCs, and altered gut microbiota was correlated with persistent symptoms after discharge, highlighting that gut microbiota may play an important role in the recovery of patients with COVID-19.

Keywords: COVID-19, recovered healthcare workers, gut microbiota, symptoms after discharge

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Introduction

COVID-19 (coronavirus disease 2019) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has rapidly spread worldwide (Chan *et al.*, 2020). Our previous studies reported data on the incubation period, clinical manifestations, virus shedding time, and complete genomic sequences of SARS-CoV-2 from healthcare workers (HCWs) with COVID-19 in the acute phase (Wang *et al.*, 2020a, 2020b). Subsequently, we found that some of these healthcare workers (HCWs) had persistent symptoms, abnormal lung function, and abnormal lung images at three months after discharge (Liang *et al.*, 2020); however, the mechanism of persistent abnormality in recovered HCWs is unclear.

The gut microbiota has a profound impact on human health and disease (Ichinohe *et al.*, 2011; Belkaid and Hand, 2014; Schirmer *et al.*, 2016), and several recent studies have suggested that the gut microbiota is altered in acute COVID-19 patients (Gu *et al.*, 2020; Zuo *et al.*, 2020a, 2020b, 2021; Yeoh *et al.*, 2021) and is associated with fecal levels of SARS-CoV-2 (Zuo *et al.*, 2020b), disease severity (Zuo *et al.*, 2020b; Yeoh *et al.*, 2021), several cytokines, and inflammatory markers (Yeoh *et al.*, 2021). However, the long-term gut microbiota of recovered patients with COVID-19 and its influence on recovery are not clear. This study compared the fecal microbiota of recovered HCWs at three months after discharge with healthy controls (HCs) to identify correlations between gut microbiota and persistent symptoms after discharge.

Materials and Methods

Study design and enrolled participants

A cohort of 29 subjects was recruited, including 15 recovered HCWs from the above 76 recovered patients (Liang *et al.*, 2020) with COVID-19 who were discharged from the Wuhan Union Hospital and 14 HCs between May and July 2020. All recovered HCWs met uniform discharge criteria according to the Chinese clinical guidance for COVID-19 pneumonia diagnosis and treatment issued by the National Health Commission (China National Health Commission). All recovered HCWs who received antibiotics, probiotics, or both within 3 months before enrollment were excluded. RNA in the stool specimens of recovered HCWs in the acute and recovery phases were extracted and tested for SARS-CoV-2 by real-time RT-PCR using a previously described protocol (Huang *et al.*, 2020). HCs were recruited from healthy HCWs without COVID-19 at the Wuhan Union Hospital. All HCs were free of malignancy or gastrointestinal disease, without

any history of antibiotics or chemotherapeutic drugs for at least 3 months before sample collection. The study was approved by the Ethics Committee of the Wuhan Union Hospital (2020-0149-02). All participants provided written informed consent prior to participation.

Sample collection

Twenty-nine stool samples from 15 recovered HCWs with COVID-19 and 14 HCs were collected (one stool sample per patient). Fresh stools from recovered HCWs with COVID-19 were collected during following-up, and fresh stools from HCs were collected during the recruitment process. Stool samples were processed in the laboratory within 30 min after collection and stored at -80°C until analysis.

DNA extraction and 16S rRNA gene sequencing

The microbiome DNA was extracted using the MagPure Stool DNA KF kit B (Magen) following the manufacturer's instructions. DNA was quantified with a Qubit Fluorometer using the Qubit® dsDNA BR Assay kit (Invitrogen), and the quality was checked by running an aliquot on 1% agarose gel. Variable regions V3–V4 of the bacterial 16S rRNA gene were amplified using degenerate PCR primers 341F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Forward and reverse primers were tagged with Illumina adapter, pad, and linker sequences. PCR enrichment was performed in a 50 μl reaction containing 30 ng template, fusion PCR primer, and PCR master mix. PCR cycling conditions were as follows: 94°C for 3 min; 30 cycles of 94°C for 30 sec, 56°C for 45 sec, 72°C for 45 sec, and a final extension for 10 min at 72°C for 10 min. The PCR products were purified using AmpureXP beads and eluted in elution buffer. Libraries were qualified using the Agilent 2100 Bioanalyzer (Agilent). The validated libraries were used for sequencing on the Illumina MiSeq service (BGI) following the standard pipelines of Illumina, and two 300 bp paired-end reads were generated.

Bioinformatic analysis of 16S rRNA sequencing

To obtain fragments of the target region, we used cutadapt v2.6 to cut the primer and adaptor contamination for reads that could match the primers. We set a window length of 30 bp, if the average quality value of the window was less than 20, the end sequence of the read was cut from the beginning of the window, and removed the reads whose final read length was less than 75% of the original read length. To obtain the final clean data, we also removed reads containing "N" and low-complexity reads (10 consecutive ATCGs). FLASH (Fast Length Adjustment of Short reads, v1.2.11) was used for sequence splicing. The paired reads obtained by paired-end sequencing were assembled into a sequence by using the overlap relationship to obtain the tags of the hypervariable region. The splicing conditions were as follows: (1) the minimum matching length was 15 bp, and (2) the allowable mismatch rate in the overlapping area was 0.1. The DADA2 (divisive amplicon denoising algorithm) method in QIIME2 was used to denoise and obtain Amplicon Sequence Variants (ASVs). The ASV is a 100% similar sequence. Thereafter, ASV representative sequences were taxonomically classified using the Ribosomal Database Project (RDP) Classifier v.2.2, with a minimum confidence threshold of 0.6. Alpha diversity and beta diversity were estimated using MOTHUR (v1.31.2) and QIIME (v1.8.0). Significantly different species were determined using R (v3.4.1) based on the Wilcoxon test.

