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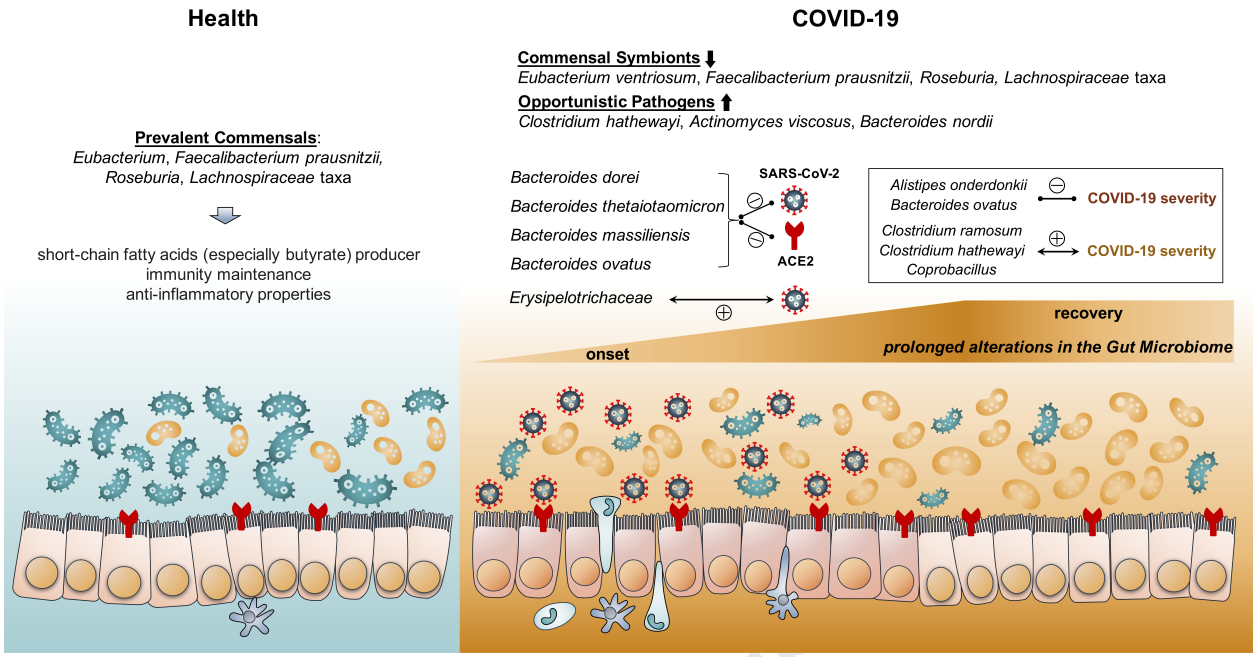

Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization

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Author contribution: TZ and FZ performed the experiments, data analyses and drafted the manuscript. YKY revised the manuscript and provided critical intellectual contribution. AC, HZ, YW, CPC, and NC assisted in experiments and metagenomics sequencing. AYLL collected the human specimens and data. FKLC, GCYL, GJ, CKLL, ZC and DSCH provided critical comments. GCYL, EYKT, KSCF, VC, and LL recruited study subjects. SN, PKSC, and GCYL designed and supervised the study.

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Abstract

Abstract

BACKGROUD & AIMS: Although SARS-CoV-2 infects gastrointestinal tissues, little is known about the roles of gut commensal microbes in susceptibility to and severity of infection. We investigated changes in fecal microbiomes of patients with SARS-CoV-2 infection during hospitalization and associations with severity and fecal shedding of virus.

METHODS: We performed shotgun metagenomic sequencing analyses of fecal samples from 15 patients with COVID-19 in Hong Kong, from February 5 through March 17, 2020. Fecal samples were collected 2 or 3 times per week from time of hospitalization until discharge; disease was categorized as mild (no radiographic evidence of pneumonia), moderate (pneumonia was present), severe (respiratory rate ≥30/min, or oxygen saturation ≤93% when breathing ambient air), or critical (respiratory failure requiring mechanical ventilation, shock, or organ failure requiring intensive care). We compared microbiome data with those from 6 subjects with community-acquired pneumonia and 15 healthy individuals (controls). We assessed gut microbiome profiles in association with disease severity and changes in fecal shedding of SARS-CoV-2.

RESULTS: Patients with COVID-19 had significant alterations in fecal microbiomes compared with controls, characterized by enrichment of opportunistic pathogens and depletion of beneficial commensals, at time of hospitalization and at all timepoints during hospitalization. Depleted symbionts and gut dysbiosis persisted even after clearance of SARS-CoV-2 (determined from throat swabs) and resolution of respiratory symptoms. The baseline abundance of *Coprobacillus*, *Clostridium ramosum*, and *Clostridium hathewayi* correlated with COVID-19 severity; there was an inverse correlation between abundance of *Faecalibacterium prausnitzii* (an anti-inflammatory bacterium) and disease severity. Over the course of hospitalization, *Bacteroides dorei*, *Bacteroides thetaiotaomicron*, *Bacteroides massiliensis*, and *Bacteroides ovatus*, which downregulate expression of ACE2 in murine gut, correlated inversely with SARS-CoV-2 load in fecal samples from patients.

CONCLUSIONS: In a pilot study of 15 patients with COVID-19, we found persistent alterations in the fecal microbiome during the time of hospitalization, compared with controls. Fecal microbiota alterations were associated with fecal levels of SARS-CoV-2 and COVID-19 severity. Strategies to alter the intestinal microbiota might reduce disease severity.

KEY WORDS: coronavirus, bacteria, gut microbiome, fecal nucleic acid

COVID-19 is a respiratory illness caused by a novel coronavirus (SARS-CoV-2) and over 3.3 million people worldwide have been infected as of May 1, 2020. Although most cases of COVID-19 are mild, disease can be severe resulting in hospitalization, respiratory failure, or death [1]. Early reports from Wuhan showed that 2-10% of COVID-19 patients had gastrointestinal (GI) symptoms including diarrhea but a recent meta-analysis reported that up to 20% had GI symptoms [2-5]. Studies have detected SARS-CoV-2 virus in anal swabs and stool samples in almost 50% of COVID-19 patients, suggesting that the digestive tract might be an extra-pulmonary site for virus replication and activity[6, 7]. Moreover, fecal calprotectin were found to be elevated in COVID-19 patients with diarrhea[8], an indicator of inflammatory responses in the gut. SARS-CoV-2 uses the angiotensin converting enzyme 2 (ACE2) receptor to enter the host and this receptor is highly expressed in both the respiratory and gastrointestinal tract[9, 10, 11]. ACE2 is important in controlling intestinal inflammation and gut microbial ecology[12]. With trillions of diverse bacteria dwelling in our gut, the gut microbiome has a myriad of effects on gene regulation of immune response and metabolism. The commensal microbiota ecosystem in the gut is dynamic and can be regulated by invading viruses to facilitate a stimulatory or suppressive response [13]. Studies have shown that respiratory viral infections may be associated with altered gut microbiome, which predispose patients to secondary bacterial infections [14, 15]. Recent meta-transcriptome sequencing of bronchoalveolar lavage fluid showed that the microbiota in SARS-CoV-2 infected patients was dominated by pathogens or oral and upper respiratory commensal bacteria[16]. In addition, co-morbidities commonly associated with severe COVID-19 are known to be associated with alterations in bacteria taxa from the phyla Bacteroidetes and Firmicutes [17-20], which were reported to regulate ACE2 expression in rodents [21]. There is an urgent need to understand host microbial perturbations that underlies SARS-CoV-2 infection, which may impact response to infection and efficacy of various future immune interventions, such as vaccines [22].

