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Enterococcus and *Eggerthella* species are enriched in the gut microbiomes of COVID-19 cases in Uganda

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Abstract

Background Infection with the COVID-19-causing pathogen SARS-CoV-2 is associated with disruption in the human gut microbiome. The gut microbiome enables protection against diverse pathogens and exhibits dysbiosis during infectious and autoimmune disease. Studies based in the United States and China have found that severe COVID-19 cases have altered gut microbiome composition when compared to mild COVID-19 cases. We present the first study to investigate the gut microbiome composition of COVID-19 cases in a population from Sub-Saharan Africa. Given the impact of geography and cultural traditions on microbiome composition, it is important to investigate the microbiome globally and not draw broad conclusions from homogenous populations.

Results We used stool samples in a Ugandan biobank collected from COVID-19 cases during 2020–2022. We profiled the gut microbiomes of 83 symptomatic individuals who tested positive for SARS-CoV-2 along with 43 household contacts who did not present any symptoms of COVID-19. The inclusion of healthy controls enables us to generate hypotheses about bacterial strains potentially related to susceptibility to COVID-19 disease, which is highly heterogeneous. Comparison of the COVID-19 patients and their household contacts revealed decreased alpha diversity and blooms of *Enterococcus* and *Eggerthella* in COVID-19 cases.

Conclusions Our study finds that the microbiome of COVID-19 individuals is more likely to be disrupted, as indicated by decreased diversity and increased pathobiont levels. This is either a consequence of the disease or may indicate that certain microbiome states increase susceptibility to COVID-19 disease. Our findings enable comparison with cohorts previously published in the Global North, as well as support new hypotheses about the interaction between the gut microbiome and SARS-CoV-2 infection.

Keywords COVID-19, SARS-CoV-2, Gut microbiome, Enterococcus, Uganda

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Background

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has extremely variable presentation, ranging from asymptomatic to fatal [1]. The resulting disease COVID-19 arose in December 2019 and has caused a global pandemic. The World Health Organization reports 775 million SARS-CoV-2 infections and 7 million deaths worldwide, including 9.6 million infections and 175 thousand deaths in the African continent [2]. SARS-CoV-2 infection results in highly heterogeneous disease, marked by fever, cough, fatigue, and other flu-like clinical symptoms. Underlying conditions increasing risk for severe disease include chronic lung problems, heart disease, diabetes, obesity, advanced age and pregnancy [1]. However, even young, healthy individuals have died from COVID-19, and what drives disease heterogeneity remains largely unexplained. Sub-Saharan Africa experienced a lower disease burden of COVID-19 than expected for reasons that remain underexplored [3, 4], although the microbiome is hypothesized to play a role.

The mammalian gut microbiome influences susceptibility to diverse pathogens and varies substantially across geographies. The protection of the gut microbiota against pathogens can occur directly, through bacterial competition in the gut, and indirectly, through modulation of the host immune system. In mouse models, the gut microbiome impacts transmission and pathogenesis of lung pathogens including *Klebsiella spp.* and *Burkholderia thailandensis* [5, 6]. Gut helminths have also been shown to alter the microbiome and immune signaling to impact respiratory syncytial virus (RSV) [7, 8].

The gut microbiome of COVID-19 patients has been described previously, but published studies have significant limitations in the numbers of patients evaluated and are primarily restricted to individuals in the United States, Europe, and China [9–12]. Many of these studies lack samples from asymptomatic controls and none include individuals living in sub-Saharan Africa. These descriptive studies have been powerful to describe how the gut microbiome changes during the course of COVID-19 and also how it compares between severe and less severe COVID-19 cases. However, this research is limited in its ability to generate hypotheses about the role of the gut microbiome in protecting against SARS-CoV-2 infection or COVID-19 disease severity.

A systematic review [13] identified 22 articles exploring 16S rRNA gene amplicon sequencing or metagenomic sequencing of patients with COVID-19. These studies identified alterations of diverse bacterial genera in COVID-19 patients including: depletion of *Ruminococcus*, *Alistipes*, *Eubacterium*, *Bifidobacterium*, *Faecalibacterium*, *Roseburia*, *Fusicatenibacter*, and *Blautia* and enrichment of *Eggerthella*, *Bacteroides*, *Actinomyces*, *Clostridium*, *Streptococcus*, *Rothia*, and *Collinsella*. An increase in *Enterococcus* abundance was identified in severe COVID-19 cases in studies conducted at NYU Langone Health and Yale New Haven Hospital, the University of Chicago Medical Center, in Hong Kong, and at the Technical University Hospital of Munich [11, 12, 14, 15]. The majority of these study sizes were small (less than 100 individuals), and just one was from the African continent (Egypt) [16]. Given the known heterogeneity of the gut microbiome across geographic regions, it is important to study the interaction of COVID-19 and the gut microbiome in diverse populations and locations.

There are various hypotheses as to why Uganda and other African countries had lower COVID-19 case fatality rates (CFR). The lower CFR in Uganda may be due to the relatively young population (median age ~ 17 years according to the 2024 national census), as compared to high-income countries, for example, the United States (median age ~ 38 years) [17, 18]. Countries with a higher burden of individuals who have survived cardiovascular disease also had a higher mortality rate, and underreporting may contribute [18]. The gut microbiome differs substantially between individuals in Uganda and those in previously studied contexts like the United States and China. Given the role of the microbiome in immune maturation and protection from diverse infectious diseases, we sought to study the role of the gut microbiome in COVID-19 cases in Uganda.

Here we describe the gut microbiomes of individuals from Uganda with symptomatic COVID-19 and a set of healthy asymptomatic controls, whose SARS-CoV-2 infection status was not verified by PCR test, from similar geographic locations. We find that the microbiomes of COVID-19 cases are more dispersed than controls, and that a subset of individuals with COVID-19 have substantial Enterococcal blooms. Future investigations may be able to determine whether these microbiome disruptions impact the course of COVID-19 disease or recovery.

Methods

Sample collection

The study received ethical approval from the Mulago Hospital Research & Ethics Committee and the Uganda National Council for Science and Technology in June 2020 (approval #s MHREC1868 & HS869ES). We used stool samples from the protocol "Establishment of a Quality Assured COVID-19 Specimen Repository to Support Research in Diagnosis, Prevention and Management of SARS CoV-2 in Uganda" [19]. The study recruited symptomatic, PCR-positive COVID-19 patients, and their household contacts in Uganda from June 2020 to December 2022. PCR-positive participants were enrolled from government-designated COVID-19

treatment centers around the country, with most study participants recruited in the capital city, Kampala. All study participants provided written informed consent before being included in the study.

