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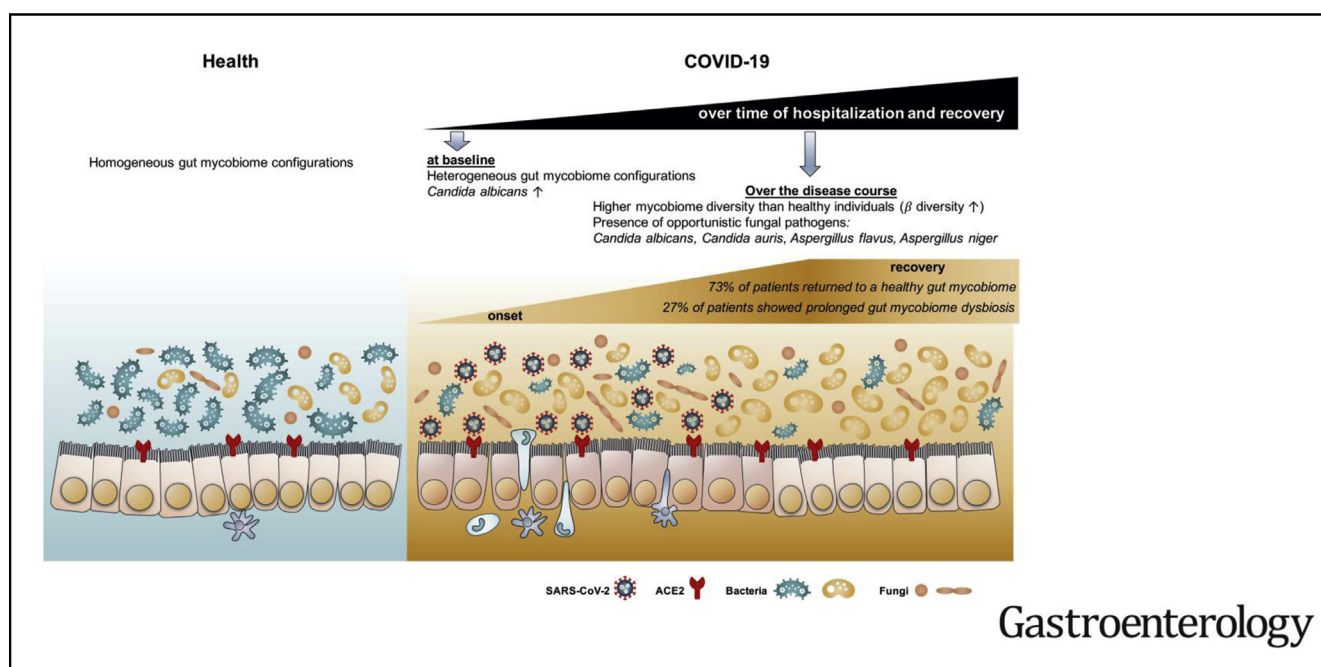
# BASIC AND TRANSLATIONAL—ALIMENTARY TRACT

## Alterations in Fecal Fungal Microbiome of Patients With COVID-19 During Time of Hospitalization until Discharge



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**BACKGROUND & AIMS:** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects intestinal cells, and might affect the intestinal microbiota. We investigated changes in the fecal fungal microbiomes (mycobiome) of patients with SARS-CoV-2 infection during hospitalization and on recovery. **METHODS:** We performed deep shotgun metagenomic sequencing analysis of fecal samples from 30 patients with coronavirus disease 2019 (COVID-19) in Hong Kong, from February 5 through May 12, 2020. Fecal samples were collected 2 to 3 times per week from time of hospitalization until discharge. We compared fecal mycobiome compositions of patients with COVID-19 with those from 9 subjects with community-acquired pneumonia and 30 healthy individuals (controls). We assessed fecal mycobiome profiles throughout

time of hospitalization until clearance of SARS-CoV-2 from nasopharyngeal samples. **RESULTS:** Patients with COVID-19 had significant alterations in their fecal mycobiomes compared with controls, characterized by enrichment of *Candida albicans* and a highly heterogeneous mycobiome configuration, at time of hospitalization. Although fecal mycobiomes of 22 patients with COVID-19 did not differ significantly from those of controls during times of hospitalization, 8 of 30 patients with COVID-19 had continued significant differences in fecal mycobiome composition, through the last sample collected. The diversity of the fecal mycobiome of the last sample collected from patients with COVID-19 was 2.5-fold higher than that of controls ( $P < .05$ ). Samples collected at all timepoints from patients with COVID-19 had increased proportions of opportunistic fungal pathogens, *Candida albicans*, *Candida auris*, and *Aspergillus flavus* compared with controls. Two respiratory-associated fungal pathogens, *A. flavus* and

*Aspergillus niger*, were detected in fecal samples from a subset of patients with COVID-19, even after clearance of SARS-CoV-2 from nasopharyngeal samples and resolution of respiratory symptoms. **CONCLUSIONS:** In a pilot study, we found heterogeneous configurations of the fecal mycobiome, with enrichment of fungal pathogens from the genera *Candida* and *Aspergillus*, during hospitalization of 30 patients with COVID-19 compared with controls. Unstable gut mycobiomes and prolonged dysbiosis persisted in a subset of patients with COVID-19 up to 12 days after nasopharyngeal clearance of SARS-CoV-2. Studies are needed to determine whether alterations in intestinal fungi contribute to or result from SARS-CoV-2 infection, and the effects of these changes in disease progression.

**Keywords:** Coronavirus; Microbe; Yeast; Intestine.

Coronavirus disease 2019 (COVID-19) is caused by a novel coronavirus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]), which primarily affects the respiratory system. Patients with COVID-19 present with variable disease symptoms and severity, some can be severe, resulting in hospitalization, respiratory failure, or even death.<sup>1</sup> A recent meta-analysis of 35 COVID-19 studies reported that the pooled prevalence of digestive symptoms was 15% and pooled prevalence of digestive system comorbidities was 4%.<sup>2</sup> Patients with gastrointestinal (GI) involvement tended to have a poor disease course.<sup>2</sup> In addition, SARS-CoV-2 virus was detected in the feces and anal swabs in patients with COVID-19 and can infect human intestinal epithelium.<sup>3-5</sup> Altogether these data suggest that the GI tract is an important extrapulmonary site for SARS-CoV-2 infection.

Bacterial and fungal infections are common complications of viral pneumonia, which may influence disease course and clinical manifestations, especially in critically ill patients.<sup>6</sup> However, data regarding bacterial or fungal coinfections in viral pneumonia led by coronavirus are lacking. Our recent study showed that the gut bacterial microbiome was significantly altered in patients with COVID-19 with a significant expansion of opportunistic pathogens in the gut, which was associated with disease severity and fecal SARS-CoV-2 shedding.<sup>7</sup> Beyond bacteria, the GI tract also harbors a large number of fungi, collectively known as the mycobiome. Intestinal fungi have been shown to be causally implicated in microbiome assembly and immune development.<sup>8</sup> Accumulating findings highlighted that the gut mycobiota can strongly influence the host immune system and this interaction is linked to bacteria activities.<sup>9,10</sup> Recent observations of the direct and indirect effects of fungal microbiota on various GI diseases have stimulated further investigation of the mycobiota composition and ways of regulating its diversity.<sup>11</sup> Although the magnitude of the number of fungal cells is smaller than that of the bacterial microbiota, their impact on health is significant, especially as a reservoir for blooms of pathogenic microbes when the host is immunocompromised and as a cofactor in driving severe