In this pilot study, we hypothesize that the intestinal microbiota is altered in SARS-CoV-2 infection and is associated with susceptibility to severe disease. We prospectively included 15 hospitalised COVID-19 patients admitted between February 16, 2020 and March 2, 2020 in Hong Kong, China, followed from hospital admission until discharge. Through the application of deep shotgun metagenomics, we investigated longitudinal changes of the gut microbiome in COVID-19.

Methods

Study subject and design

This prospective study involved 15 COVID-19 patients hospitalized with laboratory-confirmed SARS-CoV-2 infection, six patients hospitalized with community acquired pneumonia (pneumonia controls) and fifteen healthy individuals (healthy controls) (**Table 1, Supplementary Table 1, Figure 1**). SARS-CoV-2 infection was confirmed by two consecutive RT-PCR test 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 two respiratory samples. COVID-19 patients and pneumonia controls were admitted to the Prince of Wales Hospital or the United Christian Hospital, Hong Kong. Healthy controls were 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. All subjects were recruited between Feb 5 and Mar 17, 2020. Severity of COVID-19 infection was categorized as (i) mild, if there was no radiographic evidence of pneumonia; (ii) moderate, if pneumonia was present along with fever and respiratory tract symptoms ; (iii) severe, if respiratory rate ≥30/min, oxygen saturation ≤93% when breathing ambient air, or PaO_2 / FiO_2 ⩽ 300 mmHg (1mmHg = 0.133 kPa); or (iv) critical, if there was respiratory failure requiring mechanical ventilation, shock, or organ failure requiring intensive care[23]. 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 medical therapy were extracted from the electronic medical records in the Hong Kong Hospital Authority clinical management system. Fecal samples from COVID-19 patients were collected serially 2-3 times per week until discharge. This study was conducted in accordance with the Declaration of Helsinki.

Detection of fecal SARS-CoV-2 viral load

SARS-CoV-2 viral loads in stool were measured using real-time reverse-transcriptase-polymerase chain-reaction (RT-PCR) assay. Viral RNA from stool samples was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). 0.1g of stool was suspended in 1 mL of viral transport medium (in 1:10 dilution) and centrifuged for 20 min at 4000g. A 140-µL aliquot of the filtrate was used as starting material following the manufacturer's protocol. SARS-CoV-2 RNA was quantified using real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR). The primer-probe set N1 (2019-nCoV_N1-F: 5'-GAC CCC AAA ATC AGC GAA AT-3', 2019-nCoV_N1-R: 5'-TCT GGT TAC TGC CAG TTG AAT

CTG-3' and 2019-nCoV_N1-P: 5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3') designed by US Centers for Disease Control and Prevention (CDC) were purchased from Integrated DNA Technologies, USA. The one-step real-time RT-PCR reaction contained 10 µL of the extracted preparation, 4 µL T*aq*Man™ Fast Virus 1-Step Master Mix (Applied Biosystems, USA) in a final reaction volume of 20 µL. The primer and probe concentration were 0.5 µM and 0.125 µM, respectively. The cycling conditions, 25°C for 2 min, 50°C for 15 min, 95 °C for 2 min, followed by 45 cycles of 95 °C for 15 s, and 55 °C for 30 s, were performed with the StepOnePlus Real-Time PCR System (Applied Biosystems, USA). The cycle threshold (Ct) values of real time RT-PCR were converted into viral RNA copies based on a standard curve prepared from 10-fold serial dilutions of know copies of plasmid containing the full N gene (2019-nCoV_N_Positive Control, Integrated DNA Technologies, USA). Samples were considered as negative if the Ct values exceeded 39.9 cycles. The detection limit of real-time RT PCR was 347 copies/mL.

Microbial profiling of fecal samples with metagenomic sequencing

Approximately 0.1g fecal sample was prewashed with 1 ml ddH_2O and pelleted by centrifugation at 13,000×g for 1 min. The fecal DNA was subsequently extracted from the pellet using Maxwell® RSC PureFood GMO and Authentication Kit (Promega, Madison, Wisconsin) following manufacturer's instructions. Briefly, fecal pellet was added 1ml of CTAB buffer and vortexed for 30 seconds, then heating sample at 95°C for 5 minutes. After that, the samples were vortexed thoroughly with beads at maximum speed for 15 min. Then 40µl of proteinase K and 20µl 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 13,000×g for 5 min and was added in Maxwell® RSC machine for DNA extraction. 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). Libraries were subsequently sequenced on our in-house sequencer Illumina NextSeq 550 (150bp paired-end) at Center for microbiota research, The Chinese University of Hong Kong. Raw sequence reads were filtered and quality-trimmed using Trimmomatic v0.36[24] as follows: 1) Trimming low quality base (quality score < 20); 2) Removing reads shorter than 50bp; 3) removing sequencing adapters. Contaminating human reads were filtering using Kneaddata (Reference database: GRCh38 p12) with default parameters. Profiling of bacterial communities was performed using MetaPhlAn2 (*V2.9*) by mapping reads to clade-specific markers[25].

Statistical analysis

Relative data from MetaPhlAn2 were imported into R v3.5.1. Non-metric Multi-dimensional Scaling (NMDS) analyses were performed on all baseline fecal microbiomes between groups, and serial fecal microbiomes in each COVID-19 case during the disease course, based on Bray-Curtis dissimilarities using vegan package (v2.5-3). Differential bacterial taxa between COVID-19 patients (with or without antibiotics treatment at inclusion), community-acquired pneumonia patients, and healthy controls were identified using Multivariate Association with Linear Models (MaAsLin)[26]. Spearman correlation analyses were conducted to associate baseline microbiome profiles of 7 antibiotics naïve patients at baseline with COVID-19 severity, and to associate longitudinal fecal SARS-CoV-2 loads with timepoint-matched bacterial profiles across all 15 COVID-19 patients, while adjusting for confounding factors.

Data availability

Metagenomics Sequencing dataset was deposited to the NCBI Sequence Read Archive under BioProject accession number PRJNA624223.

Results

Fecal microbiome alterations in COVID-19

Among the fifteen COVID-19 patients, 7 were antibiotics-naïve [COVID-19(abx-)] and 8 received empirical antibiotics [COVID-19(abx+)] at baseline (defined as date of the first stool collection after hospitalization). The median age of COVID-19 patients, pneumonia controls and healthy controls were 55, 50 and 48 years old, respectively. 40% and 100% of COVID-19 patients and pneumonia controls, respectively, had underlying co-morbidities **(Table 1; supplementary table 1).** All COVID-19 patients presented with respiratory symptoms but only one had diarrhea at presentation. None of the patients developed GI symptoms during hospitalization. Median duration of hospitalization was 21±2.4 days (mean±s.e.) in COVID-19 and pneumonia cases.

To understand alterations of the gut microbiome that underlies SARS-CoV-2 infection, we compared baseline fecal microbiome of COVID-19 patients with healthy controls and pneumonia controls adjusting for age, gender, antibiotic use and co-morbidities. Antibiotic-naïve COVID-19 patients were enriched in opportunistic pathogens known to cause bacteremia[27, 28], including *Clostridium hathewayi*, *Actinomyces viscosus* and *Bacteroides nordii* compared with controls (**Table 2**). COVID-19 (abx+) patients demonstrated a further depletion of multiple bacterial species, which are symbionts beneficial to host immunity including *Fecalibacterium prausnitzii*, *Lachnospiraceae bacterium 5_1_63FAA, Eubacterium rectale*, *Ruminococcus obeum*, and *Dorea formicigenerans* compared to COVID-19 (abx-) patients **(Table 2).** Regardless of antibiotic use, underrepresented bacterial species in COVID-19 patients were consistently absent or present at very low abundance during the disease course, even when SARS-CoV-2 virus was cleared from the nasopharyngeal swab and stool, and respiratory symptoms had resolved (**Supplementary Figure 1-6**).