Samples for SARS-CoV-2 PCR-positive patients were on average collected within four days of a positive PCR result. PCR-positive patients were further consented to recruit their household contacts, who were included as asymptomatic controls if they were free of COVID-19 symptoms (these individuals were not PCR tested due to regulations governing use of scarce testing supplies at the time). Blood (plasma, serum), saliva, urine, stool, and oropharyngeal/nasopharyngeal swabs were collected from each participant at baseline. Samples collected were processed and stored at the Integrated Biorepository of H3Africa Uganda (IBRH3AU). Aliquots of stool from 190 participants were used for this study.

Clinical data
Data was collected using an electronic questionnaire as described in Kamulegeya et al. [19]. This questionnaire collected data on participant sociodemographic factors, occupation, marital status, education background, travel history, and current and past health conditions. In addition to the questionnaire, an interview was also administered by a trained nurse. Available metadata is compiled in Supplemental Table 1.

DNA isolation and 16S rRNA gene amplicon library preparation
Genomic DNA (gDNA) was isolated using the DNeasy PowerLyzer PowerSoil Kit from Qiagen (Cat. No./ID: 12855-50). Following the manufacturer's protocol, one scoop of stool was taken from each stool aliquot for gDNA extraction. gDNA was quantified using a Nanodrop and diluted to 10 ng/μl. 16S rRNA amplification was conducted using the KAPA HiFi Hotstart kit (Cat. No. 50-196-5215) and the primers targeting the V4 region as described by Gohl et al. [20] (Supplemental Table 2). Cycling conditions were as follows: 95 °C for 5 min, followed by 25 cycles of 98 °C for 20 s, 55 °C for 15 s, and 72 °C for 1 min. Final extension for 10 min at 72 °C. A second PCR followed to add sample-specific indices and Illumina compatible flow cell adaptors (Supplemental Table 2). Cycle was as follows: 95 °C for 5 min; ten cycles of 98 °C for 20 s, 55 °C for 15 s, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. A left-side selection (0.8×) using SPRI beads followed the PCR using the manufacturer's protocol. The PCR product was quantified using a Qubit and pooled for sequencing on an Illumina MiSeq to generate 2 × 250 bp paired end reads.

Microbiome data analysis
Each of the 126 samples included in analysis contained a minimum number of 1000 reads per sample (Supplemental Table 1). Demultiplexed reads were pooled from multiple sequencing runs. Reads were demultiplexed using standard Illumina software or the iu-demultiplex package [21]. The Quantitative Insights into Microbial Ecology 2 (Qiime2) pipeline version 2023.5.1 was used for downstream analyses [22]. Potential chimeric reads were removed using consensus-based methods. Amplicon sequence variants (ASVs) were inferred using DADA2 [23] and a phylogenetic tree was built using MAFFT alignment [24, 25]. Taxonomic assignment was performed using the SILVA138 database [26]. For alpha and beta diversity analyses, samples were rarefied to 1000 reads per sample using the Qiime2 pipeline.

Statistical analysis
Alpha diversity was calculated using Faith's Phylogenetic Diversity (PD) and the Shannon Index, with between group significance determined using a Wilcoxon rank sum test. Using the Qiime2 package, beta diversity was calculated using the Weighted UniFrac, Bray–Curtis, and Jaccard distance matrices. PERMANOVA tests were done to see if the distributions were different. The Weighted UniFrac value was set as the dependent variable, while SARS-CoV2 infection, participant location, antibiotic use, *Enterococcus* spp. presence, age, or sex were used as the independent variables. PERMDISP was used to calculate whether the variances between two groups were significantly different. The metadata file and Qiime2 files were imported into R version 4.2.1 and merged into a single Phyloseq object. Analysis of composition of microbiomes with bias correction (ANCOM-BC) [27] and microbiome multivariable associations with linear models (MaAsLin2) [28] were performed in R using the "ANCOMBC" [27, 29] and "Maaslin2" [28] packages. Bacterial taxa were determined to be differentially present based on an adjusted p-value of < 0.05. Plots were generated using the "ggplot2" [30] package and base R functions and edited in Adobe Illustrator.

Shotgun whole genome sequencing
The Qiime2 taxonomy feature table was used to determine which positive samples had *Enterococcus* species present. gDNA from these samples was used for shotgun whole genome sequencing using the published Hackflex protocol [31]. Samples were pooled and sequenced on an Illumina NovaSeq to obtain 2 × 150 bp reads. Sequences were uploaded onto Chan Zuckerberg's ID (CZ ID) online pipeline and reads per million calculated using the NT database [32]. Sequences were

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analyzed using the metagenomics Illumina pipeline 8.3.0 and the antimicrobial resistance pipeline 1.3.2.

USA metagenomes meta-analysis

A meta-analysis of six metagenomic inflammatory bowel disease (IBD) studies from the United States was performed to evaluate the presence of *Enterococcus* species in additional patient populations and healthy controls [33–38] (see Supplemental Table 4). All data was retrieved from the Sequencing Read Archive as raw fastq files and processed collectively through the CZ ID metagenomic pipeline which maps short read sequencing files to NCBI's bacterial nucleotide database. Sequences were uploaded onto the CZ ID online pipeline and analyzed as described above. Prior to analysis, samples were filtered to remove any IBD subjects in remission (non-active disease states) and any time points where subjects were undergoing a clinical intervention. Patients and controls in these studies were separated into antibiotic status based on whether antibiotics had been used within 3 months.

Results

Development of a biobank of COVID-19 cases and controls in Uganda

Participants were recruited from three COVID-19 treatment centers: Mulago National Referral Hospital, Entebbe Grade B Referral Hospital, and Masaka Regional Referral Hospital from June 2020 to December 2022 as described in Kamulegeya et al. [19] Individuals who tested PCR positive for SARS-CoV-2 and their asymptomatic household members were recruited to the study. The resulting COVID-19 biobank managed by the Integrated Biorepository of H3Africa Uganda (IBRH3AU) contains blood (plasma, serum), saliva, urine, stool, and nasal swabs. Participants also completed an electronic questionnaire for demographic data. Asymptomatic control individuals were not PCR tested for SARS-CoV-2 due to the global shortage of tests at the time, so their infection status is unknown.