Statistical analysis

Median values with interquartile range (IQR) or mean ± standard deviation (SD) were used to describe continuous variables, and absolute or relative frequencies were used to de-

Table 1. Demographic of recovered healthcare workers (HCWs) with COVID-19 and healthy controls (HCs)

Demographic	Recovered HCWs (n = 15)	HCs (n = 14)	*p*-value
Age, years	29.0 (28.0–41.0)	37.5 (31.0–48.5)	0.155
Gender			0.427
Male	3 (20.0)	5 (35.7)	
Female	12 (80.0)	9 (64.3)	
BMI, kg/m^2	22.7 (20.9–24.8)	24.1 (21.0–26.3)	0.432
Comorbidities			
Hypertension	2 (13.3)	0 (0.0)	0.483

BMI, body mass index.
Data are n (%) or median (IQR). P values were calculated by Fisher's exact test or Mann-Whitney U test. *p*-value < 0.05 is considered to be statistically significant.

Table 2. Symptoms, lung function, and lung images of 15 recovered HCWs with COVID-19 at three months after discharge

Characteristics	Recovered HCWs (n = 15)
Symptoms	
Any one of the following symptoms	12 (80.0)
Cough	8 (53.3)
Sputum	3 (20.0)
Chest tightness after activity	5 (33.3)
Anorexia	3 (20.0)
Diarrhea	3 (20.0)
Fatigue	6 (40.0)
Myalgia	4 (26.7)
Lung function	
FVC (pred %)	109.8 ± 14.8
FEV1 (pred %)	101.1 ± 11.1
FEV1/FVC (%)	79.3 ± 5.9
TLC (pred %)	102.0 ± 10.0
RV (pred %)	108.4 ± 20.9
FRC (pred %)	105.1 ± 15.0
VC (pred %)	107.2 ± 13.8
DLCO (pred %)	92.3 ± 5.5
DLCO/VA (pred %)	93.7 ± 9.0
Chest CT	
Normal	13 (86.7)
Subpleural line	1 (6.7)
Interlobular septal thickening	2 (13.3)
GGO	1 (6.7)

FVC, forced vital capacity; FEV1, forced expiratory volume in one second; TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; VC, vital capacity; DLCO, diffusion capacity for carbon; VA, alveolar volume; CT, computed tomography; GGO, ground glass opacity.
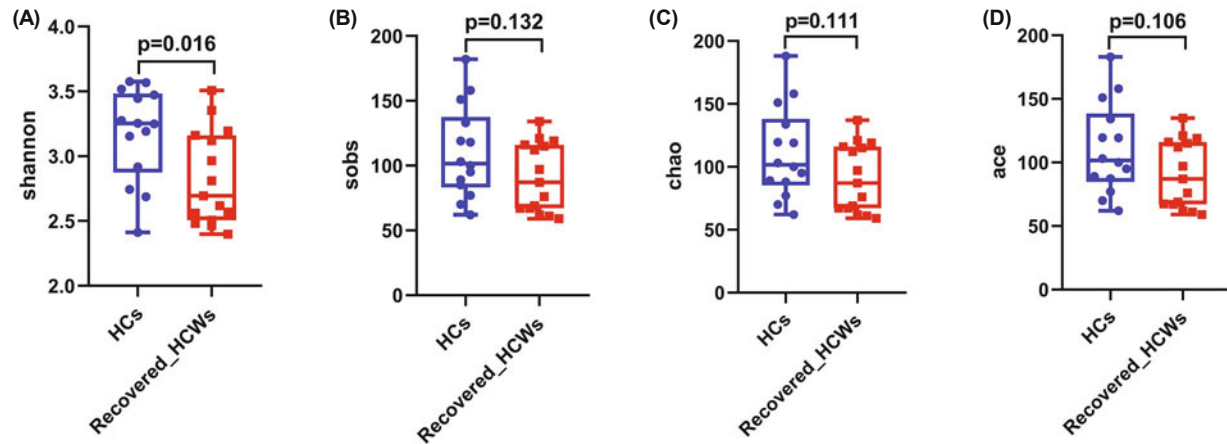Data are n (%), or mean ± SD.

Fig. 1. Comparison of alpha diversity communities between recovered healthcare workers (HCWs) and healthy controls (HCs). (A) shannon, (B) sobs, (C) chao, and (D) ace. *p*-value < 0.05 is considered to be statistically significant.

scribe categorical variables. We used the Mann-Whitney U test or independent t-test to analyze continuous variables and the χ2 test or Fisher's exact test for the analysis of categorical variables. For correlation analysis, Spearman's rank test was performed. Statistical analysis was performed using SPSS version 25.0 (IBM). A two-sided *p*-value of < 0.05 was considered statistically significant.