**Table 1.** Clinical Characteristics of Study Subjects

Variables	COVID-19 patients	Pneumonia patients	Healthy controls
Number	30	9	30
Male	16 (53)	5 (56)	15 (50)
Age, y	46 (29, 63)	63 (47,69)	34 (23, 48)
Comorbidities	11 (37)	9 (100)	0 (0)
Recent exposure history			
Tztravel to Wuhan City	0 (0)	0 (0)	
Travel to other cities of Hubei province	1 (7)	0 (0)	
Contact with person with COVID-19	9 (30)	0 (0)	
Have family cluster outbreak	4 (13)	0 (0)	
Symptoms at admission			
Fever	17 (57)	6 (67)	
Gastrointestinal symptoms			
Diarrhea	4 (13)	2 (22)	
Respiratory symptoms			
Cough	20 (67)	6 (67)	
Sputum	9 (30)	5 (56)	
Sore throat	4 (13)	0 (0)	
Rhinorrhea	5 (17)	1 (11)	
Shortness of breath	4 (13)	3 (33)	
Blood result			
Lymphocytes (x 10 <sup>9</sup> /L, normal range 1.1–2.9)	1 (0.8, 1.375)	1 (0.6, 1.2)	
Antibiotics therapy	16 (53)	100 (100)	
1 type of antibiotics	6 (20)	2 (22)	
2 types of antibiotics	7 (23)	4 (44)	
3 types of antibiotics	3 (10)	3 (33)	
Antiviral therapy	20 (67)	0 (0)	
Kaletra	19 (63)	0 (0)	
Ribavirin	12 (40)	0 (0)	
Interferon beta-1b	3 (10)	0 (0)	
Death	0 (0)	0 (0)	


Values are expressed in number (percentage) and median (interquartile range).

infectious diseases. It is unclear if the gut mycobiome is also altered and whether fungal pathogens bloom in COVID-19 and underlie disease course.

In this pilot study, we hypothesize that the intestinal fungal microbiome (mycobiome) is altered in SARS-CoV-2 infection and COVID-19 is associated with blooms of certain fungi in the gut. We prospectively included 30 patients with COVID-19 (Table 1, Figure 1, Supplementary Figure 1), admitted between February 16, 2020, and April

\* Authors contributed equally to this work.

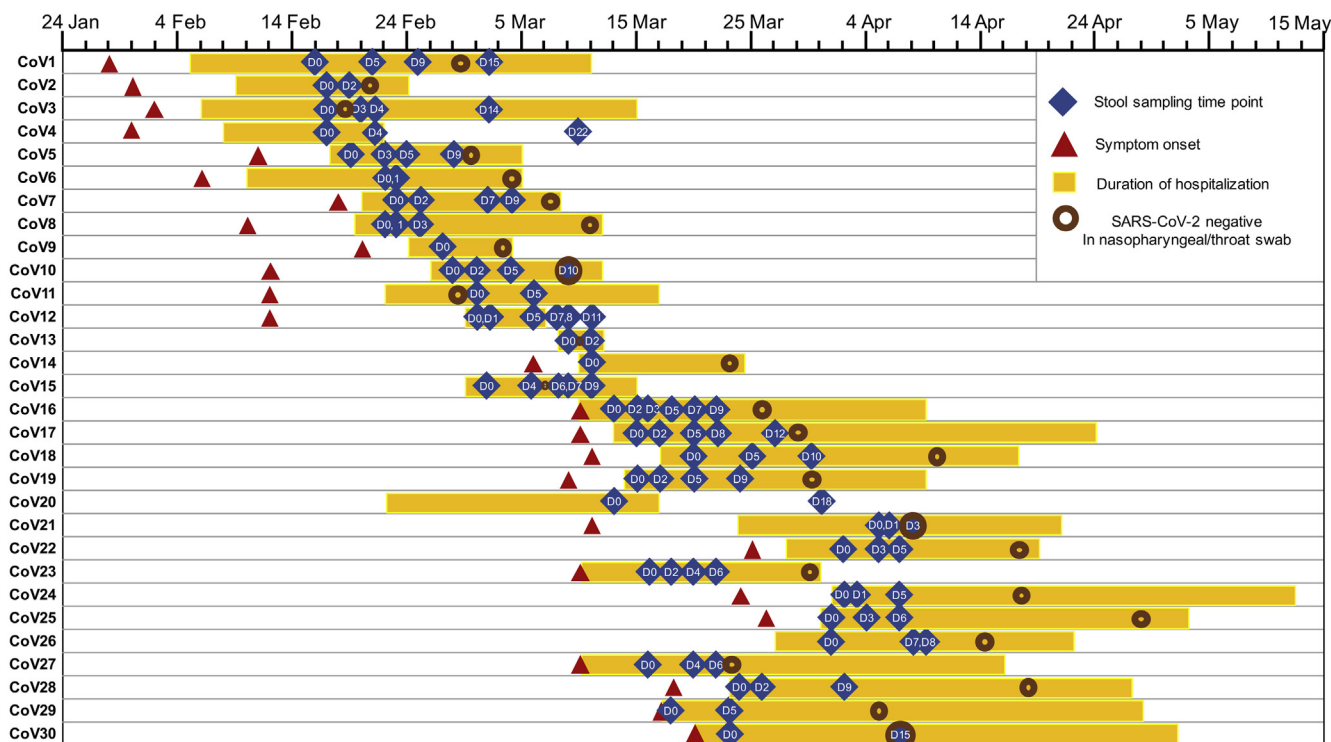
**Abbreviations used in this paper:** COVID-19, coronavirus disease 2019; GI, gastrointestinal; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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**Figure 1.** Schematic diagram of stool specimen collection and duration of hospitalization in patients with COVID-19 (n = 30). “CoV” denotes patient with COVID-19. Stool specimens were serially collected for shotgun metagenomics sequencing. “D0” denotes baseline date when the first stool was collected after hospitalization; the following time points starting with “D” represent days since baseline stool collection. “-ve nasopharyngeal/throat swab”: the first negative result for SARS-CoV-2 virus in 2 consecutive negative nasopharyngeal/throat/pooled swab tests, on which patient was then discharged.

1, 2020, in Hong Kong, China, followed from hospital admission until discharge. We investigated intestinal fungal compositions in patients with COVID-19 as compared with healthy individuals and patients with community-acquired pneumonia, and their temporal changes over time of hospitalization, via broad-target deep shotgun metagenomics sequencing.