We extracted DNA and generated 16S rRNA gene amplicon sequencing libraries from 190 stool samples. 126 libraries passed quality checks and were used for subsequent analysis (Supplemental Table 1). From these filtered samples, 101 samples had demographic metadata that allowed for more specific analyses, such as geographic location, sex/gender, age, and antibiotic exposure at the time of sample collection (Fig. 1, Table 1).
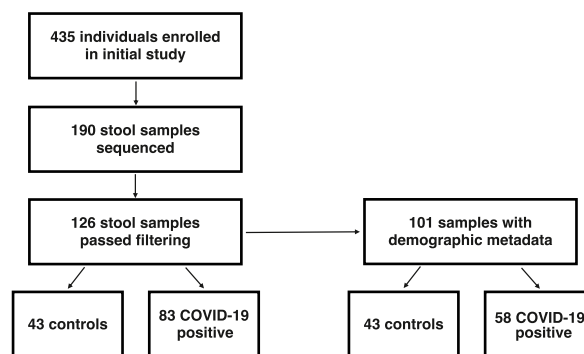

Fig. 1 Flowchart of participants and analysed stool samples

Diversity of the gut microbiome during SARS-CoV-2 infection

We evaluated the composition of the gut microbiome of individuals positive for SARS-CoV-2 infection and asymptomatic controls using 16S rRNA gene amplicon sequencing of DNA isolated from stool samples. This allowed identification of most amplicon sequence variants (ASVs) at the genus level. Based on the abundances of these ASVs, we calculated alpha diversity for each sample using a metric that includes phylogenetic information (Faith's Phylogenetic Diversity (PD)) and a metric that is phylogeny independent (Shannon Index). We identified significantly lower alpha diversity in the gut microbiomes of COVID-19 cases compared to those of asymptomatic controls using both metrics, suggesting that COVID-19 cases have less complex microbiomes than controls (Fig. 2A, B, Faith's PD $p*** < 0.001$, Shannon $p**** < 0.0001$, Wilcoxon).

We next investigated the similarity of gut microbiomes across individuals using beta diversity. We used the Weighted UniFrac distance metric which takes into account both ASV abundance and taxonomy. The gut microbiomes of individuals infected with SARS-CoV-2 formed an overlapping, but distinct distribution by principal coordinate analysis when compared to asymptomatic controls (Fig. 3A, PERMANOVA $p < 0.01$). Microbiomes of asymptomatic individuals were more similar to each other, and those of infected individuals were significantly more dispersed ($p < 0.01$, PERMDISP). We also compared microbiome diversity using the Bray–Curtis and Jaccard distance metrics, which consider abundance and presence/absence respectively (Supplemental Fig. 1A, B). We see similar results using these different distances metrics as we do when using Weighted UniFrac (Supplemental Fig. 1A, B, $p < 0.01$, PERMANOVA; $p < 0.01$ PERMDISP). No differences were observed between the microbiomes of male and female subjects (Supplemental Fig. 1C, $p < 0.01$). Age of

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Table 1 Sample characteristics with available metadata (N = 101)

Characteristic		n (%) or Median (IQR)	
COVID-19 result		COVID-19 Positive	Asymptomatic control
		58 (57.4)	43 (42.6)
Sex	Female	21 (20.8)	23 (22.8)
	Male	37 (36.6)	19 (18.8)
	Unknown	0 (0.0)	1 (1.0)
Age		49 years (33-60)	24.5 years (19-35.75)
	<20	1 (1.0)	12 (11.9)
	20-29	10 (9.9)	16 (15.8)
	30-39	8 (7.9)	6 (5.9)
	40-49	10 (9.9)	5 (5.0)
	50-59	13 (12.9)	2 (2.0)
	60-69	8 (7.9)	0 (0.0)
	>=70	8 (7.9)	1 (1.0)
	Unknown	1 (1.0)	1 (1.0)
Antibiotics	Yes	30 (29.7)	1 (1.0)
	No	28 (27.7)	14 (13.9)
	Unknown	0 (0.0)	28 (27.7)

subjects, however, was linked to increased dispersion (Supplemental Fig. 1D). This may be in part due to the fact that the median age of asymptomatic controls was much lower than that of the COVID-19 cases.

We overlaid clinical and geographic data on the PCoA plot to see if these groupings drove the differences observed. We first wanted to test if antibiotic exposure explained the increased dispersion in COVID-19 cases. Comparing the distribution and dispersion of microbiomes from SARS-CoV-2 positive individuals with and without antibiotic exposure did not yield any significant differences (Fig. 3B, p = 0.577, PERMANOVA; p = 0.915, PERMDISP). We next tested the role of geography in microbiome composition. Both controls and COVID-19 cases were sampled from geographic regions around Uganda. Samples from individuals in the same district did not cluster with each other (Supplement Fig. 1E). Districts were classified into major cities (urban) or rural (all other areas) based on country data [39]. Using these criteria, gut microbiome beta diversity also did not differ significantly based on district urbanization (Fig. 3C, p = 0.127, PERMANOVA; p = 0.515, PERMDISP). Given that these variables are not significantly related to dispersion between microbiome samples, we conclude that COVID-19 cases are correlated with increased dispersion independent of the recorded metadata.

Taxa enriched in COVID-19 cases or controls

We next explored whether specific taxa are enriched in the microbiomes of COVID-19 cases or controls. Analysis of microbiome composition with bias correction (ANCOM-BC) [27] identified genera that were significantly enriched in asymptomatic controls. Species significantly enriched in controls with a log2(fold change) greater than 1.5 include *Lactobacillus*, *Clostridium sensu stricto 1*, *Haemophilus*, *Romboutsia*, *Agathobacter*, and *Akkermansia* (Fig. 4A, Supplemental Fig. 2). Additional differential abundance testing using microbiome multivariable associations with linear models (MaAsLin2) [28] identified *Turicibacter* and *Lachnospira* as species that are also significantly enriched in controls (Fig. 4B, adjusted p-value < 0.05). *Akkermansia* and *Romboutsia*, in particular, have been shown to be associated with beneficial outcomes in the context of inflammatory conditions such as diabetes and ulcerative colitis [40–42].