Among all host factors, COVID-19 infection showed the largest effect size in affecting the gut microbiome (PERMANOVA test, R^2=0.066, p=0.002, **Figure 2a**), followed by hyperlipidemia, pneumonia, and antibiotics, while age and gender showed no significant effects on gut microbiome alterations (**Figure 2a**). At the whole microbiome community level, healthy subjects' fecal microbiome clustered together whereas that of COVID-19 (Abx-) patients clustered separately (PERMANOVA test, p=0.001) and were more heterogeneous (**Figure 2b**). Antibiotic treatment in COVID-19 patients was associated with a more heterogenous microbiome configuration and accompanied by further shift of the gut microbiome away from a healthy microbiome (**Figure 2b**).

We next explored whether recovery from SAR-CoV-2 infection was associated with restoration of gut microbiome to a community level similar to that of healthy individuals. Overall, the gut microbiome of all COVID-19 patients remained stable but were markedly disparate from that of healthy controls, both during the disease course and after clearance of SARS-CoV-2 (**Figure 2c**). While five COVID-19 patients' microbiome (CoV1, 4, 7, 11, 15) showed closer proximity to healthy microbiomes over time, patients CoV3, 5, 8, 10, and 12 became more disparate from healthy microbiomes over time (**Supplementary Figure 7**). At the last follow-up, the gut microbiome of these ten patients remained substantially different from that of healthy controls, despite clearance of SARS-CoV-2 infection as defined by negative SARS-CoV-2 tests on nasopharyngeal swab or deep throat saliva (**Figure 2c, Supplementary Figure7**). Of note, patient CoV4 was discharged on Day 5 but his gut microbiome on Day 22 were persistently different from that of healthy individuals.

Baseline gut microbiome and disease severity of COVID-19

To understand whether baseline gut microbiome impacts the severity of COVID-19, we assessed association between baseline fecal microbiome and COVID-19 severity (mild, moderate, severe, or critical) in seven antibiotic-naïve COVID-19 cases. A total of 23 bacterial taxa were found to be significantly associated with COVID-19 disease severity, most of which (15 out of 23) were from the Firmicutes phylum (**Table 3**). Among them, 8 and 7 Firmicutes members, respectively, showed positive and negative correlation with disease severity. These data are in line with a report showing that different Firmicutes bacteria have diverse roles in up-regulating or down-regulating ACE2 expression in the murine gut [21]. Our finding of the association of gut Firmicutes bacteria with COVID-19 severity highlights the potential importance of bacterial membership in modulating human response to SARS-CoV-2 infection.

Three bacterial members from the Firmicutes phylum, the genus *Coprobacillus*, the species *Clostridium ramosum* and *Clostridium hathewayi*, were the top bacteria positively associated with COVID-19 disease severity (Spearman correlation coefficient $Rho>0.9$, $p<0.01$, **Table 3**). Both *Clostridium ramosum* and *Clostridium hathewayi* have been associated with human infection and bacteremia[27, 29]. Importantly, *Coprobacillus* bacterium has been shown to strongly up-regulate colonic expression of ACE2 in the murine gut [21]. In contrast, two beneficial species *Alistipes onderdonkii* and *Faecalibacterium prausnitzii* were top bacterial species to show a negative correlation with COVID-19 severity (**Table 3**). *Alistipes* species are indole positive, involved in the serotonin precursor tryptophan metabolism and in maintaining gut immune homeostasis[30, 31], while *Faecalibacterium prausnitzii* has anti-inflammatory properties [32] . Although we cannot assign a causative or preventive role of

these bacteria in disease pathogenesis or severity, our data underscore a potential role for bacteria in determining response to SARS-CoV-2 infection and intensity of the infection in the host.

Fecal SARS-CoV-2 virus load and gut bacterial abundance

Eleven of the 15 patients had SARS-CoV-2 nucleic acid detected in feces at hospitalization (median 3.86×10^3 copies per mL inoculum, as determined by RT-PCR) and five of them cleared the SARS-CoV-2 virus over time (**Figure 3a**). We investigated whether gut bacteria were associated with fecal SARS-CoV-2 load over the course of hospitalization. A total of 14 bacterial species were identified to be significantly associated with fecal viral load of SARS-CoV-2 across all fecal samples (**Figure 3b**). Among them, 6 species were from the Bacteroidetes phylum. Four Bacteroides species, including *Bacteroides dorei*, *Bacteroides thetaiotaomicron*, *Bacteroides massiliensis*, and *Bacteroides ovatus*, showed significant inverse correlation with fecal SARS-CoV-2 load (all Spearman correlation coefficient *Rho*<-0.2, *p*<0.05, **Figure 3b**). Interestingly, all these four species were associated with down-regulation of ACE2 expression in the murine colon [21]. Taken together, these data suggest that *Bacteroides* species may have a potential protective role in combating SARS-CoV-2 infection by hampering host entry through ACE2. In contrast, *Erysipelotrichaceae bacterium 2_2_44A*, a Firmicutes species, showed the strongest positive correlation with fecal SARS-CoV-2 load (Spearman correlation coefficient *Rho*=0.89, *p*=0.006, **Figure 3b**). *Erysipelotrichaceae* has been implicated in inflammation-related disorders of the GI tract[33]. Considering the strong association of baseline abundance of *Erysipelotrichaceae* with COVID-19 severity (Spearman correlation *Rho*=0.89, *p*=0.006, **Table 3**), gut *Erysipelotrichaceae* may play a role in augmenting SARS-CoV-2 infection in the host gut.

Discussion

We showed for the first time that the gut microbiome was disturbed in patients with COVID-19. The alterations, observed even in COVID-19 patients naïve to antibiotic therapy, were characterized by enrichment of opportunistic pathogens and depletion of beneficial commensals (**Figure 4**). Loss of salutary species in COVID-19 persisted in the majority of patients despite clearance of SARS-CoV-2 virus, suggesting that exposure to SARS-CoV-2 infection and/or hospitalization may be associated with a more long-lasting detrimental effect to the gut microbiome.

Studies have shown that respiratory viral infections can alter the gut microbiome, such as pulmonary infections by influenza and respiratory syncytial virus (RSV) [15, 34-36]. Viral infections predispose patients to secondary bacterial infections, which often have a more severe clinical course[14, 37]. We found that a number of pathogens and opportunistic pathogens were enriched in the gut microbiome of COVID-19 patients including *Clostridium hathewayi*, *Bacteroides nordii*, *Actinomyces viscosus* and a higher baseline abundance of *Clostridium hathewayi* correlated with more severe COVID-19. Most of these bacteria are bacteraemia-associated bacteria, indicating susceptibility for severe disease course due to potential secondary bacterial infection. We also identified an opportunistic pathogen of the oral cavity and upper respiratory tract, *Actinomyces viscosus,* in the gut of COVID-19 patients [38]. Its presence suggests the passage or transmission of extra-intestinal microbes into the gut.