## Methods

### Study Subject and Design

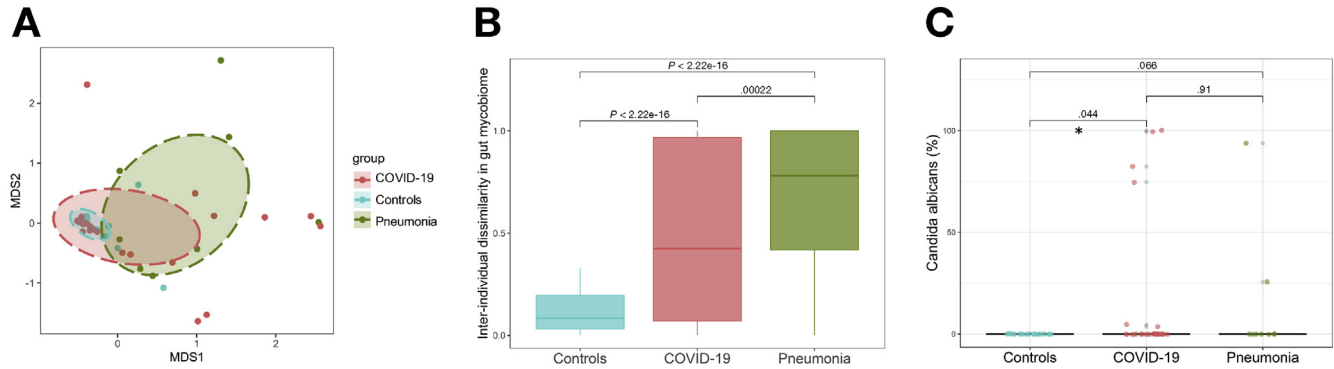
This prospective study involved 30 patients with COVID-19 hospitalized with laboratory-confirmed SARS-CoV-2 infection, 9 patients hospitalized with community-acquired pneumonia (pneumonia controls), and 30 healthy individuals (controls) (Table 1). Thirty hospitalized patients with COVID-19 were admitted between February 16, 2020, and April 1, 2020, in Hong Kong, China, and were followed from hospital admission until discharge (Figure 1). SARS-CoV-2 infection was confirmed by 2 consecutive reverse-transcriptase polymerase chain reaction (PCR) tests targeting different regions of the RdRp gene performed by the local hospital and Public Health Laboratory Service. Pneumonia controls were patients admitted with community-acquired pneumonia tested negative for SARS-CoV-2 PCR on 2 respiratory samples. Patients with COVID-19 and pneumonia controls were admitted to the Prince of Wales Hospital or the United Christian Hospital, Hong Kong. Controls were healthy individuals with no past medical history or history of antibiotic intake in the past 3 months recruited via

advertisement from the general population and tested negative for SARS-CoV-2. Severity of COVID-19 infection was categorized as (1) mild, if there was no radiographic evidence of pneumonia; (2) moderate, if pneumonia was present along with fever and respiratory tract symptoms; (3) severe, if respiratory rate  $\geq 30$ /min, oxygen saturation  $\leq 93\%$  when breathing ambient air, or  $\text{PaO}_2 / \text{FiO}_2 \leq 300$  mm Hg (1 mm Hg = 0.133 kPa); or (4) critical, if there was respiratory failure requiring mechanical ventilation, shock, or organ failure requiring intensive care.<sup>12</sup> This study was approved by the Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committees (2020.076). All patients provided informed consent to participate in this study. Data including demographics, laboratory results, imaging results and drug therapy were obtained from the electronic medical records in the Hong Kong Hospital Authority clinical management system. Fecal samples from patients with COVID-19 were collected serially 2 to 3 times per week until discharge. This study was conducted in accordance with the Declaration of Helsinki.

### Fecal DNA Extraction

Approximately 0.1-g fecal sample was prewashed with 1 mL double-distilled  $\text{H}_2\text{O}$  and pelleted by centrifugation at 13,000g for 1 minute. The fecal DNA was subsequently extracted from the pellet using Maxwell RSC PureFood GMO and Authentication Kit (Promega, Madison, WI) following the manufacturer’s instructions. Briefly, the fecal pellet was added to 1 mL of CTAB buffer and vortexed for 30 seconds, then the sample was heated at 95°C for 5 minutes. After that, the samples were vortexed





**Figure 2.** Gut mycobiome (fungal community) alterations in patients with COVID-19. (A) Fecal mycobiome alterations in COVID-19, viewed by NMDS (nonmetric multidimensional scaling) plot based on Bray-Curtis dissimilarities. The fecal mycobiome was compared among healthy controls (n = 30), COVID-19 (n = 30), and pneumonia patient controls (n = 9). (B) Interindividual dissimilarities between fecal mycobiomes within each group. The mycobiome dissimilarity was calculated as Bray-Curtis dissimilarity. Between-group comparison was conducted by *t* test. (C) The relative abundance of *Candida albicans* in the fecal mycobiome. Between-group comparison was conducted by Wilcoxon rank sum test.

thoroughly with beads at maximum speed for 15 minutes. Then 40  $\mu$ L of proteinase K and 20  $\mu$ L of RNase A was added into sample and the mixture was incubated at 70°C for 10 minutes. The supernatant was then obtained by centrifuging at 13,000g for 5 minutes and was added to the Maxwell RSC machine for DNA extraction.

### Shotgun Metagenomics Sequencing and Fungal Profiling

Extracted DNA was subject DNA libraries construction, completed through the processes of end repairing, adding A to tails, purification and PCR amplification, using Nextera DNA Flex Library Preparation kit (Illumina, San Diego, CA). Libraries were subsequently sequenced on our in-house sequencer Illumina Novaseq 6000 (150 base pairs paired-end) at the Center for Microbiota Research, The Chinese University of Hong Kong.

Raw sequence reads were filtered and quality-trimmed using Trimmomatic v0.36<sup>13</sup> as follows: (1) trimming low-quality base (quality score <20); (2) removing reads shorter than 50 base pairs; (3) removing sequencing adapters. Contaminating human reads were filtering using Kneaddata (Reference database: GRCh38 p12) with default parameters. Profiling of the fungal community was performed using MiCoP. The fungal abundance profile of each sample was expressed in relative abundance (%) among the fecal fungal community.<sup>14</sup>

### Statistical Analysis

Data on the relative abundance of fecal fungi was imported into R v3.5.1. Nonmetric multidimensional scaling analyses were performed on all baseline fecal mycobiomes between groups, and serial fecal mycobiomes in each COVID-19 case during the disease course, based on Bray-Curtis dissimilarities using vegan package (v2.5–3). Differential fungal taxa between patients with COVID-19 (at baseline or across all time points during hospitalization) and healthy controls were identified using LefSE.<sup>15</sup>