Results

Demographics and characteristics of the participants

The demographic data of 15 recovered HCWs and 14 HCs are summarized in Table 1. In addition, symptoms, lung function, and lung images of recovered HCWs at three months after discharge are summarized in Table 2 (Supplementary data Tables S1 and 2). The median age of recovered HCWs was 29.0 (28.0–41.0) years, with three (20%) men and 12 (80%) women. In comparison, the HCs comprised nine women and five men with a median age of 37.5 (31.0–48.5) years. Two recovered HCWs had hypertension, and there were no comorbidities in the HCs. There was no difference between the two groups for age, sex, body mass index (BMI), and comorbidity. At least one symptom was reported by 80% of the recovered HCWs (12 of 15) at three months after discharge. The most common symptoms after discharge were cough (53.3%, 8 of 15) and fatigue (40%, 6 of 15), followed by chest tightness after activity (33.3%, 5 of 15) and myalgia (26.7%, 4 of 15). Although the mean values of all pulmonary function measurements were within normal limits in recovered HCWs (Table 2), recovered HCW 1 with abnormalities in total lung capacity (TLC), residual volume (RV), functional residual capacity (FRC), and recovered HCW 15 with abnormalities in forced expiratory volume in one second (FEV1)/forced vital capacity (FVC) were detected. Supplementary data Table S1 shows the pulmonary function data of the 15 recovered HCWs. Additionally, persistent lung computed tomography (CT) injuries at three months after discharge were seen in 2 recovered HCWs.

Taxonomic differences of gut microbiota between recovered HCWs and HCs

In total, 29 stool samples (15 stool samples from recovered HCWs and 14 stool samples from HCs) were sequenced, generating an average of 134,410 clean reads per sample (Supplementary data Table S3). Alpha diversity measured by four different methods were all lower in the recovered HCWs than in HCs, only the difference in shannon index reached statistical significance by Wilcoxon rank sum test (shannon, p = 0.016; sobs, p = 0.132; chao, p = 0.111; ace, p = 0.106) (Fig. 1). Principal coordinates analysis of Bray-Curtis distances indicated difference in the fecal microbiota between recovered HCWs and HCs (P = 0.009) (Fig. 2). To investigate changes in the microbiota of recovered HCWs compared with HCs, we subsequently assessed relative abundance in the two groups at the phylum, family, genus, and species levels. At the phylum level, Firmicutes (mean 47.78% *vs.* 62.34%), Bacteroidetes (mean 38.27% *vs.* 30.65%), and Proteobacteria (mean 9.48%
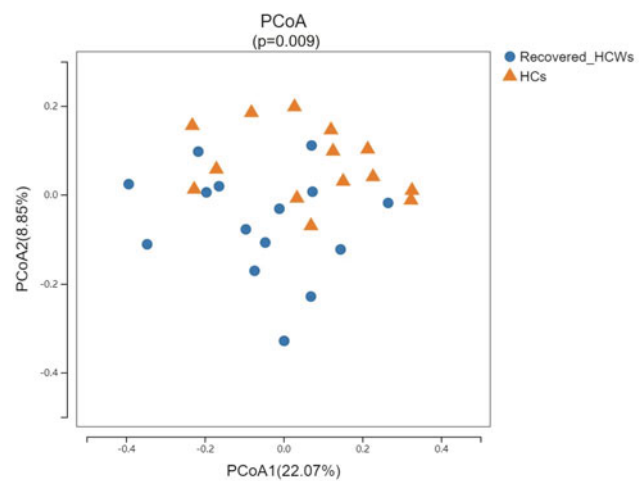

Fig. 2. The beta diversity results were assessed by principal coordinates analysis (PCoA) of Bray-Curtis distances between recovered HCWs and HCs.
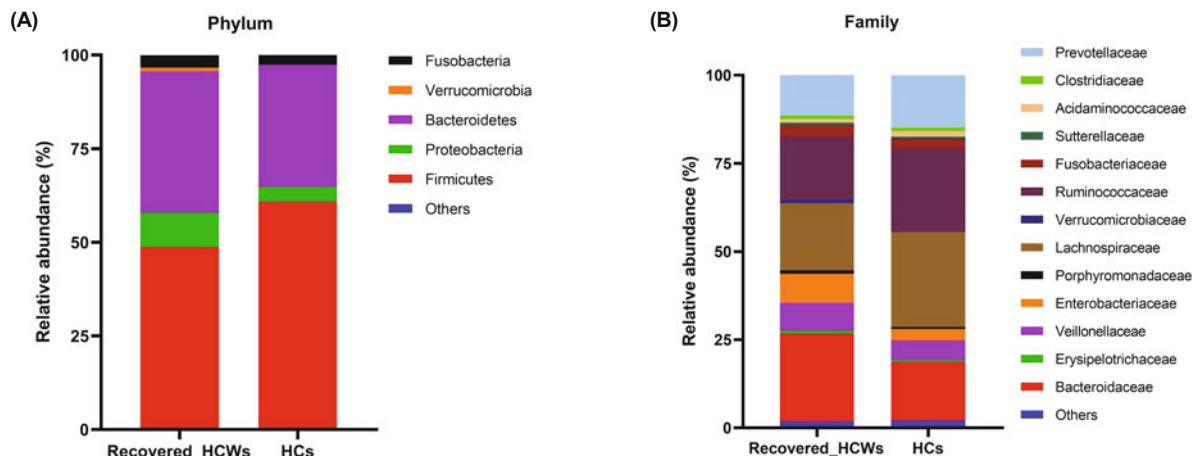
Fig. 3. Taxonomic distribution between recovered HCWs and HCs described by mean relative abundance. (A) Taxonomic distribution at the phylum level, (B) Taxonomic distribution at the family level.