Recently, a study provided direct evidence that SARS-CoV-2 can bind to human ACE2 as host entry point [9]. ACE2 is highly expressed in the intestine especially in colonocytes of healthy subjects and in patients with inflammatory bowel disease [10], and can regulate amino acid transport, microbial ecology and inflammation in the gut [12]. Interestingly, Bacteroidetes species have been shown to down-regulate ACE2 expression in the murine colon, whereas Firmicutes species showed variable effects in modulating ACE2 expression [21]. We found that baseline abundance of Bacteroidetes species, *Alistipes onderdonkii* and *Bacteroides ovatus*, negatively correlated with COVID-19 severity, and four species from the genus Bacteroides of the phylum Bacteroidetes (*Bacteroides dorei*, *Bacteroides thetaiotaomicron*, *Bacteroides massiliensis*, and *Bacteroides ovatus*) showed inverse correlation with fecal viral load of SARS-CoV-2 (**Figure 4**). Among them, *Bacteroides dorei* has been reported to suppress colonic ACE2 expression[21] and to calibrate host immune response [39, 40]. The highest SARS-CoV-2 mortality and morbidity has been reported in older patients and in those with underlying chronic diseases that are associated with inflammation such as hypertension, obesity, diabetes mellitus and coronary artery disease[41-43]. Interestingly, these

subjects were also reported to have a lower abundance of Bacteroides species than healthy individuals [17-20]. These findings altogether suggest that an individual's gut microbiome configuration may affect the subject's susceptibility and response to SARS-CoV-2 infection.

Cytokine profile associated with hyperinflammation state in severe COVID-19 has been characterized by increased interferon-γ inducible protein and other cytokines. Given limited proven treatment for COVID-19, understanding host cytokine pathways and microbiota interactions with cytokine responses in SARS-CoV-2 infection is essential in developing new treatment approaches[44].

One major limitation of this exploratory study is the modest sample size. Although assigning a causative relationship between COVID-19 and gut dysbiosis requires larger validation studies, this pilot study presents the first data to examine the influence of SARS-CoV2 infection on gut microbiome composition and dynamics. We attempted to adjust for factors, such as age, gender, therapy, comorbidities, which may explain the observed variance in the data. As we included only hospitalized patients with moderate/severe disease, such findings may not be generalizable to all COVID-19 cases, including those with mild or asymptomatic COVID-19. Stool collected after hospitalization for microbiome analysis does not represent the *bona fide* baseline microbiome at COVID-19 onset, nor the baseline microbiome before disease onset. Further studies should prospectively include asymptomatic subjects, and if infected with SARS-CoV-2, followed up at disease onset, during disease course, and long term after discovery to delineate the role of microbiome changes in SARS-CoV-2 infection and post-infection recovery.

The use of empirical antibiotics (which was common in the initial outbreak of SARS-CoV-2 when secondary bacterial infection was a concern) led to further loss of salutary symbionts and exacerbation of gut dysbiosis in COVID-19 patients and our data support avoidance of unnecessary antibiotics use in the treatment of viral pneumonitis, as antibiotics can eliminate beneficial bacteria and weaken the gut barrier[45]. In addition, antibiotics-driven gut microbiome perturbation can alter immunity to vaccines in humans[46]. Improving efficacy of future immune interventions such as vaccines, through modulating the gut microbiome, in combating COVID-19 should be considered. One approach for promoting a healthy microbiome may include measures to enhance intestinal butyrate production through the promotion of microbial interactions by dietary changes, and reduction of pro-inflammatory states.

In conclusion, our study provides evidence of prolonged gut microbiome dysbiosis in COVID-19 and its association with fecal SARS-CoV-2 virus shedding and disease severity. These data highlight a new concept that novel and targeted approach of modulation of the gut microbiota may represent a therapeutic avenue for COVID-19 and its co-morbidities.

Reference

1. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. Jama 2020.
2. **Huang C, Wang Y, Li X**, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet 2020;395:497-506.
3. C**hen N, Zhou M, Dong X**, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet 2020;395:507-513.
4. **Liang W, Feng Z, Rao S**, et al. Diarrhoea may be underestimated: a missing link in 2019 novel coronavirus. Gut 2020.
5. **Cheung KS, Hung IFN**, Chan PPY, et al. Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from the Hong Kong cohort and systematic review and meta-analysis. Gastroenterology 2020.
6. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020.
7. **Xu Y, Li X, Zhu B**, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nature Medicine 2020:1-4.
8. Effenberger M, Grabherr F, Mayr L, et al. Faecal calprotectin indicates intestinal inflammation in COVID-19. Gut 2020.
9. **Shang J, Ye G, Shi K**, et al. Structural basis of receptor recognition by SARS-CoV-2. Nature 2020:1-8.
10. Wang J, Zhao S, Liu M, et al. ACE2 expression by colonic epithelial cells is associated with viral infection, immunity and energy metabolism. medRxiv 2020.
11. **Xiao F, Tang M, Zheng X**, et al. Evidence for gastrointestinal infection of SARS-CoV-2. Gastroenterology 2020.
12. **Hashimoto T, Perlot T,** Rehman A, et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature 2012;487:477-481.
13. Ma W-T, Pang M, Fan Q-L, et al. The commensal microbiota and viral infection: a comprehensive review. Frontiers in immunology 2019;10:1551.
14. Hanada S, Pirzadeh M, Carver KY, et al. Respiratory viral infection-induced Microbiome alterations and secondary bacterial pneumonia. Frontiers in immunology 2018;9:2640.
15. Yildiz S, Mazel-Sanchez B, Kandasamy M, et al. Influenza A virus infection impacts systemic microbiota dynamics and causes quantitative enteric dysbiosis. Microbiome 2018;6:9.
16. **Shen Z, Xiao Y, Kang L**, et al. Genomic diversity of SARS-CoV-2 in Coronavirus Disease 2019 patients. Clinical Infectious Diseases 2020.
17. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. nature 2006;444:1027.
18. Emoto T, Yamashita T, Sasaki N, et al. Analysis of gut microbiota in coronary artery disease patients: a possible link between gut microbiota and coronary artery disease. Journal of atherosclerosis and thrombosis 2016:32672.
19. Yang T, Santisteban MM, Rodriguez V, et al. Gut dysbiosis is linked to hypertension. Hypertension 2015;65:1331-1340.
20. Ley RE, Turnbaugh PJ, Klein S, et al. Human gut microbes associated with obesity. nature 2006;444:1022-1023.
21. **Geva-Zatorsky N, Sefik E**, Kua L, et al. Mining the human gut microbiota for immunomodulatory organisms. Cell 2017;168:928-943. e11.