## Results

### Fecal Mycobiome Alterations in COVID-19

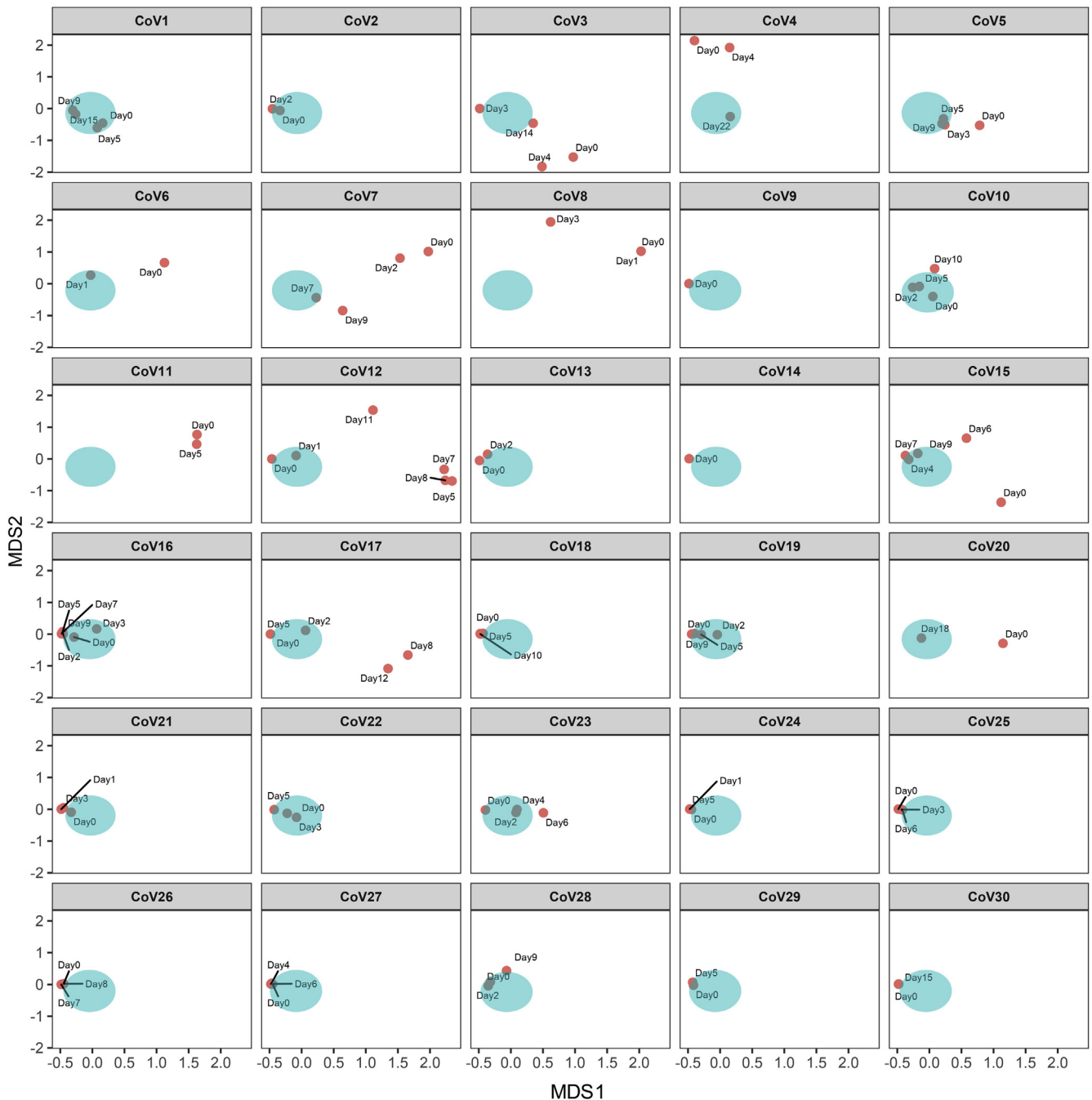
A total of 30 patients hospitalized with COVID-19, 30 healthy controls, and 9 patients hospitalized with

community-acquired pneumonia were included (Table 1; Supplementary Table 1). All patients with COVID-19 presented with respiratory symptoms and 4 also had diarrhea at presentation. To understand alterations of the gut mycobiome that underlies SARS-CoV-2 infection, we compared baseline fecal mycobiome composition (first time point of stool sampling after hospitalization) between patients with COVID-19, healthy controls, and pneumonia controls. At the whole fungal community level, fecal mycobiome of healthy controls densely clustered together, whereas that of patients with COVID-19 formed a substantially broader cluster significantly different from that of controls (PERMANOVA test,  $P = .001$ , Figure 2A). Fecal mycobiome was more heterogeneous across patients with COVID-19 than controls, as demonstrated by an approximately 6-fold increase in interindividual mycobiome dissimilarity in patients with COVID-19 relative to controls ( $P < 2.22e-16$ , Figure 2B). Patients with community-acquired pneumonia also showed heterogeneous fecal mycobiome configurations (Figure 2B), indicating that both community-acquired pneumonia and COVID-19-associated pneumonia were associated with fungal dysbiosis in the gut.

We next identified differential fungal species in fecal samples between patients with COVID-19 and healthy controls adjusting for antibiotics use and comorbidities. We found that *Candida albicans* was significantly enriched in 6 (20%) of 30 patients with COVID-19 but was absent in healthy controls (false discovery rate  $P = .04$ , Figure 2C). These data suggest that gut mycobiome was significantly altered in patients with COVID-19 with an increase in *Candida* species.

### Temporal Changes in Gut Mycobiome Over Time of Hospitalization

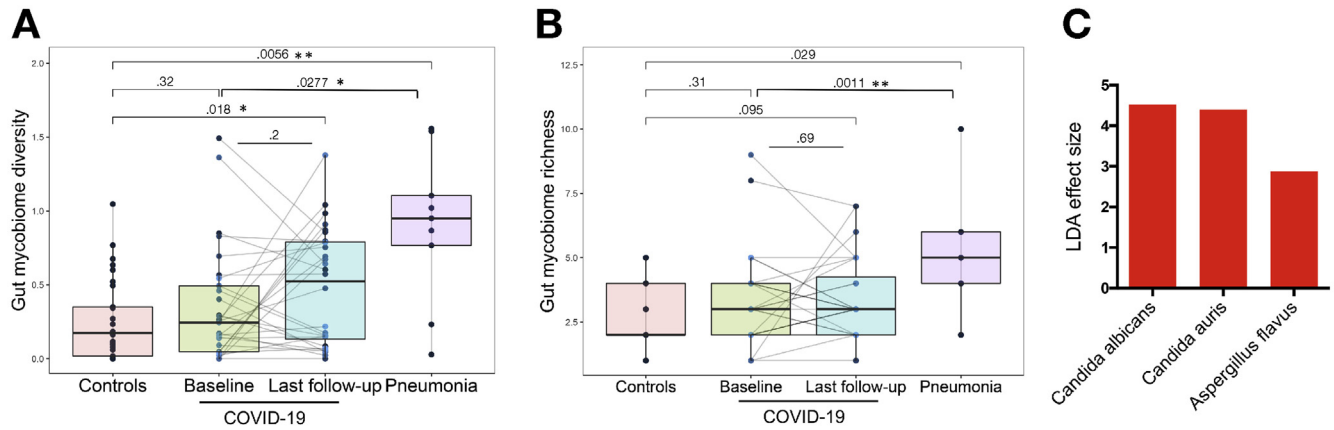
We investigated longitudinal dynamics of the gut mycobiome in COVID-19 over time of hospitalization and explored whether recovery from SAR-CoV-2 infection was associated with restoration of gut mycobiome to a



**Figure 3.** Gut mycobiome (fungal community) alterations in patients with COVID-19 and longitudinal changes during time of hospitalization. Temporal compositional changes in fecal mycobiome with respect to each COVID-19 case were viewed by NMDS (nonmetric multidimensional scaling) plot based upon Bray-Curtis dissimilarities. The aqua cluster denotes the fecal mycobiome cluster of healthy controls. “CoV” denotes patient with COVID-19. “Day0” denotes baseline date when the first stool was collected after hospitalization; the following time points starting with “Day” represents days since baseline stool collection.

community similar to that of healthy individuals. The gut mycobiome in most (22 of 30) patients with COVID-19 was stable over the course of hospitalization and remained similar to that of healthy controls at the latest follow-up (Figure 3). However, fecal mycobiome of 8 of 30 patients (patients CoV3, 7, 8, 10, 11, 12, 17, and 23) showed drastic changes over the course of hospitalization and had a different fecal mycobiome composition compared with that

of healthy controls at the last follow-up (Figure 3). The diversity of the fecal mycobiome did not differ between healthy controls and patients with COVID-19 at baseline (Figure 4A). In contrast, the diversity and richness of the gut mycobiome were both significantly higher in patients with community-acquired pneumonia than patients with COVID-19 at baseline (Figure 4A and B,  $P < .05$  and  $< .01$ , respectively). At the last follow-up after hospitalization,