vs. 4.13%) were the most dominant phyla in both recovered HCWs and HCs (Fig. 3A). The relative abundance of Actinobacteria was significantly increased in recovered HCWs with COVID-19 compared with HCs (mean 0.31% *vs.* 0.08%, p = 0.035, Mann-Whitney test) (Fig. 4A). At the family level, Lachnospiraceae and Desulfovibrionaceae were depleted in recovered HCWs (Figs. 3B and 4B). At the genus level, *Faecalibacterium, Roseburia, Fusicatenibacter, Ruminococcus,*

Clostridium XVIII, Dorea, Butyricicoccus, Romboutsia, Intestinimonas, and *Bilophila* were depleted, and *Escherichia, Flavonifractor,* and *Intestinibacter* were enriched in recovered HCWs compared to HCs (Fig. 4C). At the species level, there were 20 bacterial taxa with a mean relative abundance > 10^{-5}, showing distinct relative abundances between the two groups. Several opportunistic pathogens, including *Escherichia unclassified, Intestinibacter bartlettii, Clostridium aldenense,*
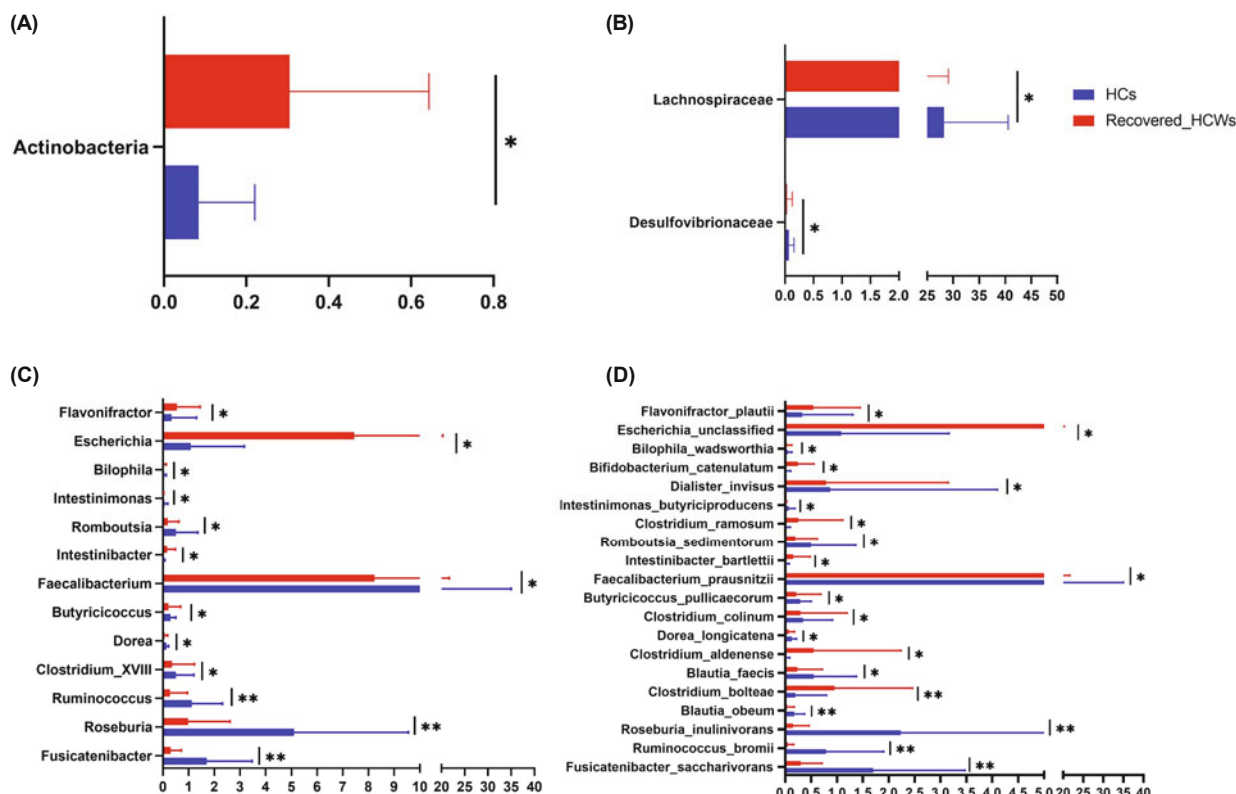

Fig. 4. Taxonomic differences of gut microbiota between recovered HCWs and HCs. Comparison of relative abundance at the bacterial phylum (A), family (B), genus (C), and species (D) between the two groups. *p*-value < 0.05 is considered to be statistically significant, *p < 0.05, **p < 0.01.

inflammation-related pathogens including *Clostridium bolteae*, *Flavonifractor plautii*, and COVID-19 severity-related pathogen (*Clostridium ramosum*) were enriched in recovered HCWs. In addition, recovered HCWs demonstrated a further depletion of multiple bacterial species, including *Faecalibacterium prausnitzii*, *Roseburia inulinivorans*, *Fusicatenibacter saccharivorans*, *Ruminococcus bromii*, *Blautia faecis*, *Butyricicoccus pullicaecorum*, and *Intestinimonas butyriciproducens* (Fig. 4D). These results indicated that recovered HCWs with COVID-19 had significant alterations in fecal microbiota compared with HCs, characterized by enrichment of opportunistic pathogens and depletion of beneficial commensals (Supplementary data Table S4–7).