22. Kourosh Kalantar-Zadeh, Stephanie A. Ward, Kamyar Kalantar-Zadeh, et al. Considering the Effects of Microbiome and Diet on SARS-CoV‑2 Infection: Nanotechnology Roles. ACS Nano 2020.
23. **Wu J, Liu J, Zhao X**, et al. Clinical characteristics of imported cases of COVID-19 in Jiangsu province: a multicenter descriptive study. Clinical Infectious Diseases 2020.
24. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114-2120.
25. Segata N, Waldron L, Ballarini A, et al. Metagenomic microbial community profiling using unique clade-specific marker genes. Nature methods 2012;9:811.
26. Morgan XC, Tickle TL, Sokol H, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome biology 2012;13:R79.
27. Elsayed S, Zhang K. Human infection caused by Clostridium hathewayi. Emerging infectious diseases 2004;10:1950.
28. Dakshinamoorthy M, Venkatesh A, Arumugam K. A Literature Review on Dental Caries Vaccine-A Prevention Strategy. Indian Journal of Public Health Research & Development 2019;10:3041-3043.
29. Forrester JD, Spain DA. Clostridium ramosum bacteremia: case report and literature review. Surgical infections 2014;15:343-346.
30. Gao J, Xu K, Liu H, et al. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. Frontiers in cellular and infection microbiology 2018;8:13.
31. Verdu EF, Hayes CL, O'Mahony SM. Importance of the microbiota in early life and influence on future health. The Gut-Brain Axis: Elsevier, 2016:159-184.
32. **Miquel S**, Martin R, Rossi O, **Wells J, Langella P**, et al. Faecalibacterium prausnitzii and human intestinal health. Current opinion in microbiology 2013;16:255-261.
33. Kaakoush NO. Insights into the role of Erysipelotrichaceae in the human host. Frontiers in cellular and infection microbiology 2015;5:84.
34. **Wang J, Li F**, Wei H, et al. Respiratory influenza virus infection induces intestinal immune injury via microbiota-mediated Th17 cell–dependent inflammation. Journal of Experimental Medicine 2014;211:2397-2410.
35. Deriu E, Boxx GM, He X, et al. Influenza virus affects intestinal microbiota and secondary salmonella infection in the gut through type I interferons. PLoS pathogens 2016;12:e1005572.
36. **Groves HT**, Cuthbertson L, James P, **Moffatt M, J. Cox M, Tregoning J, e**t al. Respiratory disease following viral lung infection alters the murine gut microbiota. Frontiers in immunology 2018;9:182.
37. Brundage JF. Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. The Lancet infectious diseases 2006;6:303-312.
38. Habib S, Siddiqui AH, Azam M, et al. Actinomyces viscosus causing disseminated disease in a patient on methotrexate. Respiratory medicine case reports 2018;25:158-160.
39. **Vatanen T, Kostic AD, d'Hennezel E,** et al. Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. Cell 2016;165:842-853.

40. **Yoshida N, Emoto T, Yamashita T**, et al. Bacteroides vulgatus and Bacteroides dorei reduce gut microbial lipopolysaccharide production and inhibit atherosclerosis. Circulation 2018;138:2486-2498.
41. Qingxian C, Fengjuan C, Fang L, et al. Obesity and COVID-19 Severity in a Designated Hospital in Shenzhen, China. China (3/13/2020) 2020.
42. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? The Lancet Respiratory Medicine 2020;8:e21.
43. Hill MA, Mantzoros C, Sowers JR. Commentary: COVID-19 in Patients with Diabetes. Metabolism 2020.
44. Mendes V, Galvao I, Vieira AT. Mechanisms by which the gut microbiota influences cytokine production and modulates host inflammatory responses. Journal of Interferon & Cytokine Research 2019;39:393-409.
45. Tulstrup MV-L, Christensen EG, Carvalho V, et al. Antibiotic treatment affects intestinal permeability and gut microbial composition in Wistar rats dependent on antibiotic class. PLoS One 2015;10.
46. **Hagan T, Cortese M, Rouphael N**, et al. Antibiotics-driven gut microbiome perturbation alters immunity to vaccines in humans. Cell 2019;178:1313-1328. e13.

Author names in bold designate shared co-first authorship

Figure legends

Table 1. Subjects characteristics

Table 2. Gut microbiome features in patients with COVID-19

Table 3. Gut bacteria correlated with COVID-19 severity

Figure 1. Schematic diagram of stool sample collection, SARS-CoV-2 PCR test results and hospitalization duration in patients with COVID-19 (n=15). “CoV” denotes patient with COVID-19. Stool specimens were serially collected for shotgun metagenomics sequencing and RT-qPCR test for SARS-CoV-2 virus; “D0” denotes baseline date when the first stool was collected after hospitalization; the following timepoints starting with ‘D’ represents days since baseline stool collection. “+ve throat swab”: the first positive result for SARS-CoV-2 virus in nasopharyngeal/throat/pooled swabs; “-ve throat swab”: the first negative result for SARS-CoV-2 virus in two consecutive negative nasopharyngeal/throat/pooled swab tests, upon which patient was then discharged.

Figure 2. Gut microbiome alterations in COVID-19 patients and longitudinal changes over the disease course. **(A),** the effect size of subject metadata in gut microbiome composition, as determined by PERMANOVA test. $^{**}p<0.01$; $^{*}p<0.05$. **(B)**, microbiome community alterations in COVID-19, viewed by NMDS (Non-metric multidimensional scaling) plot based upon Bray-Curtis dissimilarities. The microbiomes were compared between healthy controls (n=15), COVID-19 (abx-, n=7), COVID-19 (abx+, n=8), and pneumonia controls (n=6). **(C)**, Dissimilarity of the gut microbiome of COVID-19 patients to that of healthy controls during the disease course. The microbiome dissimilarity was calculated as bray-curtis dissimilarity. The grey area denotes the rang of bray-curtis dissimilarities among gut microbiomes of healthy controls, with the solid black line indicates the median dissimilarity among healthy individuals. “CoV” denotes patient with COVID-19. “D0” denotes baseline date when the first stool was collected after hospitalization; the following timepoints starting with ‘D’ represents days since baseline stool collection.

Figure 3. Correlation between gut bacteria and fecal SARS-CoV-2 shedding in COVID-19 patients over the disease course. **(A)**, longitudinal changes in fecal viral loads of COVID-19 patients. **(B)**, Bacteria significantly associated with fecal viral load during disease course, as determined by spearman correlation test.

Figure 4. Schematic summary of the gut microbiome alterations in COVID-19. In healthy individuals, *Eubacterium*, *Faecalibacterium prausnitzii, Roseburia,* and *Lachnospiraceae* taxa are prevalent in their gut microbiome. However, the gut microbiome of COVID-19 patients is characterized by enrichment of opportunistic pathogens and depletion of commensals in the gut. Such gut dysbiosis persists during the COVID-19 disease course, even after clearance/recovery of SARS-CoV-2 infection. Baseline fecal abundance of the bacteria *Coprobacillus*, *Clostridium ramosum*, and *Clostridium hathewayi* showed significant correlation with COVID-19 severity whereas an anti-inflammatory bacterium *Faecalibacterium prausnitzii* showed an inverse correlation. Four Bacteroidetes members, including *Bacteroides dorei*, *Bacteroides thetaiotaomicron*, *Bacteroides massiliensis*, and *Bacteroides ovatus*, known to down-regulate ACE2 expression in the murine gut, showed significant inverse correlation with fecal SARS-CoV-2 viral load in patients with COVID-19.

Table 1. Subjects characteristics

Variables	COVID-19 cases	Pneumonia controls	Healthy controls
Number	15	6	15
Male	7 (47%)	4 (67%)	9 (60%)
Median Age, years (IQR)	55 (44, 67.5)	50 (44, 65)	48 (45, 48)
Co-morbidities	6 (40%)	6 (100%)	0 (0%)
Recent exposure history			
Travel to cities of Hubei province	1 (7%)	0 (0%)	0 (0%)
Contact with person with COVID19	5 (33%)	0 (0%)	0 (0%)
Have family cluster outbreak	4 (27%)	0 (0%)	0 (0%)
Symptoms at admission			
Fever	9 (60%)	4 (67%)	
Gastrointestinal symptoms			
Diarrhea	1 (7%)	2(33%)	
Respiratory symptoms			
Cough	11 (73%)	4 (67%)	
Sputum	5 (33%)	3 (50%)	
Rhinorrhea	3 (20%)	1 (17%)	
Shortness of breath	4 (27%)	3 (50%)	
Blood result			
Lymphocyte counts ($x10^9$/L, normal range 1.1-2.9)	0.9 (0.7, 1.1)	1.1 (0.9, 1.2)	
Antibiotic therapy at presentation	7 (47%)	6 (100%)	