**Figure 4.** Bloom of gut fungi in patients with COVID-19 during time of hospitalization. (A) The diversity of fecal mycobiome (fungi) in patients with COVID-19 over time of hospitalization (plotted as the baseline time point and the last follow-up time point after hospitalization), compared with healthy controls ( $n = 30$ ) and pneumonia patient controls ( $n = 9$ ). Between-group comparison was conducted by  $t$  test, last follow-up versus baseline comparison for the hospitalized patients with COVID-19 were conducted by paired  $t$  test. (B) The richness of fecal mycobiome (fungi) in patients with COVID-19 over time of hospitalization (plotted as the baseline time point and the last follow-up time point after hospitalization), compared with healthy controls ( $n = 30$ ) and pneumonia patient controls ( $n = 9$ ). Between-group comparison was conducted by  $t$  test, last follow-up versus baseline comparison for the hospitalized patients with COVID-19 were conducted by paired  $t$  test. (C) Overrepresented fungal species in feces in patients with COVID-19 during time of hospitalization, compared with healthy controls. LefSE analysis was conducted to identify differential species, only species with LDA effect size  $> 2$  and false discovery rate  $P < .1$  were plotted.

patients with COVID-19 showed a 2.5-fold higher diversity of the fecal mycobiome compared with healthy controls ( $P < .05$ , Figure 4A).

Overall, during disease course, the diversity of the fecal mycobiome showed constant changes in 53% (16 of 30) of patients with COVID-19 (Supplementary Figure 1). Altogether these data suggest that the gut mycobiome was unstable in a subset of patients with COVID-19 during the time of hospitalization. Patients CoV3, CoV10, and CoV11 who cleared SARS-CoV-2 virus from nasopharyngeal swab and had resolution of respiratory symptoms continued to display a different gut mycobiome composition with that of healthy controls in later time points up to the last follow-up (12, 0, and 5 days after nasopharyngeal clearance of SARS-CoV-2 respectively for patients CoV3, CoV10, and Cov11, Figure 3). These data indicate persistent gut fungal dysbiosis despite disease resolution in a subset of patients with COVID-19.

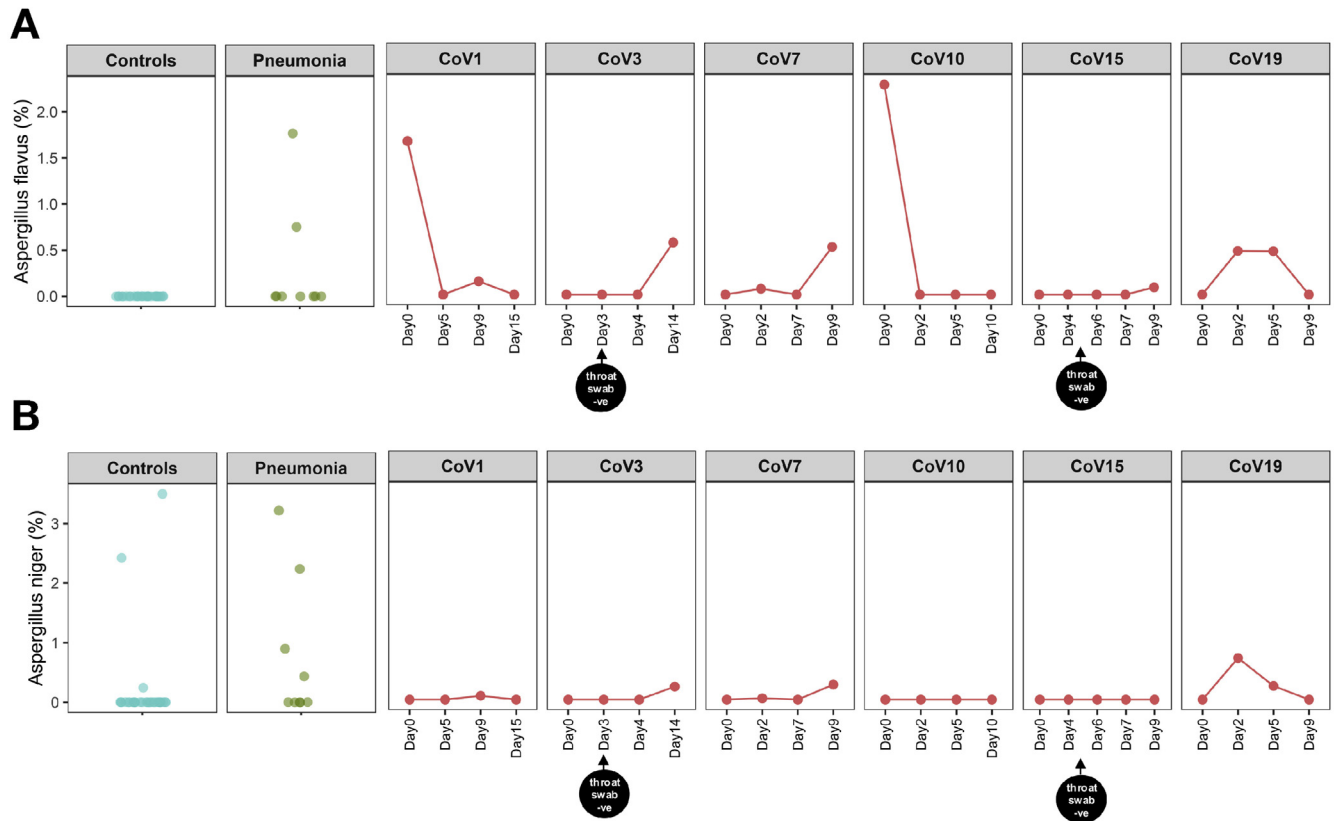
Across all time points during hospitalization, *C. albicans*, *Candida auris* and *Aspergillus flavus* were overrepresented in fecal samples of patients with COVID-19; these species however were absent in healthy controls (Figure 4C, Supplementary Figures 2 and 3, and Figure 5A). Patients (CoV6, 7, 8 and 11) who showed high fecal abundance of *C. albicans* ( $>50\%$  in relative abundance) had a substantial decrease of *C. albicans* over time of hospitalization (Supplementary Figure 2A and B). Blooms of *C. albicans*, *C. auris*, and *A. flavus*, were also seen in patients with community-acquired pneumonia (Figures 2C and 5, and Supplementary Figures 2C and 3C).

Two fungal species from the genus *Aspergillus*, *A. flavus* and *Aspergillus niger*, known to cause pulmonary aspergillosis and respiratory illnesses (particularly cough),<sup>16,17</sup> were detected in serial fecal samples of 6 and 4 patients

with COVID-19, respectively, during hospitalization (Figure 5). All 6 patients presented with cough and varying degree of COVID-19 severity. Among the detected *Aspergillus* species, *A. flavus* was the most abundant and prevalent member (in 6/30 patients; relative abundance up to 2.28%, Figure 5A). *A. niger* was absent at baseline but showed a low relative abundance of  $<1\%$  in 4 of 30 patients with COVID-19 over time of hospitalization (Figure 5B). Importantly, patients CoV15 and CoV19 who presented with a mild disease course showed presence of *A. flavus* and *A. niger* in fecal samples over time. *A. flavus* and *A. niger* were also detected at various time points after nasopharyngeal swab turned negative for SARS-CoV-2 in patients CoV3 and CoV15 (after 11 days and 4 days, respectively, Figure 5). These data indicate cobloom of opportunistic fungal pathogens, *Candida* species and *Aspergillus* species, in the gut of patients with COVID-19 over the disease course. Their presence after nasopharyngeal clearance of SARS-CoV-2 may pose a long-term threat to human health beyond SARS-CoV-2 infection.