Relationship between different species and symptoms after discharge

Spearman's rank-based correlation test was performed to identify microbial species associated with persistent symptoms in recovered HCWs with COVID-19 at three months after discharge. We found that *F. prausnitzii* was negatively correlated with chest tightness after activity (r = -0.591, p = 0.02), and *I. butyriciproducens* was negatively correlated with

cough (r = -0.635, p = 0.011). *Escherichia unclassified* was positively correlated with fatigue (r = 0.567, p = 0.028), chest tightness after activity (r = 0.687, p = 0.005), and myalgia (r = 0.523, p = 0.045). *Intestinibacter bartlettii* was positively correlated with anorexia (r = 0.629, p = 0.012) and fatigue (r = 0.545, p = 0.036) (Fig. 5).

Discussion

As far as we known this was the first study to show the gut microbiota of recovered HCWs with COVID-19 at three months after discharge was different from that of HCs, and altered gut microbiota was correlated with persistent symptoms after discharge. These data suggest that depletion of salutary species may persist in most recovered patients despite SARS-CoV-2 virus clearance, suggesting exposure to SARS-CoV-2 infection may be associated with a more long-lasting detrimental effect on the gut microbiota.

Several studies on the gut microbiota of COVID-19 patients have mainly focused on the acute phase of COVID-19. However, the composition of the gut microbiota at long
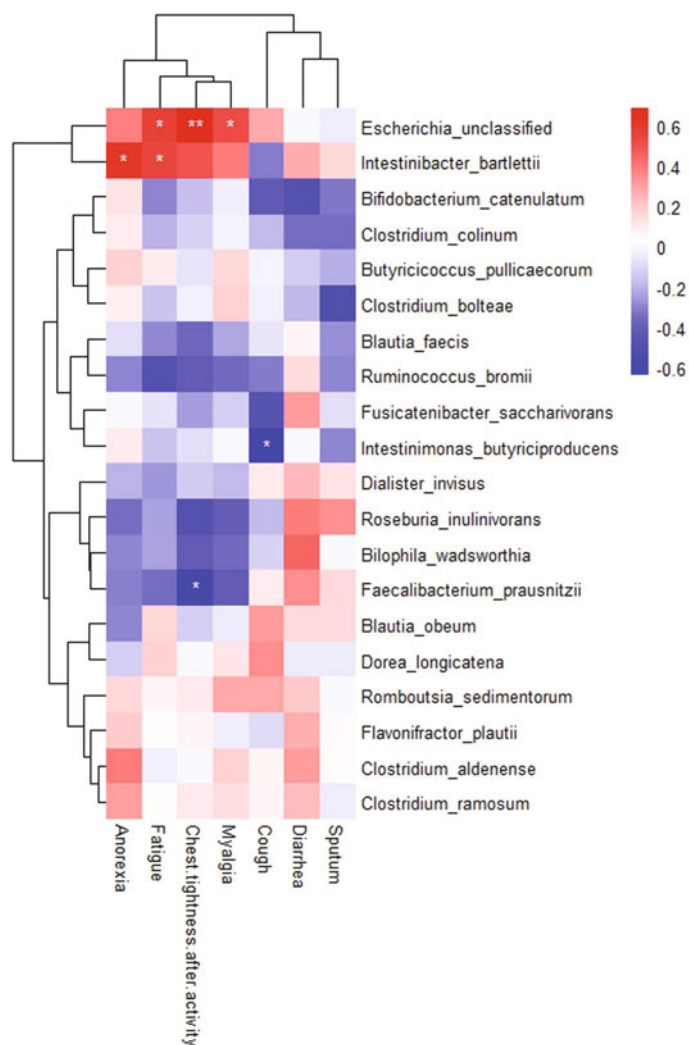

Fig. 5. **Relationship between different species and persistent symptoms of recovered HCWs with COVID-19 at three months after discharge.** Spearman's rank correlation coefficient is indicated using a color gradient: red indicates positive correlation; blue indicates negative correlation. *p*-value < 0.05 is considered to be statistically significant, *p < 0.05, **p < 0.01.

term after discharge is not clear. Gu *et al.* (2020) have found that COVID-19 patients had a significantly higher relative abundance of *Streptococcus*, *Rothia*, *Veillonella*, *Erysipelatoclostridium*, and *Actinomyces* compared with HCs. These results were consistent with our previous study (Zhou *et al.*, 2021) on gut microbiota metagenomics of COVD-19 in the acute phase, in which we also found that the relative abundances of *Streptococcus*, *Rothia*, *Veillonella*, and *Actinomyces* were significantly higher in COVID-19 patients than in HCs, however, this study did not find these taxa differences at three months after discharge between survivors of COVID-19 and HCs, indicating the relative abundance of these taxa gradually reduced or returned to normal at long term after discharge in survivors of COVID-19. All of these results suggest that the gut microbiota of COVID-19 dynamically changes.