Amoxycillin Clavulanate	4 (27%)	3 (50%)	
Cephalosporin	5 (33%)	6 (100%)	
Tetracycline	4 (27%)	0 (0%)	
Antiviral therapy	13 (87%)	0 (0%)	
Lopinavir-Ritonavir	13 (87%)	0 (0%)	
Ribavirin	7 (47%)	0 (0%)	
Interferon beta-1b	1 (7%)	0 (0%)	
Death	0 (0%)	0 (0%)	

#Values are express in number (percentage) and median (interquartile range)

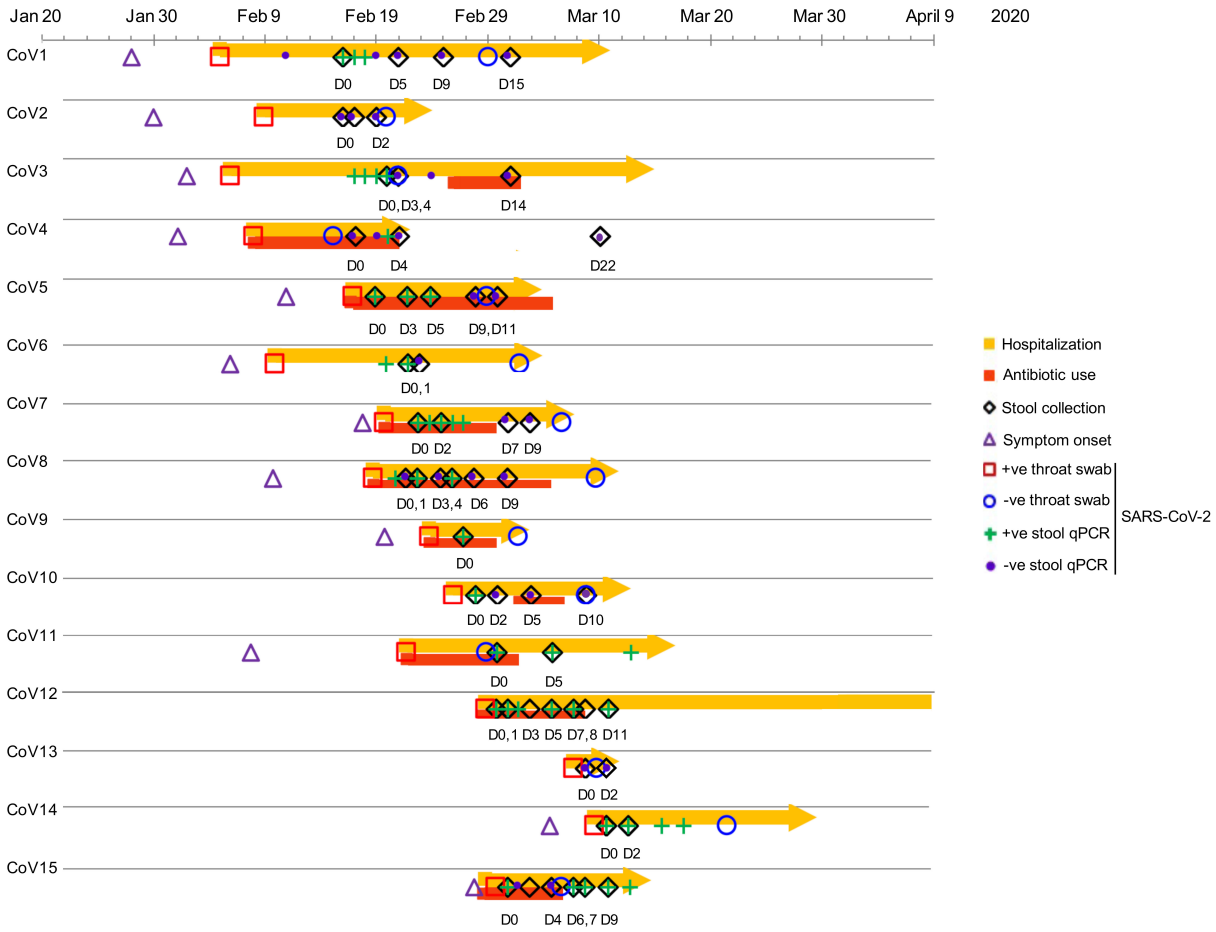
Table 2 Gut microbiome features in patients with COVID-19

Gut microbiome feature	Taxon	Group	Coefficient	*P* value	*Q* value
COVID-19 (antibiotics naïve: abx-)					
Specifically enriched in COVID-19(Abx-)	p_Actinobacteria|c_Actinobacteria|o_Actinomycetales|f_Actinomycetaceae|g_Actinomyces|s_Actinomyces_viscosus	COVID-19 (Abx-)	0.243	8.2E-	6.4E-05
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Clostridiaceae|g_Clostridium|s_Clostridium_hathewayi	COVID-19 (Abx-)	1.130	4.8E-	2.5E-03
	p_Bacteroidetes|c_Bacteroidia|o_Bacteroidales|f_Bacteroidaceae|g_Bacteroides|s_Bacteroides_nordii	COVID-19 (Abx-)	0.164	2.3E-	8.5E-03
Underrepresented in both COVID-19 and pneumonia	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Eubacteriaceae|g_Eubacterium|s_Eubacterium_ventriosum	COVID-19 (Abx-)	-0.280	1.2E-04	2.5E-02
COVID-19 (antibiotics exposed: abx+)					
Underrepresented in COVID-19(Abx+)	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Lachnospiraceae|g_Dorea|s_Dorea_formicigenerans	COVID-19 (Abx+)	-0.812	2.6E-	0.00853
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Lachnospiraceae|g_Blautia	COVID-19 (Abx+)	-0.441	6.1E-	0.01491
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Ruminococcaceae|g_Faecalibacterium	COVID-19 (Abx+)	-0.537	3.2E-	0.00924
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Ruminococcaceae|g_Faecalibacterium|s_Faecalibacterium_prausnitzii	COVID-19 (Abx+)	-0.537	3.2E-	0.00924
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Eubacteriaceae	COVID-19 (Abx+)	-0.289	4.6E-	0.01236

	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Eubacteriaceae|g_Eubacterium	COVID-19 (Abx+)	-0.289	4.6E-	0.01236
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Eubacteriaceae|g_Eubacterium|s_Eubacterium_rectale	COVID-19 (Abx+)	-0.903	1.7E-	0.03316
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Ruminococcaceae	COVID-19 (Abx+)	-0.300	2.0E-	0.03709
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Lachnospiraceae|g_Roseburia	COVID-19 (Abx+)	-0.598	2.3E-	0.04018
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Lachnospiraceae|g_Coprococcus	COVID-19 (Abx+)	-0.447	1.6E-	0.03239
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Lachnospiraceae|g_Blautia|s_Ruminococcus_obeum	COVID-19 (Abx+)	-0.623	4.3E-	0.00243
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Lachnospiraceae|g_Lachnospiraceae_noname|s_Lachnospiraceae_bacterium_5_1_63FAA	COVID-19 (Abx+)	-0.341	2.5E-	0.00853
Underrepresented in both COVID-19 and pneumonia	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Eubacteriaceae|g_Eubacterium|s_Eubacterium_ventriosum	COVID-19 (Abx+)	-0.307	8.6E-06	0.00376
Pneumonia patients					
Underrepresented in pneumonia	p_Firmicutes|c_Bacilli|o_Lactobacillales|f_Enterococcaceae|g_Enterococcus|s_Enterococcus_faecium	Pneumonia	0.228	5.0E-	0.01261
	p_Firmicutes|c_Erysipelotrichia|o_Erysipelotrichales|f_Erysipelotrichaceae|g_Erysipelotrichaceae_noname|s_Clostridium_ramosum	Pneumonia	0.195	3.1E-	0.00190
	p_Firmicutes|c_Erysipelotrichia|o_Erysipelotrichales|f_Erysipelotrichaceae|g_Coprobacillus	Pneumonia	0.402	5.2E-	0.00257
	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Lachnospiraceae|g_Lachnospiraceae_noname|s_Lachnospiraceae_bacterium_5_1_63FAA	Pneumonia	-0.348	7.6E-	0.01752
Underrepresented in both COVID-19 and pneumonia	p_Firmicutes|c_Clostridia|o_Clostridiales|f_Eubacteriaceae|g_Eubacterium|s_Eubacterium_ventriosum	Pneumonia	-0.256	3.5E-04	0.03539