The gut microbiomes of COVID-19 cases were strikingly enriched for *Eggerthella* and *Enterococcus* (1.76-fold and 2.75-fold respectively, adjusted p-value < 0.05) (Fig. 4). *Enterococcus* species were completely absent from asymptomatic controls, whereas *Enterococcus* was found in 56.6% of COVID-19 cases. The range of *Enterococcus* relative abundance in the COVID-19 cases ranged from 0 to 85% (Fig. 5A). *Eggerthella* species were present in only 2% of asymptomatic individuals, whereas *Eggerthella* was found in 43.4% of COVID-19
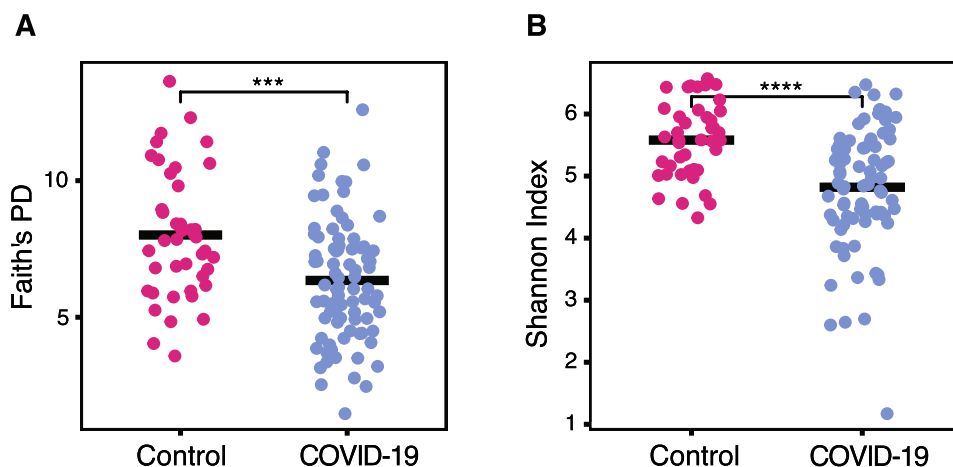
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Fig. 2 The gut microbiome of individuals with COVID-19 is less diverse. Alpha diversity is shown using (**A**) Faith's Phylogenetic diversity (PD) and (**B**) the Shannon Index (***p < 0.001 Faith's PD, ****p < 0.0001 Shannon, Wilcoxon)
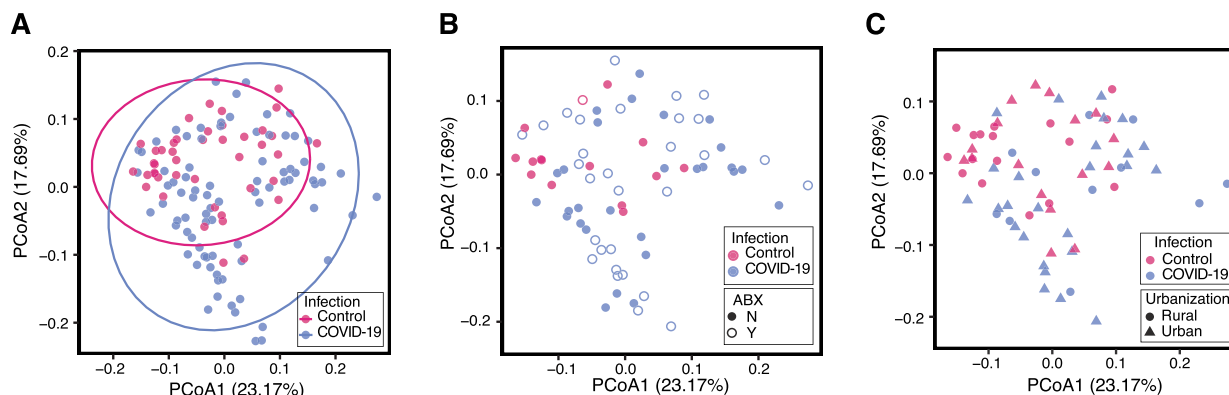

Fig. 3 Beta-diversity analysis of the gut microbiomes of COVID-19 cases and asymptomatic controls. **A** Principal coordinate analysis (PCoA) calculated using weighted UniFrac distance metric is shown for individuals who tested positive for COVID-19 and asymptomatic controls (p < 0.01, PERMANOVA; p < 0.01, PERMDISP). The same PCoA is also plotted with point fill indicating recent antibiotic exposure at the time of sample collection (**B** p = 0.577, PERMANOVA; p = 0.915, PERMDISP) or a symbol indicating whether the individual resides in a rural or urban area (**C**, p = 0.127, PERMANOVA; p = 0.515, PERMDISP). *ABX* antibiotics exposure

cases. *Eggerthella* relative abundance in COVID-19 cases ranged from 0 to 4% (Fig. 5B). MaAsLin2 analysis additionally identified *Lachnoclostridium* as enriched in COVID-19 cases (Fig. 5C).

We next tested if the presence of each species was linked to the heterogeneity in beta diversity observed above. We plotted the beta diversity PCoA and labeled the presence/absence of *Enterococcus* (Fig. 5D). The microbiomes of COVID-19 cases that had *Enterococcus* did not cluster together, but formed a distinct distribution to the microbiomes of COVID-19 cases with no *Enterococcus* (p < 0.01, PERMANOVA). Microbiomes with *Enterococcus* were quite dispersed. While this suggests that *Enterococcus* presence contributes to the broader dispersion seen in the microbiomes of COVID-19 cases,

it was not sufficient to explain this increased dispersion. *Enterococcus* positive microbiomes were not significantly more dispersed than COVID-19 positive microbiomes without *Enterococcus* (p = 0.639, PERMDISP). *Eggerthella* positive COVID-19 cases also formed a statistically distinct distribution from positive COVID-19 cases without *Eggerthella* (Fig. 5E), while changes attributed to *Lachnoclostridium* did not reach significance by PERMANOVA (Fig. 5F).