## Discussion

We showed for the first time that the gut mycobiome was disturbed in patients with COVID-19. Patients hospitalized with SARS-CoV-2 infection showed more heterogeneous gut mycobiome configurations (higher interindividual mycobiome dissimilarities) compared with healthy individuals. These data suggest that gut microbiota changes induced by SARS-CoV-2, might at least in part, be stochastic, therefore leading to transition from a stable to unstable microbial community state in COVID-19.<sup>18</sup> In line with our findings in patients with COVID-19, a highly heterogeneous assembly of microbial community in response to disease



**Figure 5.** The presence of *Aspergillus flavus* (A) and *Aspergillus niger* (B) in patients with COVID-19 over time of hospitalization. “CoV” denotes patient with COVID-19. “Controls” denotes healthy controls (n = 30). “Pneumonia” denotes patient controls with pneumonia (n = 9). “Day0” denotes baseline date when the first stool was collected after hospitalization; the following time points starting with “Day” represent days since baseline stool collection. “throat swab -ve” indicates the first negative result for SARS-CoV-2 virus in 2 consecutive negative nasopharyngeal/throat/pooled swab tests, on which patient was then discharged.

stressors have been reported in the gut of patients with inflammatory bowel disease<sup>19</sup> and in the lungs of patients with human immunodeficiency virus/AIDs.<sup>18,20,21</sup> Human immunodeficiency virus/AIDs is associated with decreased CD4<sup>+</sup> T-cell counts, which increase an individual’s susceptibility to a wide range of bacterial, viral, and fungal opportunistic pathogens.<sup>20</sup> Similarly, SARS-CoV-2 infection was associated with a significant reduction in the number of T cells,<sup>22,23</sup> which could compromise host immune homeostasis and stability of the microbial communities residing in the human gut. In favor of this hypothesis, the overall gut mycobiome was unstable in a subset of patients with COVID-19 over time of hospitalization, and these individuals ended up with a different gut mycobiome composition compared with that of healthy individuals. In addition, gut mycobiome diversity of patients with COVID-19 at the last follow-up during hospitalization was significantly higher than that of healthy individuals. These data indicate expansion of fungi in the gut of patients with COVID-19 and SARS-CoV-2 infection may be associated with a persistent gut mycobiome dysbiosis in some patients.

Secondary fungal infection or coinfection in patients with COVID-19 during the pandemic is garnering increased attention.<sup>24</sup> Specific enrichments of opportunistic fungal pathogens, *Candida* and *Aspergillus* lineages, were observed

in patients with COVID-19 during the disease course. Among them, *C. albicans* was overrepresented in COVID-19. *C. albicans* has been shown to impair holistic gut microbiome assembly in humans and mice.<sup>25,26</sup> Moreover, gut colonization by *C. albicans* aggravated inflammation in the gut and non-gut tissues.<sup>27,28</sup> *Aspergillus* infections were recently reported in respiratory tract secretions and tracheal aspirates in patients with COVID-19 from 2 studies (detection rate of 10% and 20%, respectively, in cohorts from China and France).<sup>29,30</sup> Population-based studies have reported 19% to 33% incidence of pulmonary aspergillosis in patients with COVID-19.<sup>31-33</sup> *Aspergillus* is a genus of ubiquitous fungi that cause a variety of pulmonary and respiratory symptoms.<sup>17</sup> *Aspergillus* may captivate on the immune-compromised host and affect the clinical features, disease course, and prognosis.<sup>17</sup> To date, there are a lack of data on whether fungal pathogens exist in the gut of patients with COVID-19. In this case series, we provide the first evidence demonstrating the presence of opportunistic *Aspergillus* pathogens in the feces of patients with COVID-19; *A. flavus* were detected in the feces of 20% of patients with COVID-19 via metagenomics sequencing. Cough was previously reported to be more frequent in subjects infected by *Aspergillus* species than those who were not infected.<sup>16</sup> All 6 patients with COVID-19 who had fecal *A. flavus*



presented with cough during hospitalization, suggestive of a systemic effect of *Aspergillus* infection and the intricate link between the GI and respiratory systems. Two of the 3 patients (CoV1 and 3) who had both fungal species *A. flavus* and *A. niger* in their feces presented with critical COVID-19 and were admitted to intensive care unit, and the third case (CoV7) had high fever and moderate COVID-19 severity. Patient CoV19 who had mild COVID-19 showed clearance of *A. flavus* and *A. niger* over time of hospitalization. Although the overall gut mycobiome structure in patients with COVID-19 gradually resembled that of healthy individuals over time of hospitalization (Figure 3), *Aspergillus* species continued to be present in a subset of patients with COVID-19 (CoV3 and CoV15) even after nasopharyngeal clearance of SARS-CoV-2 (Figure 5). The post-recovery existence of *Aspergillus* species underscores a potential prolonged detrimental effect of secondary fungal infection on host health after SARS-CoV-2 infection, cautioning on long-term monitoring of patients with COVID-19 after recovery.

Pneumonia is one of the leading infectious causes of death.<sup>34</sup> Fungal coinfections in the respiratory tract and alterations in the lung mycobiome have been reported in pneumonia and pulmonary diseases.<sup>17,35,36</sup> However, to our knowledge, changes in the gut mycobiome were not reported in patients with pneumonia to date. In the present study, we observed that patients with community-acquired pneumonia also showed a significant alteration in the gut mycobiome compared with healthy controls. Similar to patients with COVID-19, blooms of opportunistic fungal pathogens, *Candida* species and *Aspergillus* species, were also seen in patients with community-acquired pneumonia. In contrast, patients with community-acquired pneumonia showed more heterogeneous gut mycobiome configurations than patients with COVID-19, indicating that the gut mycobiome in community-acquired pneumonia may be more “dysbiotic” than that in COVID-19. This could in part relate to the use of antibiotics in these individuals. In addition, the diversity and richness of the gut mycobiome were both significantly lower in patients with COVID-19 than patients with community-acquired pneumonia. These data collectively suggest that patients with COVID-19 have a similar but less severe dysbiosis of gut mycobiome compared with patients with community-acquired pneumonia.

One limitation of this exploratory study is the modest sample size. Although assigning a causative relationship between COVID-19 and gut fungal dysbiosis requires larger validation studies, this pilot study presents the first to examine the influence of SARS-CoV-2 infection on gut mycobiome composition and dynamics. Stool collected after hospitalization for mycobiome analysis does not represent the bona fide baseline mycobiome at COVID-19 onset, nor the baseline mycobiome before disease onset. In addition, it is unclear whether blooms of gut fungi are a result of SARS-CoV-2 infection or of coinfection during hospitalization. The contribution of gut fungi in the pathogenesis and disease progression of COVID-19 is also unknown. Future studies should prospectively include asymptomatic SARS-CoV-2-infected subjects without hospitalization. In addition, patients with symptomatic SARS-CoV-2 infection should be

followed from disease onset until after recovery to thoroughly delineate the role of gut fungi during SARS-CoV-2 infection and the effects of these changes in disease progression and long-term health.