Table 3. Correlation of gut bacteria with COVID-19 severity

Correlation	Bacteria taxa	Correlation coefficient Rho	p value
Positive correlation with COVID-19 severity	p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales|f__Erysipelotrichaceae|g__Coprobacillus	0.92	0.003
	p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales|f__Erysipelotrichaceae|g__Erysipelotrichaceae_noname|s__Clostridium_ramosum	0.92	0.003
	p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae|g__Clostridium|s__Clostridium_hathewayi	0.90	0.005
	p__Firmicutes|c__Erysipelotrichia	0.90	0.006
	p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales	0.90	0.006
	p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales|f__Erysipelotrichaceae	0.90	0.006
	p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales|f__Erysipelotrichaceae|g__Erysipelotrichaceae_noname	0.90	0.006
	p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Actinomycetaceae|g__Actinomyces|s__Actinomyces_odontolyticus	0.87	0.011
	p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales|f__Erysipelotrichaceae|g__Erysipelotrichaceae_noname|s__Erysipelotrichaceae_bacterium_6_1_45	0.87	0.011
	p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacteriales|f__Enterobacteriaceae|g__Enterobacter	0.87	0.011
	p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacteriales|f__Enterobacteriaceae|g__Enterobacter|s__Enterobacter_cloacae	0.87	0.011
	p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Porphyromonadaceae|g__Parabacteroides|s__Parabacteroides_unclassified	0.81	0.029
	p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Rikenellaceae|g__Alistipes|s__Alistipes_indistinctus	0.81	0.029
Negative correlation with COVID-19 severity	p__Actinobacteria|c__Actinobacteria|o__Bifidobacteriales|f__Bifidobacteriaceae|g__Bifidobacterium|s__Bifidobacterium_pseudocatenulatum	-0.81	0.026
	p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae|g__Dorea	-0.81	0.026
	p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae|g__Dorea|s__Dorea_longicatena	-0.81	0.026
	p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacteroides|s__Bacteroides_ovatus	-0.84	0.019
	p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae|g__Anaerostipes|s__Anaerostipes_hadrus	-0.87	0.011
	p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae|g__Lachnospiraceae_noname|s__Lachnospiraceae_bacterium_5_1_63FAA	-0.87	0.011
	p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae|g__Roseburia	-0.87	0.011
	p__Firmicutes|c__Clostridia|o__Clostridiales|f__Ruminococcaceae|g__Faecalibacterium	-0.87	0.011
	p__Firmicutes|c__Clostridia|o__Clostridiales|f__Ruminococcaceae|g__Faecalibacterium|s__Faecalibacterium_prausnitzii	-0.87	0.011
	p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Rikenellaceae|g__Alistipes|s__Alistipes_onderdonkii	-0.90	0.005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April 9
2020
CoV1
D0 D5 D9 D15
CoV2
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CoV3
D0,D3,4 D14
CoV4
D0 D4 D22
CoV5
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CoV6
D0,1
CoV7
D0 D2 D7 D9
CoV8
D0,1 D3,4 D6 D9
CoV9
D0
CoV10
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CoV11
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CoV12
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CoV13
D0 D2
CoV14
D0 D2
CoV15
D0 D4 D6,7 D9
Hospitalization
Antibiotic use
Stool collection
Symptom onset
+ve throat swab
-ve throat swab
+ve stool qPCR
-ve stool qPCR
SARS-CoV-2