Exploring the role of *Enterococcus* and *Eggerthella* species in COVID-19

Antibiotic exposure has been previously shown to cause blooms in *Enterococcus* abundance in hospitalized patients with diarrhea in Vietnam and cancer in
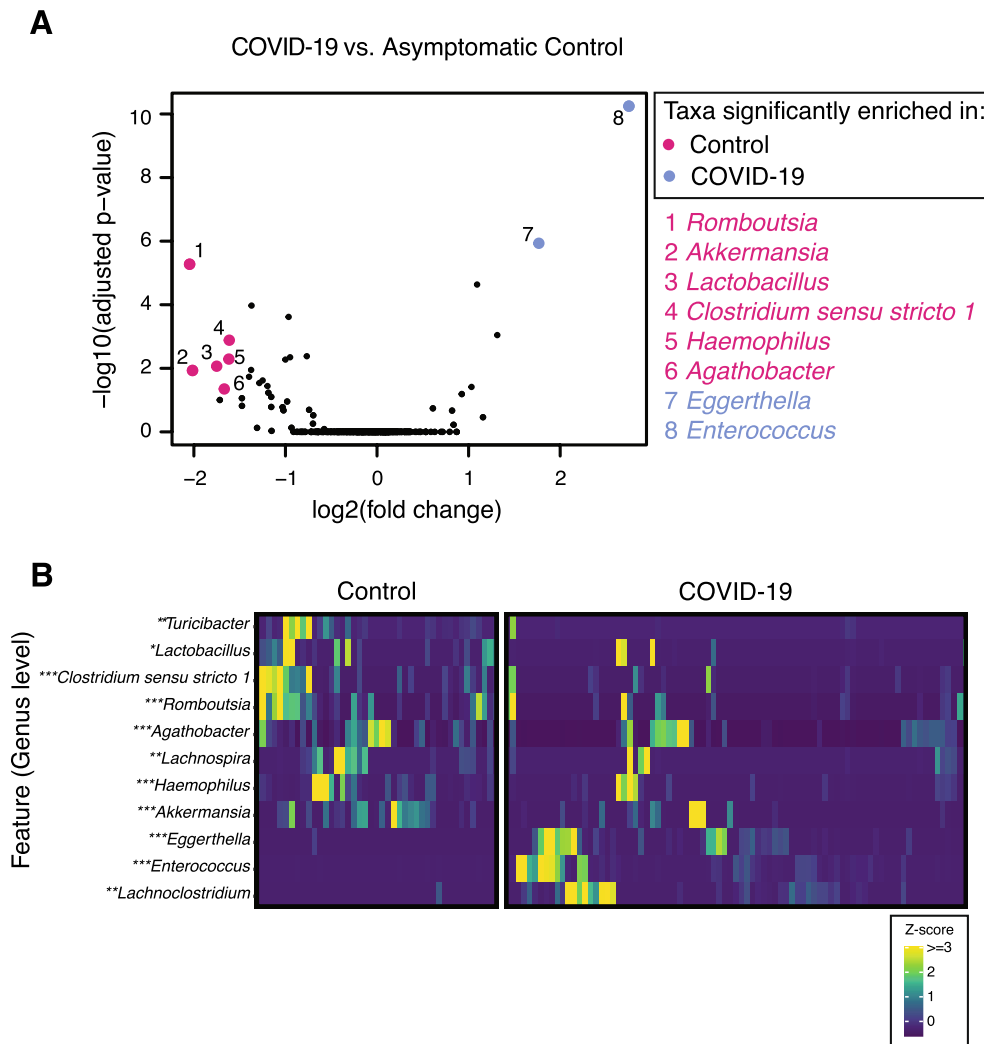
Fig. 4 Genera differentially abundant between COVID-19 cases and controls. **A** The log2(fold change) of each genera is plotted versus the −log(adj p-value) as calculated by analysis of composition of microbiomes with bias correction (ANCOM-BC) (pink and blue dots represent species with an absolute log2(fold change) greater than 1.5 and an adjusted p-value less than 0.05). **B** Relative abundance for ANCOM-BC and MaAsLin2 selected taxa (padj < 0.05) (* taxa only selected by ANCOM-BC, **taxa only selected by MaAsLin2, *** taxa selected by both methods)

the United States [43, 44]. Available metadata on antibiotic use allowed for us to test this hypothesis in the context of COVID-19 and Uganda. Individuals positive for SARS-CoV-2 were compared based on whether or not they received antibiotics at the time of sample collection. The relative abundance was also not significantly different based on antibiotic exposure within COVID-19 cases (Fig. 6A, p = 0.242 Wilcoxon test) suggesting that an increased risk of *Enterococcus* bloom in COVID-19 cases is not due to increased antibiotic exposure alone (16.8% of COVID-19 cases without antibiotics contained *Enterococcus* vs. 13.9% of cases exposed to antibiotics, Fig. 6B). Reported antibiotic exposure also did not result

in significant changes in alpha diversity among COVID-19 positive individuals (Supplemental Fig. 3 A, B). Antibiotic exposure based on sex and geography also did not result in significant changes in alpha and beta diversity among COVID-19 positive individuals (Supplemental Fig. 3 C–H). Interestingly, the vast majority of *Enterococcus* positive microbiomes were sampled from individuals at Mulago hospital and antibiotic exposure within that particular site is not correlated with the abundance of *Enterococcus* (Supplemental Fig. 4).

To determine the *Enterococcus* species contributing to these blooms, we performed shotgun metagenomic sequencing on five *Enterococcus*-containing samples.
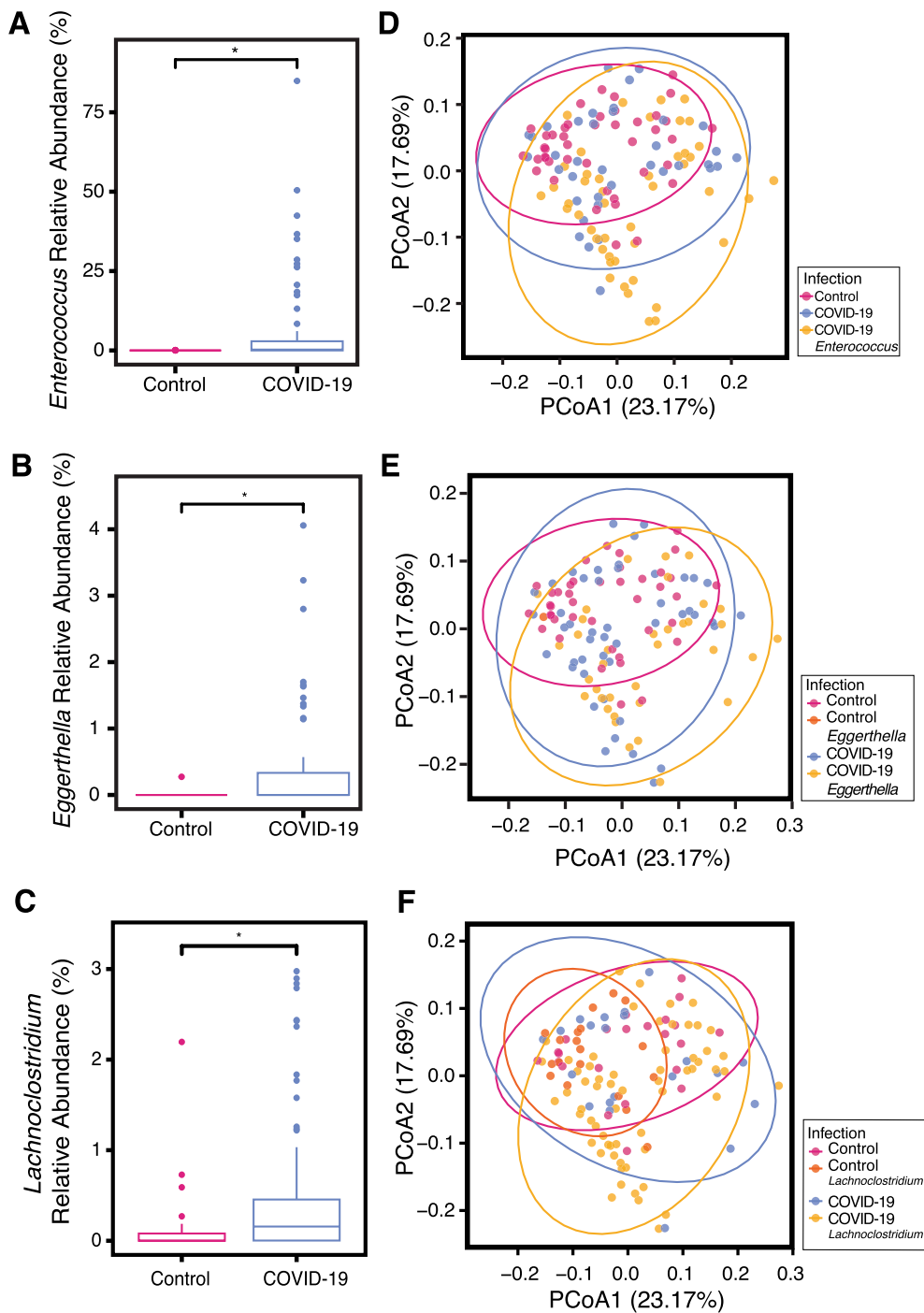
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Fig. 5 *Enterococcus* species were highly abundant in COVID-19 cases. The relative abundance of (**A**) *Enterococcus* species (padj* < 0.05, Holm test), **B** *Eggerthella* species (padj* < 0.05, Holm test), and (**C**) *Lachnoclostridium* species (padj* < 0.05 MaAsLin2) in positive COVID-19 individual and asymptomatic individuals. PCoA identifying positive COVID-19 individuals with (**D**) *Enterococcus* (p < 0.01, PERMANOVA; p = 0.639, PERMDISP), **E** *Eggerthella* (p < 0.05, PERMANOVA; p = 0.821, PERMDISP), and **F** *Lachnoclostridium* (p = 0.095, PERMANOVA; p = 0.634, PERMDISP) present in their gut microbiome