In conclusion, our study provides evidence of numerous blooms of fungal species in the gut of patients with COVID-19. These data highlight an important role of gut mycobiome in COVID-19. The effects of gut fungi in disease pathogenesis and long-term health after recovery from SARS-CoV-2 infection warrant further investigation.

## Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at [www.gastrojournal.org](http://www.gastrojournal.org), and at <https://doi.org/10.1053/j.gastro.2020.06.048>.

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#### CRedit Authorship Contributions

Tao Zuo, PhD (Conceptualization: Lead; Formal analysis: Equal; Investigation: Lead; Methodology: Equal; Writing – original draft: Lead). Hui Zhan, PhD (Data curation: Lead; Formal analysis: Equal; Methodology: Lead; Writing – review & editing: Equal). Fen Zhang, PhD (Data curation: Supporting; Formal analysis: Supporting). Qin Liu, PhD (Data curation: Supporting; Formal analysis: Supporting). Eugene Y.K. Tso, MD (Resources: Lead). Grace Lui, Doctor (Resources: Equal). Nan Chen, Master (Methodology: Equal; Validation: Equal). Amy Li, NA (Data curation: Lead; Investigation: Equal). Wenqi Lu, NA (Methodology: Equal). Francis K.L. Chan, MD (Investigation: Supporting; Project administration: Lead). Paul K.S. Chan, PhD (Resources: Supporting). Siew C Ng, PhD (Conceptualization: Supporting; Funding acquisition: Lead; Writing – review & editing: Lead).

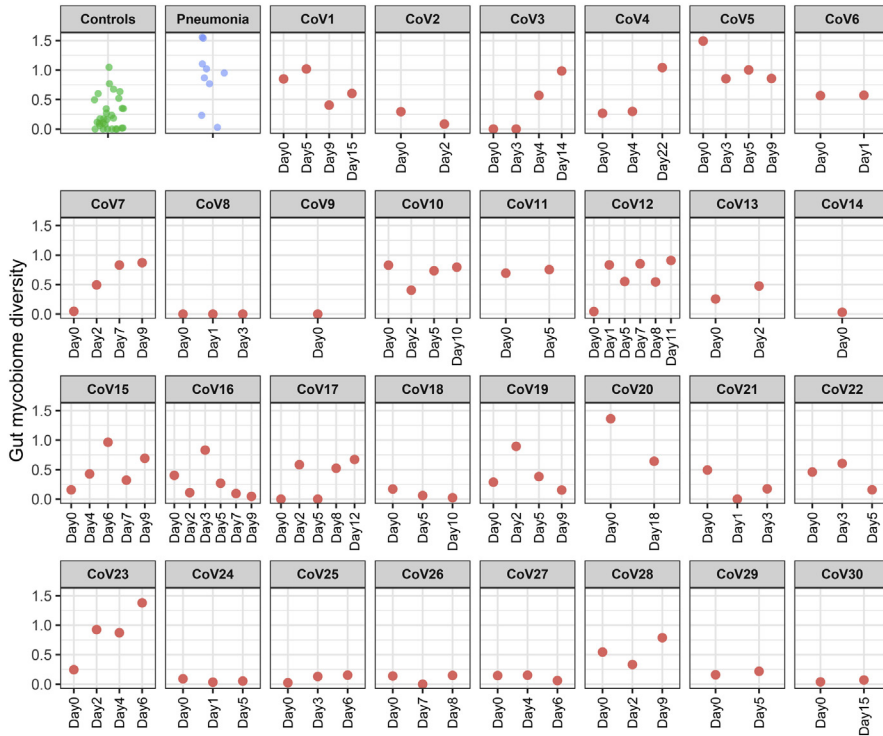
#### Conflict of interest

The authors disclose no conflicts.

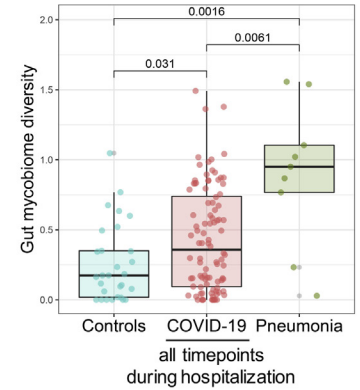
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**A**

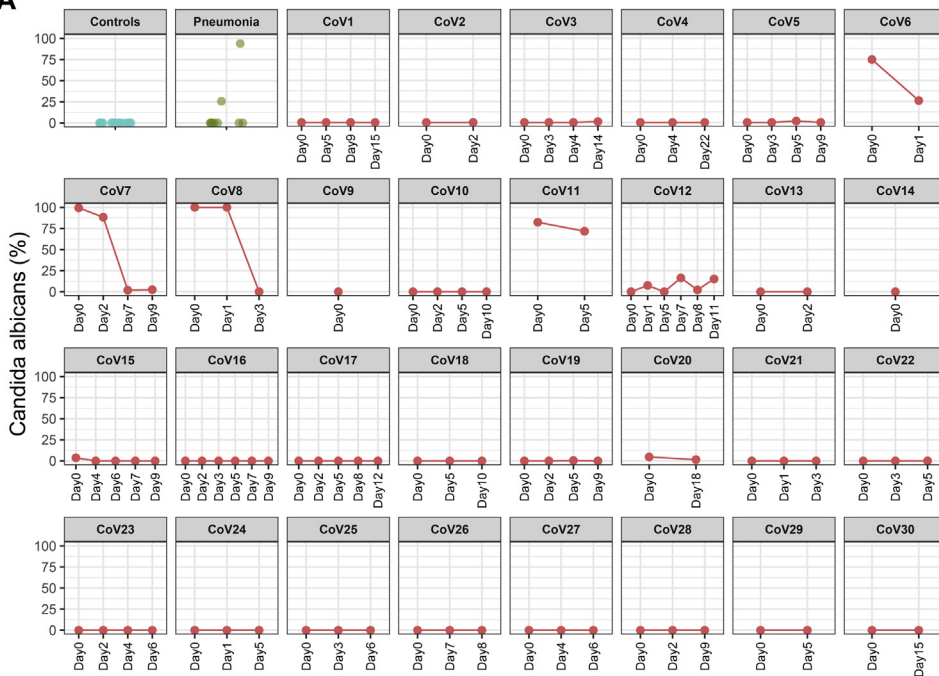


**B**

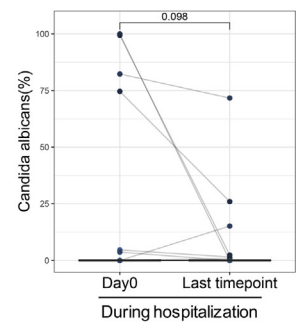


**Supplementary Figure 1.** Temporal changes in the diversity of gut mycobiome (fungal) in COVID-19 patients over time of hospitalization.

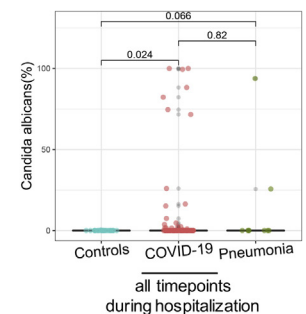
**A**



**B**



**C**



**Supplementary Figure 2.** Temporal changes in the relative abundance of *Candida albicans* in the gut mycobiome of COVID-19 patients over time of hospitalization. (A) Longitudinal changes of *C. albicans* in each COVID-19 case. (B) Alteration in the relative abundance of *C. albicans* compared between baseline and the last follow-up; statistical comparison was conducted by paired Wilcoxon rank sum test. (C) The relative abundance of *C. albicans* across all time points in all patients with COVID-19 during hospitalization, as compared with healthy controls. Statistical comparison was conducted by paired Wilcoxon rank sum test.