Compared with HCs, the fecal microbiota of recovered HCWs at three months after discharge exhibited decreased bacterial diversity. Although only shannon index decreased significantly (p = 0.016), sobs index, chao index, and ace index also decreased. Low bacterial diversity has been reported in various diseases (Kuang *et al.*, 2017; Yu *et al.*, 2017; Wei *et al.*, 2020), including COVID-19 in the acute phase (Gu *et al.*, 2020), implying gut microbiota dysbiosis which may be related to the severity, course and prognosis of these diseases. We also observed an increase in opportunistic pathogens and inflammation-related pathogens, including *Escherichia unclassified*, *I. bartlettii*, *C. aldenense*, *C. ramosum*, *C. bolteae*, and *F. plautii*. *Escherichia* is composed of several species and cryptic clades, including *Escherichia coli*, as a vertebrate gut commensal, and occasionally as an opportunistic pathogen involved in diarrheic and extra-intestinal diseases (Galardini *et al.*, 2020). *Intestinibacter bartlettii* was found to be more frequently present in patients with neurodevelopmental disorders patients group (Bojović *et al.*, 2020), and previously known as *Clostridium bartlettii* isolated from the feces of two autistic male children (Song *et al.*, 2004), is a strong producer of trans-3-Indolylacrylic acid (IAA), which can be transformed by glycine conjugation to Indolyl-3-acryloylglycine (IAG), a putative autistic spectrum disorder (ASD) diagnostic urinary marker (Shattock and Whiteley, 2002). *Clostridium aldenense* and *C. bolteae* are believed to cause bacteremia (Williams *et al.*, 2010), and *F. plautii* has potential significance for the diagnosis of ulcerative colitis (UC) (Li *et al.*, 2021). For the gut microbiota of COVID-19 in the acute phase, Zuo *et al.* (2020b) found *that C. ramosum* is correlated with COVID-19 severity. In addition, previous studies have shown that *F. prausnitzii* (Lopez-Siles *et al.*, 2017), *R. inulinivorans* (Ticinesi *et al.*, 2020), *F. saccharivorans* (Jin *et al.*, 2019), *R. bromii* (Rosés *et al.*, 2021), *B. faecis* (Takahashi *et al.*, 2016), *B. pullicaecorum* (Chang *et al.*, 2020), and *I. butyriciproducens* (Bui *et al.*, 2016) are all butyrate-producing species. Our research found that the above of butyrate-producing species were all reduced in recovered HCWs. Consistently, Yeoh *et al.* (2021) also found *F. prausnitzii*, *R. bromii*, and *Dorea longicatena* were reduced in COVID-19 patients and remained low in samples collected up to 30 days. *Faecalibacterium prausnitzii* is the most abundant bacterium in the human intestinal microbiota of healthy adults, representing more than 5% of the total bacterial population (Miquel *et al.*, 2013; Lopez-Siles *et al.*, 2017; Leylabadlo *et al.*, 2020), which has anti-inflammatory properties and produces short-chain fatty acids (SCFA) and stimulates the secretion of IL-10 through peripheral blood mononuclear cells, mucosal dendritic cells (DCs), and macrophages (Furet *et al.*, 2010). SCFAs are end products of bacterial anaerobic fermentation of dietary fiber and can regulate immune responses in the intestines (Martin-Gallausiaux *et al.*, 2021), such as by inducing regulatory T cells in the colon (Arpaia *et al.*, 2013; Furusawa *et al.*, 2013), inhibiting inflammatory responses through NF-κB inhibition (Segain *et al.*, 2000).

In this study, another significant finding was that altered gut microbiota was correlated with persistent symptoms after discharge. In recovered HCWs, *Escherichia unclassified* as an opportunistic pathogen was positively correlated with fatigue, chest tightness after activity, and myalgia. *Intestinibacter bartlettii* was positively correlated with anorexia and fatigue. Interestingly, *F. prausnitzii* and *I. butyriciproducens* as butyrate-producing species were negatively correlated with chest tightness after activity and cough, respectively. The above information suggests that gut microbiome dysbiosis may cause persistent symptoms of survivors of COVID-19 for a long time after discharge which may through regulating production of anti-inflammatory metabolites such as SCFA and/or facilitating the enrichment of opportunistic pathogens in the patients. However, the specific mechanism by which the gut microbiota affects recovered patients requires further study.

This study has several limitations. First, this was a single-center study with small sample size. However, regardless of recovered patients with COVID-19 or healthy controls were all healthcare workers from Wuhan Union Hospital, the collection process of stool samples was very strict. Although the sample size was small, the sample quality was very high. Second, our study only described the gut microbiota of recovered patients at three months after discharge. However, we do not know how long the altered gut microbiota will last. We should continue pay attention to gut microbiota at six months or even one year after discharge. Therefore, our team is currently collecting stool samples from recovered patients with COVID-19 at one year after discharge to assess whether the gut microbiota continues to be abnormal or return to normal. Third, lifestyle and diet may affect gut microbiota, we did not have information on lifestyle, however, the diet of all healthcare workers was provided by the worker restaurant of Wuhan Union Hospital.

In conclusion, this study provides evidence of gut microbiome dysbiosis in recovered HCWs with COVID-19 at three months after discharge and its association with persistent symptoms after discharge. These data highlight that gut microbiota may play an important role in the recovery of patients with COVID-19. The mechanism on how the gut microbiota alteration impacts the recovery of survivors of COVID-19 should be further studied.

Acknowledgements

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Statement

The study was approved by the Ethics Committee of the Wuhan Union Hospital (2020-0149-02). All participants provided written informed consent prior to participation.