These samples were identified as *Enterococcus*-containing via the 16S rRNA amplicon sequencing. The metagenomes of the *Enterococcus*-containing samples were analyzed using the Chan Zuckerberg Infectious Disease (CZ ID) online platform [32]. These samples identified that the predominant species contributing to these
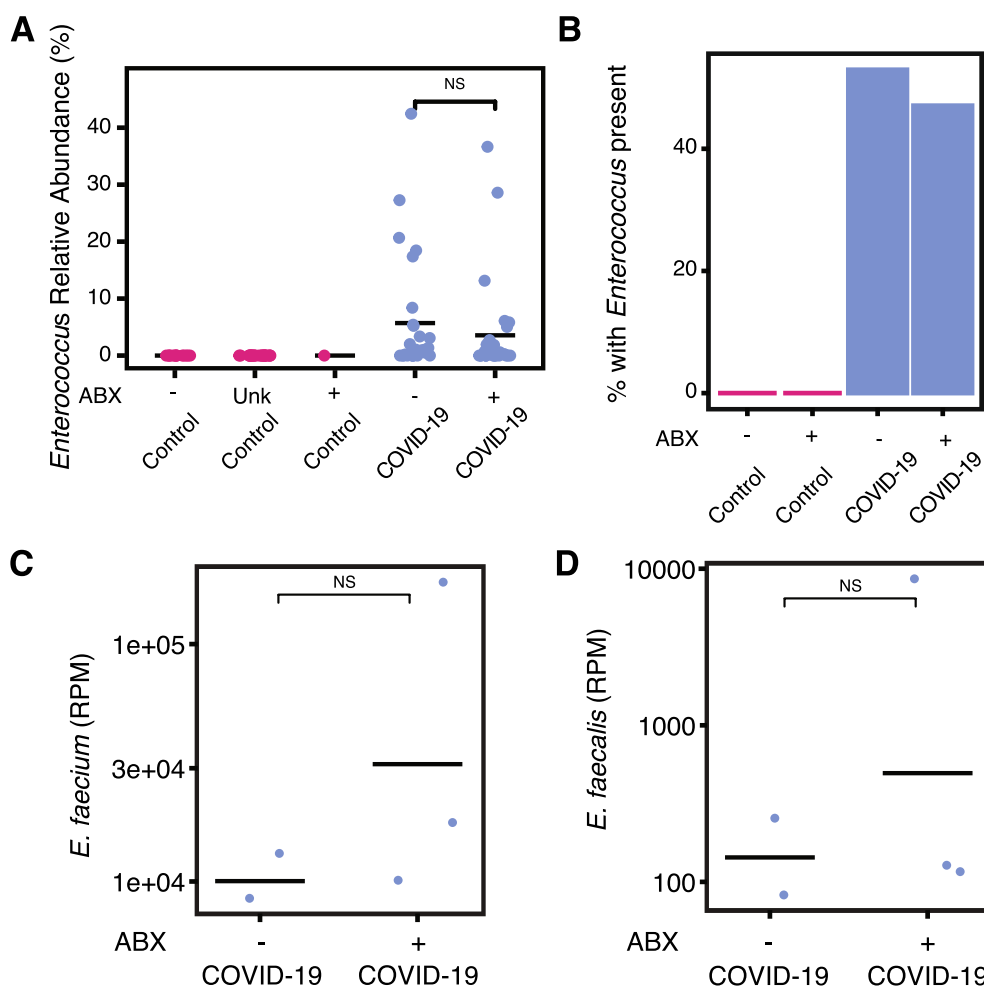
Fig. 6 The role of antibiotic exposure on *Enterococcus* species. **A** *Enterococcus* relative abundance in COVID-19 cases and controls based on antibiotic exposure status (ABX = antibiotic exposure, Unk = Unknown, *NS* not significant, Kruskal–Wallis). **B** The percent of individuals with *Enterococcus* detected at greater than 0.1% in their gut microbiome is shown for asymptomatic controls and COVID-19 positive individuals. The reads per million (RPM) of **C** *Enterococcus faecium* (p = 0.4, Wilcoxon test) and **D** *Enterococcus faecalis* (p = 0.8, Wilcoxon test) in positive COVID-19 individuals with and without antibiotic exposure

blooms was *E. faecium*. *E. faecium* was identified in five of five sequenced samples (Fig. 6C). *E. faecalis* was also identified in all samples sequenced at a lower percentage (Fig. 6D). The *Eggerthella* species was identified as *Eggerthella lenta*, which was present in all metagenome sequenced samples (Supplemental Fig. 5). The antimicrobial resistance pipeline identified aminoglycoside, tetracycline, macrolide, streptogramin, lincosamide, and fluoroquinolone resistance reads associated with *E. faecium* (Supplement Table 3). Macrolide, fluoroquinolone, diaminopyrimidine, lincosamide, and pleuromutilin resistance reads were associated with *E. faecalis* (Supplement Table 3).