**Supplementary Table 1.** Clinical Characteristics of Patients With COVID-19

Case	Sex	Age	Comorbidities	Recent exposure history	Symptoms at admission		Admitted to ICU	Medication		Blood routine lymphocytes <sup>a</sup>	Chest radiograph findings	COVID-19 severity
					Fever and respiratory	GI		Antibiotics	Antiviral			
CoV1	F	65	Hypothyroidism, hypertension, Chronic hepatitis B carrier	No	Fever, cough, sputum	Nil	Yes	Nil	Kaletra, ribavirin	1.0	Bilateral LZ haziness	Critical
CoV2	F	55	None	Contact with person with COVID-19	Fever, runny nose	Nil	No	Nil	Kaletra	1.2	Bilateral LZ haziness	Moderate
CoV3	M	42	None	Travel to Hubei province	Fever, cough	Nil	Yes	Daptomycin meropenem	Nil	0.6	Worsening Right LZ haziness, Right LL collapse re-opened	Critical
CoV4	M	70	Hyperlipidemia, duodenal ulcer	No	Sputum, shortness of breath	Nil	No	Augmentin, doxycycline	Kaletra	0.6	Bilateral lung haziness	Severe
CoV5	M	58	None	No	Fever, cough	Diarrhea	No	Ceftriaxone, augmentin, doxycycline	Kaletra, ribavirin	0.9	Slight Right LZ haziness	Moderate
CoV6	M	71	None	No	Fever, cough, shortness of breath	Nil	No	Nil	Kaletra	1.0	Bilateral lung infiltration	Severe
CoV7	M	48	Diabetes, hypertension, hyperlipidemia	No	Fever, cough	Nil	No	Augmentin	Kaletra, ribavirin	1.3	Left LZ haziness	Moderate
CoV8	F	38	None	No	Fever, cough, sputum, runny nose	Nil	No	Ceftriaxone, doxycycline	Kaletra	0.7	Bilateral LZ infiltrates	Moderate
CoV9	M	33	None	Contact with person with COVID-19	Fever, cough	Nil	No	Doxycycline	Kaletra, ribavirin	0.7	Bilateral LZ haziness	Mild
CoV10	F	70	Obesity, hypertension	No	Cough	Nil	No	Ceftriaxone, piperacillin+tazobactam	Kaletra, ribavirin	0.8	Bilateral LZ haziness	Moderate
CoV11	M	62	Diabetes, hyperlipidemia, left subclavian artery occlusion	No	Fever, cough, sputum, shortness of breath	Nil	No	Sulperazon, Ceftriaxone Disodium, Doxycycline hyclate	Oseltamivir, Kaletra	0.9	Bilateral lung infiltrates	Severe
CoV12	F	71	Hypertension, renal impairment, hyperlipidemia	Contact with person with COVID-19	Cough	Nil	No	Ceftriaxone, azithromycin	Kaletra, ribavirin	2.2	Bilateral LZ haziness	Moderate

Supplementary Table 1.Continued

Case	Sex	Age	Comorbidities	Recent exposure history	Symptoms at admission		Admitted to ICU	Medication		Blood routine lymphocytes <sup>a</sup>	Chest radiograph findings	COVID-19 severity
					Fever and respiratory	GI		Antibiotics	Antiviral			
CoV13	F	47	None	Contact with person with COVID-19	Nil	Nil	No	Nil	Nil	1.9	No definite consolidation	Moderate
CoV14	F	22	None	Contact with person with COVID-19	Fever, runny nose	Nil	No	Nil	Nil	1.8	Nil	Moderate
CoV15	F	46	None	Contact with person with COVID-19	Cough, shortness of breath	Nil	No	Augmentin	Kaletra, ribavirin, interferon beta-1b	1.0	Clear	Mild
CoV16	M	23	None	Travel to Egypt	Fever	Nil	No	Nil	Kaletra	1.4	Clear	Mild
CoV17	M	67	Hypertension	Travel to UK	Fever, cough with white sputum	Diarrhea	No	Augmentin, Azithromycin	Remdesivir, Kaletra, Ribavirin	1.6	Right LL infiltrate	Severe
CoV18	F	34	None	Travel to UK and Spain	Cough, yellowish sputum and whitish sputum, runny nose, sore throat	Nil	No	Nil	Ribavirin, Kaletra	1.7	Left LZ haziness	Mild
CoV19	M	59	Ankylosing spondylitis, Bilateral OA hip	Same building with person with COVID-19	Cough, sore throat, hoarseness, whitish sputum	Nil	No	Augmentin	Ribavirin, Kaletra, Remdesivir	0.7	Clear	Mild
CoV20	F	38	None	No	Fever, cough with whitish sputum, runny nose	Nil	No	Ceftriaxone Disodium, Doxycycline hyclate	Kaletra, Oseltamivir	0.7	Bilateral LZ infiltrates	Moderate
CoV21	F	18	None	Travel to USA	Dry cough, loss of olfactory sensation	Nausea	No	Nil	Nil	1.1	Bilateral LZ haziness	Mild
CoV22	F	38	None	No	Sore throat, cough, fever	Nil	No	Nil	Remdesivir	1.1	Bilateral lung infiltrates	Severe
CoV23	F	63	None	Contact with person with COVID-19	Fever, dry cough	Nil	No	Ceftriaxone Disodium	Kaletra, Ribavirin, Interferon Beta-1B	0.9	Bilateral LZ haziness	Mild

Supplementary Table 1. Continued

Case	Sex	Age	Comorbidities	Recent exposure history	Symptoms at admission		Admitted to ICU	Medication		Blood routine lymphocytes <sup>a</sup>	Chest radiograph findings	COVID-19 severity
					Fever and respiratory	GI		Antibiotics	Antiviral			
CoV24	M	15	None	Travel to UK	Sore throat, cough with yellowish sputum	Nil	No	Nil	Nil	1.0	Clear	Asymptomatic
CoV25	M	28	None	Travel to South Africa, Russia, and transit in UK	Nil	Nil	No	Nil	Nil	1.4	Clear	Mild
CoV26	M	66	Hypertension, hepatoma	Travel to Sydney, contact with Person with COVID-19	throat discomfort	Diarrhea	No	Piperacillin+ Tazobactam injection	Nil	0.8	Mild left LZ infiltrates	Moderate
CoV27	M	63	Cervical radiculopathy, bilateral varicose vein with bilateral Trendelenburg operation	Travel to Egypt	Fever	Nil	No	Nil	Interferon Beta-1B, Ribavirin, Kaletra	1.3	Clear	Mild
CoV28	F	16	None	Travel to UK	Nil	Abdominal pain, nausea, diarrhea	No	Nil	Nil	1.5	No pneumonic changes	Mild
CoV29	M	27	None	Travel to UK	Fever	Nil	No	Augmentin	Nil	0.7	No consolidation	Mild
CoV30	M	19	Eczema	Travel to France and Austria, contacted with person with COVID-19	dry cough, nasal congestion	Nil	No	Nil	Nil	1.2	Clear	Mild

COVID-19, coronavirus disease 2019; GI, gastrointestinal; ICU, intensive care unit; LZ, Lower Zone; LL, Lower Lobe; MZ, Middle Zone

<sup>a</sup>Value in  $\times 10^9/L$ , normal range 1.1–2.9.