Availability of Data and Materials

The sequences from our study were deposited in the NCBI Sequence Read Archive (PRJNA736160).

References

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J.R., Pfeffer, K., Coffer, P.J., *et al.* 2013. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* **504**, 451–455.

Belkaid, Y. and Hand, T.W. 2014. Role of the microbiota in immunity and inflammation. *Cell* **157**, 121–141.

Bojović, K., Ignjatović, Đ., Soković Bajić, S., Vojnović Milutinović, D., Tomić, M., Golić, N., and Tolinaćki, M. 2020. Gut microbiota dysbiosis associated with altered production of short chain fatty acids in children with neurodevelopmental disorders. *Front. Cell. Infect. Microbiol.* **10**, 223.

Bui, T.P., Shetty, S.A., Lagkouvardos, I., Ritari, J., Chamlagain, B., Douillard, F.P., Paulin, L., Piironen, V., Clavel, T., Plugge, C.M., *et al.* 2016. Comparative genomics and physiology of the butyrate-producing bacterium *Intestinimonas butyriciproducens. Environ. Microbiol. Rep.* **8**, 1024–1037.

Chan, J.F.W., Yuan, S., Kok, K.H., To, K.K.W., Chu, H., Yang, J., Xing, F., Liu, J., Yip, C.C.Y., Poon, R.W.S., *et al.* 2020. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* **395**, 514–523.

Chang, S.C., Shen, M.H., Liu, C.Y., Pu, C.M., Hu, J.M., and Huang, C.J. 2020. A gut butyrate-producing bacterium *Butyricicoccus pullicaecorum* regulates short-chain fatty acid transporter and receptor to reduce the progression of 1,2-dimethylhydrazine-associated colorectal cancer. *Oncol. Lett.* **20**, 327.

Furet, J.P., Kong, L.C., Tap, J., Poitou, C., Basdevant, A., Bouillot, J.L., Mariat, D., Corthier, G., Doré, J., Henegar, C., *et al.* 2010. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes* **59**, 3049–3057.

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T.A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T., *et al.* 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **504**, 446–450.

Galardini, M., Clermont, O., Baron, A., Busby, B., Dion, S., Schubert, S., Beltrao, P., and Denamur, E. 2020. Major role of iron uptake systems in the intrinsic extra-intestinal virulence of the genus *Escherichia* revealed by a genome-wide association study. *PLoS Genet.* **16**, e1009065.

Gu, S., Chen, Y., Wu, Z., Chen, Y., Gao, H., Lv, L., Guo, F., Zhang, X., Luo, R., Huang, C., *et al.* 2020. Alterations of the gut microbiota in patients with coronavirus disease 2019 or H1N1 influenza. *Clin. Infect. Dis.* **71**, 2669–2678.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., *et al.* 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497–506.

Ichinohe, T., Pang, I.K., Kumamoto, Y., Peaper, D.R., Ho, J.H., Murray, T.S., and Iwasaki, A. 2011. Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proc. Natl. Acad. Sci. USA* **108**, 5354–5359.

Jin, M., Kalainy, S., Baskota, N., Chiang, D., Deehan, E.C., McDougall, C., Tandon, P., Martínez, I., Cervera, C., Walter, J., *et al.* 2019. Faecal microbiota from patients with cirrhosis has a low capacity to ferment non-digestible carbohydrates into short-chain fatty acids. *Liver Int.* **39**, 1437–1447.

Kuang, Y.S., Lu, J.H., Li, S.H., Li, J.H., Yuan, M.Y., He, J.R., Chen, N.N., Xiao, W.Q., Shen, S.Y., Qiu, L., *et al.* 2017. Connections between the human gut microbiome and gestational diabetes mellitus. *GigaScience* **6**, 1–12.

Leylabadlo, H.E., Ghotaslou, R., Feizabadi, M.M., Farajnia, S., Moaddab, S.Y., Ganbarov, K., Khodadadi, E., Tanomand, A., Sheykhsaran, E., Yousefi, B., *et al.* 2020. The critical role of *Faecalibacterium prausnitzii* in human health: an overview. *Microb. Pathog.* **149**, 104344.

Li, W., Sun, Y., Dai, L., Chen, H., Yi, B., Niu, J., Wang, L., Zhang, F., Luo, J., Wang, K., *et al.* 2021. Ecological and network analyses identify four microbial species with potential significance for the diagnosis/treatment of ulcerative colitis (UC). *BMC Microbiol.* **21**, 138.

Liang, L., Yang, B., Jiang, N., Fu, W., He, X., Zhou, Y., Ma, W.L., and Wang, X. 2020. Three-month follow-up study of survivors of coronavirus disease 2019 after discharge. *J. Korean Med. Sci.* **35**, e418.

Lopez-Siles, M., Duncan, S.H., Garcia-Gil, L.J., and Martinez-Medina, M. 2017. *Faecalibacterium prausnitzii*: from microbiology to diagnostics and prognostics. *ISME J.* **11**, 841–852.

Martin-Gallausiaux, C., Marinelli, L., Blottière, H.M., Larraufie, P., and Lapaque, N. 2021. SCFA: mechanisms and functional importance in the gut. *Proc. Nutr. Soc.* **80**, 37–49.