We next sought to understand the distribution of *Enterococcus* in individuals outside Uganda to compare to what we observed there. We hypothesized that

if *Enterococcus* is linked to susceptibility to symptomatic COVID-19, we may observe more *Enterococcus* in populations with higher case fatality rates than Uganda, including the United States. We re-analyzed gut metagenome data to identify levels of *Enterococcus* species in six readily available studies from the United States which included healthy controls and patients with inflammatory bowel disease [33–38] (Supplemental Table 4). We found that *E. faecium* and *E. faecalis* were found at a low level in some healthy controls (less than 2500 reads per million, Supplemental Fig. 6A, B). Although this data is not directly comparable to our amplicon sequencing data, it provides evidence that baseline *Enterococcus* levels in the population may vary by geography. Additionally, antibiotic exposure in this cohort also did not influence *Enterococcus* abundance, supporting our finding that

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antibiotics did not play a role in Enterococcal blooms in our population (Supplemental Fig. 6A). In fact, in the US datasets there was a trend toward antibiotic exposure suppressing *Enterococcus* in healthy individuals. *Enterococcus* was more prevalent in individuals with inflammatory bowel disease, although again antibiotic history did not influence the abundance significantly (Supplemental Fig. 6, not significant, Wilcoxon test). Thus, *Enterococcus* presence, like COVID-19 disease severity, may be heavily influenced by underlying medical conditions and geography.

Discussion

During the SARS-CoV-2 pandemic, researchers reported on gut microbiome dysbiosis in cohorts of hospitalized COVID-19 patients located in the United States, Europe, and China [9–12, 14, 15]. These studies identified bacterial taxa correlated with COVID-19 disease or its severity, but specific interactions and mechanisms were not identified. Given that the gut microbiome varies substantially across geographic and cultural contexts, it is important to also explore the relationship between the gut microbiome and COVID-19 in populations in the Global South, including Africa.

In this study, we found that Ugandan individuals who tested positive for SARS-CoV-2 had a less diverse gut microbiome compared to those of asymptomatic controls and increased dispersion. Decreased alpha diversity has been observed in cohorts of diverse disease states including inflammatory bowel disease, flu, Parkinson's disease, and multiple sclerosis [45–48]. Gu et al. also found lower diversity in COVID-19 cases compared to healthy controls in a Chinese study [9]. How microbial diversity decreases as an effect of infection or disease is still poorly understood. Our study includes asymptomatic individuals as controls for a healthy gut microbiome. In contrast, many prior studies only compared COVID-19 patients at different levels of severity. Additionally, this study, to our knowledge, is the first COVID-19 microbiome cross-sectional study in sub-Saharan Africa. We saw no differences in the microbiomes between individuals in urban and rural areas. This may be because all individuals were able to access medical treatment and thus were relatively nearby to urban areas compared to other studies [49].

Asymptomatic individuals recruited from the same households as cases were used as a proxy for a healthy microbiome. However, their SARS-CoV-2 infection status was not verified by PCR test. The microbiomes of asymptomatic individuals were enriched with *Romboutsia* and *Akkermansia*. *Romboutsia* was also identified in healthy controls when compared to individuals with COVID-19 in a study by Gu et al. [9] This suggests a protective and beneficial role for *Romboutsia* in the context of SARS-CoV-2. *Romboutsia* species have also been associated with improvements in endothelial function and changes in metabolic function in diet-induced obese rats [42]. *Akkermansia*, on the other hand, is known for both its beneficial [50, 51] and detrimental roles [52] in human health. *Akkermansia muciniphila* has been associated with alleviating ulcerative colitis and reducing the incidence of diabetes in mouse studies by modulating inflammatory gene expression [40, 41]. It has also been associated with protection from infectious agents like *Listeria monocytogenes* [53]. In contrast, Bernard-Raichon et al. [11] showed in their mouse model of SARS-CoV-2 that infection led to dysbiosis in the gut led by *Akkermansia*. This suggests a context-dependent role for *Akkermansia* in COVID-19 infection and disease and requires further investigation.

Individuals who tested positive for SARS-CoV-2 had higher relative abundances of microbiome-resident pathobionts than their asymptomatic counterparts. Both *Enterococcus* and *Eggerthella* can cause or exacerbate disease opportunistically, and both were elevated in the microbiomes of COVID-19 cases. Strikingly, no asymptomatic individuals had any *Enterococcus* present in their guts and just 2% of asymptomatic individuals had *Eggerthella* present at any abundance. *Enterococcus* is a member of the intestinal flora that can cause hospital-acquired bloodstream infections and urinary tract infections [54]. It has also been associated with states of gut dysbiosis as an effect of antibiotic usage [55], although here we do not find that antibiotic use increases *Enterococcus* carriage in our COVID-19 study or a meta-analysis of individuals from the United States. Other similar studies [11–15] saw enrichment of *Enterococcus* in severe COVID-19 cases compared to less severe cases. Elevated *Enterococcus* abundance may lead to increased susceptibility to disease through immune stimulation or production of bioactive metabolites. Enterococcal blooms may also be a consequence of COVID-19 infection and related treatment. Experimental models using gnotobiotic mice with defined microbiomes, or prospective longitudinal human studies, are required to define the role of specific gut resident bacterial taxa in COVID-19 disease.

Eggerthella lenta is a gut pathobiont associated with bacteremia [56] and inflammatory diseases. *Eggerthella* is elevated in patients with IBD [57], and rheumatoid arthritis (RA) mouse models suggest *E. lenta* promotes IBD by antigen-independent Th17 activation [58]. COVID-19 disease has previously been associated with increased *Eggerthella* abundance in a study with just 13 cases and five controls [13, 59]. Our study expands this finding and validates it in a larger sample size. Interestingly, Th17 responses have been associated with severe

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COVID-19 [60, 61]. *Eggerthella* may have a role in increasing host susceptibility to COVID-19 disease, possibly through exacerbation of Th17 responses, but further studies are needed.