Fig.2

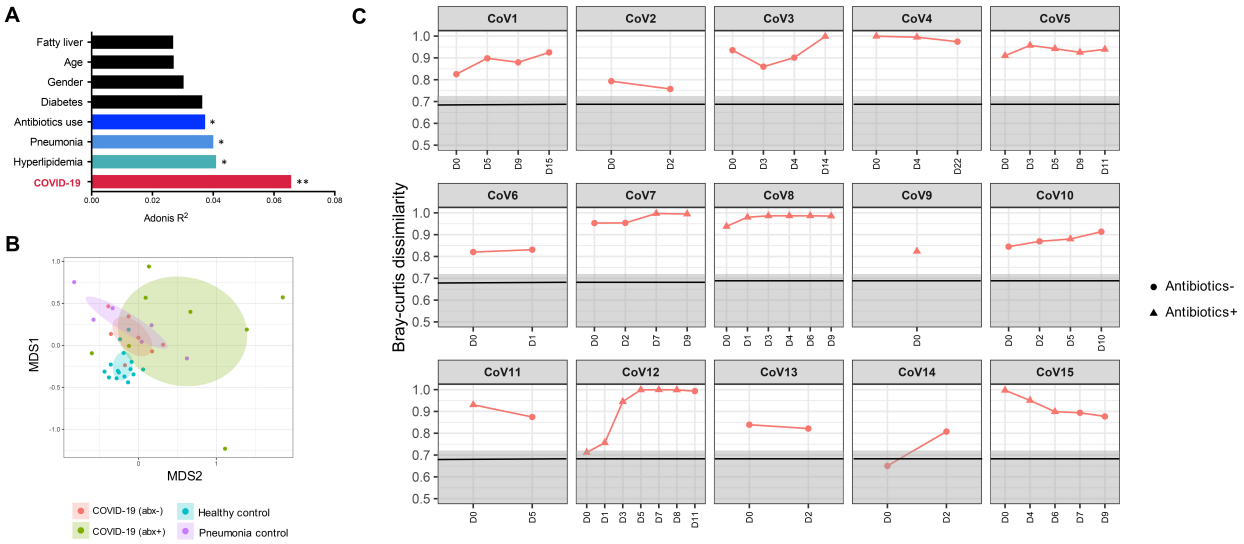
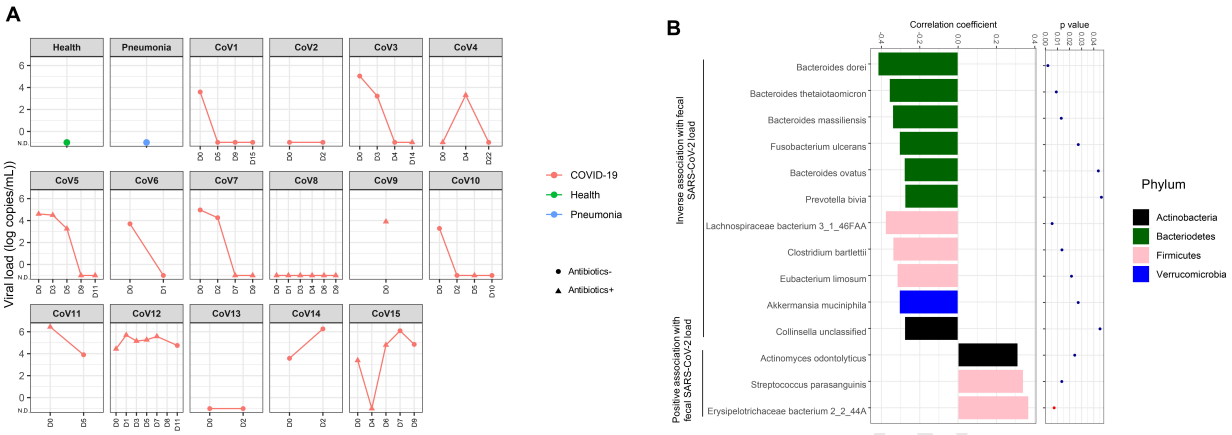
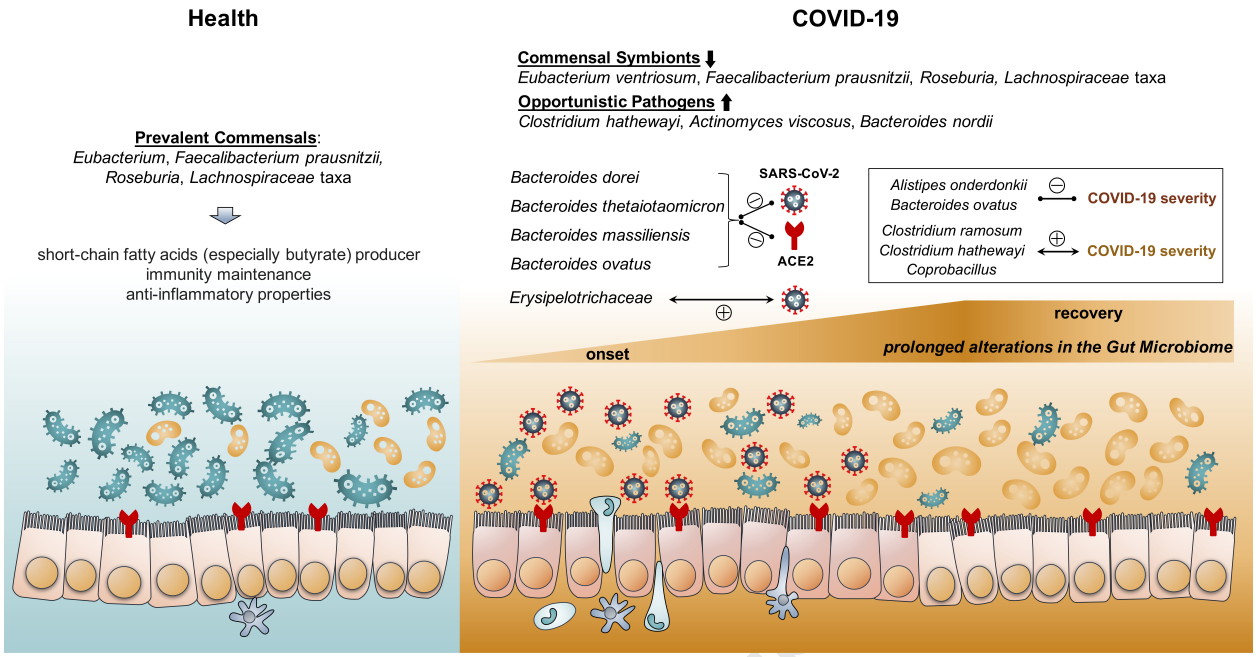
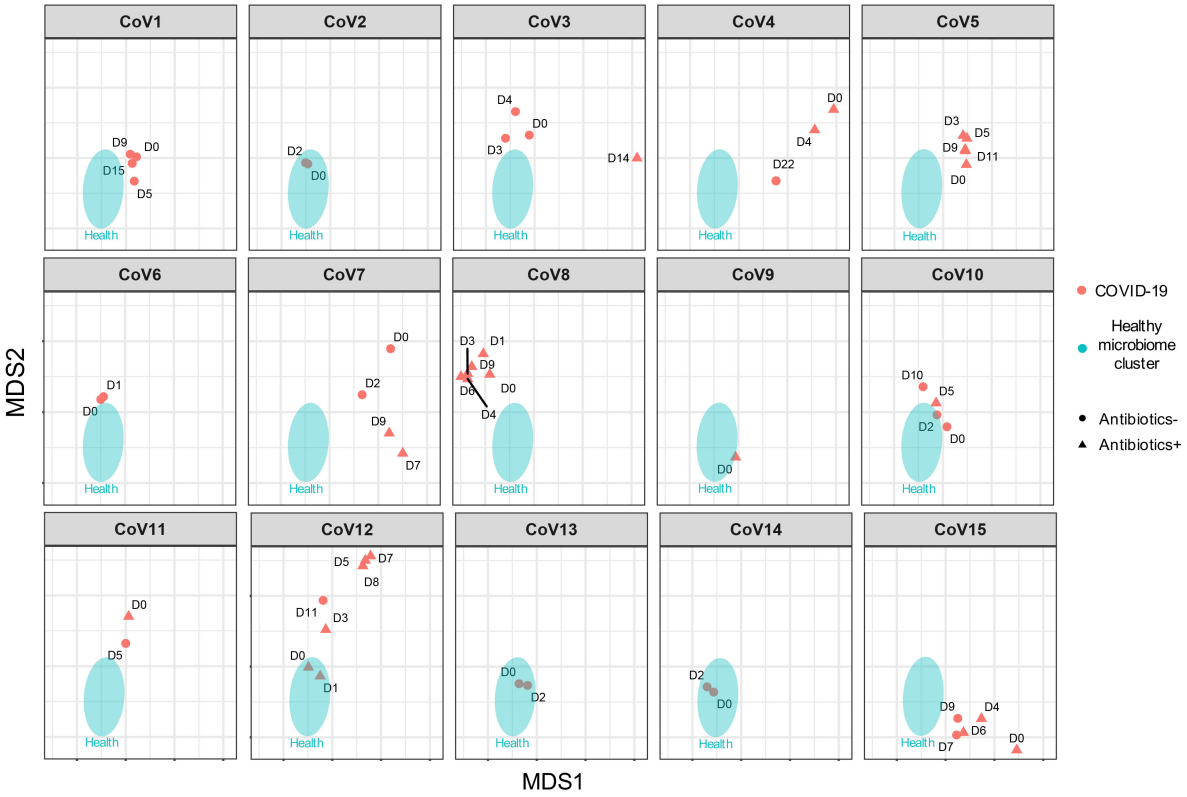


Figure. 3







What you need to know

BACKGROUD AND CONTEXT: SARS-CoV-2 infects gastrointestinal tissues. The authors investigated changes in fecal microbiomes of patients with SARS-CoV-2 infection during hospitalization and associations with severity and fecal shedding of virus.

NEW FINDINGS: Fecal microbiomes from patients with COVID-19 had depletion of symbionts and enrichment of opportunistic pathogens, which persisted after clearance of SARS-CoV-2. Baseline microbiome composition associated with COVID-19 severity. Multiple species from the Bacteroidetes phylum correlated inversely with fecal shedding of SARS-CoV-2.

LIMITATIONS: This was a pilot exploratory study of 15 patients with COVID-19; further studies are needed of alterations in intestinal microbiomes of these patients over time.

IMPACT: These findings indicate the prolonged effect of SARS-CoV-2 infection on the gut microbiomes of patients with COVID-19. Strategies to alter the gut microbiome might be developed to manage gastrointestinal effects of the virus in these patients.

Lay Summary: COVID-19 patients have altered gut microbiome with depletion of symbionts and enrichment of opportunistic pathogens, which correlate with disease severity. Such gut microbiome dysbiosis could persist even after clearance of SARS-CoV-2.