Miquel, S., Martín, R., Rossi, O., Bermúdez-Humarán, L.G., Chatel, J.M., Sokol, H., Thomas, M., Wells, J.M., and Langella, P. 2013. *Faecalibacterium prausnitzii* and human intestinal health. *Curr. Opin. Microbiol.* **16**, 255–261.

Rosés, C., Cuevas-Sierra, A., Quintana, S., Riezu-Boj, J.I., Martínez, J.A., Milagro, F.I., and Barceló, A. 2021. Gut microbiota bacterial species associated with mediterranean diet-related food groups in a northern spanish population. *Nutrients* **13**, 636.

Schirmer, M., Smeekens, S.P., Vlamakis, H., Jaeger, M., Oosting, M., Franzosa, E.A., Ter Horst, R., Jansen, T., Jacobs, L., Bonder, M.J., *et al.* 2016. Linking the human gut microbiome to inflammatory cytokine production capacity. *Cell* **167**, 1125–1136.

Segain, J.P., de la Blétière, D.R., Bourreille, A., Leray, V., Gervois, N., Rosales, C., Ferrier, L., Bonnet, C., Blottière, H.M., and Galmiche, J.P. 2000. Butyrate inhibits inflammatory responses through NFκB inhibition: implications for Crohn's disease. *Gut* **47**, 397–403.

Shattock, P. and Whiteley, P. 2002. Biochemical aspects in autism spectrum disorders: updating the opioid-excess theory and presenting new opportunities for biomedical intervention. *Expert Opin. Ther. Targets* **6**, 175–183.

Song, Y.L., Liu, C.X., McTeague, M., Summanen, P., and Finegold, S.M. 2004. *Clostridium bartlettii* sp. nov., isolated from human faeces. *Anaerobe* **10**, 179–184.

Takahashi, K., Nishida, A., Fujimoto, T., Fujii, M., Shioya, M., Imaeda, H., Inatomi, O., Bamba, S., Sugimoto, M., and Andoh, A. 2016. Reduced abundance of butyrate-producing bacteria species in the fecal microbial community in Crohn's disease. *Digestion* **93**, 59–65.

Ticinesi, A., Mancabelli, L., Tagliaferri, S., Nouvenne, A., Milani, C.,

Del Rio, D., Lauretani, F., Maggio, M.G., Ventura, M., and Meschi, T. 2020. The gut-muscle axis in older subjects with low muscle mass and performance: a proof of concept study exploring fecal microbiota composition and function with shotgun metagenomics sequencing. *Int. J. Mol. Sci.* **21**, 8946.

Wang, X., Zhou, Q., He, Y., Liu, L., Ma, X., Wei, X., Jiang, N., Liang, L., Zheng, Y., Ma, L., *et al.* 2020a. Nosocomial outbreak of COVID-19 pneumonia in Wuhan, China. *Eur. Respir. J.* **55**, 2000544.

Wang, X., Zhou, Y., Jiang, N., Zhou, Q., and Ma, W.L. 2020b. Persistence of intestinal SARS-CoV-2 infection in patients with COVID-19 leads to re-admission after pneumonia resolved. *Int. J. Infect. Dis.* **95**, 433–435.

Wei, Y., Li, Y., Yan, L., Sun, C., Miao, Q., Wang, Q., Xiao, X., Lian, M., Li, B., Chen, Y., *et al.* 2020. Alterations of gut microbiome in autoimmune hepatitis. *Gut* **69**, 569–577.

Williams, O.M., Brazier, J., Peraino, V., and Goldstein, E.J. 2010. A review of three cases of *Clostridium aldenense* bacteremia. *Anaerobe* **16**, 475–477.

Yeoh, Y.K., Zuo, T., Lui, G.C.Y., Zhang, F., Liu, Q., Li, A.Y.L., Chung, A.C.K., Cheung, C.P., Tso, E.Y.K., Fung, K.S.C., *et al.* 2021. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. *Gut* **70**, 698–706.

Yu, J., Feng, Q., Wong, S.H., Zhang, D., Liang, Q.Y., Qin, Y., Tang, L., Zhao, H., Stenvang, J., Li, Y., *et al.* 2017. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut* **66**, 70–78.

Zhou, Y., Shi, X., Fu, W., Xiang, F., He, X., Yang, B., Wang, X., and Ma, W.L. 2021. Gut microbiota dysbiosis correlates with abnormal immune response in moderate COVID-19 patients with fever. *J. Inflamm. Res.* **14**, 2619–2631.

Zuo, T., Liu, Q., Zhang, F., Lui, G.C., Tso, E.Y., Yeoh, Y.K., Chen, Z., Boon, S.S., Chan, F.K., Chan, P.K., *et al.* 2021. Depicting SARS-CoV-2 faecal viral activity in association with gut microbiota composition in patients with COVID-19. *Gut* **70**, 276–284.

Zuo, T., Zhan, H., Zhang, F., Liu, Q., Tso, E.Y.K., Lui, G.C.Y., Chen, N., Li, A., Lu, W., Chan, F.K.L., *et al.* 2020a. Alterations in fecal fungal microbiome of patients with COVID-19 during time of hospitalization until discharge. *Gastroenterology* **159**, 1302–1310.

Zuo, T., Zhang, F., Lui, G.C.Y., Yeoh, Y.K., Li, A.Y.L., Zhan, H., Wan, Y., Chung, A.C.K., Cheung, C.P., Chen, N., *et al.* 2020b. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. *Gastroenterology* **159**, 944–955.