Activation of the immune system by SARS-CoV-2 may impact gut microbiota composition, or gut microbiota composition may impact the immune response to SARS-CoV-2 infection. Much of this biology remains to be explored. SARS-CoV-2 is largely a pulmonary infection, although a significant fraction of patients also experience infection of the gastrointestinal tract. Gut T cell priming against SARS-CoV-2 infection in the gut could be influenced by the microbiome. These T cells may subsequently traffic to the lungs, contributing to severe manifestations of infection. Even in the absence of gastrointestinal infection in the gut, the propensity of T cells to traffic from one mucosal tissue to another means that T cells specific for gut antigens could in principle influence antigen specific responses to COVID-19 in the lungs [62]. Conversely, T cell responses primed in the lung could influence the composition of the gut microbiome by altering the inflammatory environment in the intestinal mucosa. COVID-19 infection is associated with the production of cytokines including type I interferon, IL-6, IL-17, IL-1, and TNF-α [61, 63–65]. Cytokines produced during infection have been shown to have an impact on the gut microbiome [66, 67]. In addition, specific gut microbes can impact production of cytokines [68, 69] that may impact the host's response to the virus.

While our study provides advances on previous reports by including controls, a larger subject pool, and an understudied geographic region, it does have some limitations imposed by implementation challenges associated with the pandemic. We do not know whether the asymptomatic controls were asymptomatic carriers of SARS-CoV-2 or not infected. At the beginning of the COVID-19 pandemic, there were many barriers to sample collection and PCR testing, including availability of supplies and access to accredited testing facilities. Only a handful of facilities were approved by the government for both the testing and collection of COVID-19 samples. Resources were also prioritized for those who had active symptoms of disease, with asymptomatic household contacts landing at the bottom of the priority list. Retesting these samples at a later date was considered, but determined to be likely to yield false negatives given the time in storage. Regardless, the microbiome changes observed are interesting whether they are associated with resistance to infection or disease presentation. Future studies may be able to delve into this in more detail.

Although we do not have clinical metadata for ~ 20% of the individuals with sequencing data, the 101 individuals we do have is larger than most published studies. For controls, antibiotic use was not reported. Although individuals were sampled as soon as possible after diagnosis, there was variable time between start of infection and sampling. Conducting this study in the early stages of the COVID-19 pandemic was extremely challenging, and it was not feasible to collect all desired clinical data from all subjects. Based on saturation of alpha rarefaction curves and to include the maximum number of subjects, we chose to rarefy our sequencing data to 1000 reads per sample for diversity analyses as described in the methods. Although we are confident this measure included the vast majority of taxa, we may have missed extremely low abundance taxa that could play a role in microbiome dysbiosis. Despite these limitations, our analysis demonstrates for the first time disruption of the gut microbiome in individuals with COVID-19 in Uganda.

Conclusion

We have shown an association with increased microbiome variability and an enrichment of *Enterococcus* and *Eggerthella* in the microbiomes of COVID-19 cases compared to asymptomatic controls in Uganda. These disruptions may be a consequence of the disease or cause increased susceptibility to SARS-CoV-2 infection and/ or COVID-19 disease presentation. Mechanistic experiments or prospective cohort studies are required to disentangle these possibilities. Understanding how the gut microbiome interacts with respiratory pathogens can better inform treatment strategies and the development of prophylactic probiotics.

Supplementary Information

The online version contains supplementary material available at https://doi. org/10.1186/s13099-025-00678-4.

Supplementary Material 1.

Supplementary Material 2.

Availability of data and materials
Sequencing reads are available through NCBI Short Read Archive under Bioproject PRJNA1131762. Analysis code is available on github: https://github.com/c-agudelo/COVID-19-analysis.

Declarations

Ethics approval and consent to participate
The study received ethical approval from the Mulago Hospital Research & Ethics Committee and the Uganda National Council for Science and Technology in June 2020 (approval #s MHREC1868 & HS869ES). All participants consented to sample storage and use of their samples and data in current and future studies related to SARS-CoV-2 infection according to the protocol "Establishment of a Quality Assured COVID-19 Specimen Repository to Support Research in Diagnosis, Prevention and Management of SARS CoV-2 in Uganda" [19].

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References
1. Mayo Clinic Staff. COVID-19: who's at higher risk of serious symptoms? https://www.mayoclinic.org/diseases-conditions/coronavirus/in-depth/coronavirus-who-is-at-risk/art-20483301.
2. World Health Organization, WHO Coronavirus (COVID-19) dashboard. https://data.who.int/dashboards/covid19/cases.
3. Zinsou B-E, et al. Main modulators of COVID-19 epidemic in sub-Saharan Africa. Heliyon. 2023;9: e12727.
4. Wolday D, et al. Interrogating the impact of intestinal parasite–microbiome on pathogenesis of COVID-19 in Sub-Saharan Africa. Front Microbiol. 2021;12: 614522.
5. Schieber AMP, et al. Disease tolerance mediated by microbiome *E. coli* involves inflammasome and IGF-1 signaling. Science. 2015;350:558–63.
6. Sequeira RP, McDonald JAK, Marchesi JR, Clarke TB. Commensal bacteroidetes protect against *Klebsiella pneumoniae* colonization and transmission through IL-36 signalling. Nat Microbiol. 2020;5:304–13.
7. McFarlane AJ, et al. Enteric helminth-induced type I interferon signaling protects against pulmonary virus infection through interaction with the microbiota. J Allergy Clin Immunol. 2017;140:1068-1078.e6.
8. Karo-Atar D, Khan N, Divangahi M, King IL. Helminth-mediated disease tolerance in TB: a role for microbiota? PLOS Pathog. 2021;17: e1009690.
9. Gu S, et al. Alterations of the gut microbiota in patients with coronavirus disease 2019 or H1N1 influenza. Clin Infect Dis. 2020;71:2669–78.
10. Lai P, et al. Metagenomic assessment of gut microbial communities and risk of severe COVID-19. https://www.researchsquare.com/article/rs-1717624/v1. 2022. https://doi.org/10.21203/rs.3.rs-1717624/v1.
11. Bernard-Raichon L, et al. Gut microbiome dysbiosis in antibiotic-treated COVID-19 patients is associated with microbial translocation and bacteremia. Nat Commun. 2022;13:5926.
12. Schult D, et al. Gut bacterial dysbiosis and instability is associated with the onset of complications and mortality in COVID-19. Gut Microbes. 2022;14:2031840.
13. Farsi Y, et al. Diagnostic, prognostic, and therapeutic roles of gut microbiota in COVID-19: a comprehensive systematic review. Front Cell Infect Microbiol. 2022;12: 804644.
14. Stutz MR, et al. Immunomodulatory fecal metabolites are associated with mortality in COVID-19 patients with respiratory failure. Nat Commun. 2022;13:6615.
15. Zuo T, et al. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. Gastroenterology. 2020;159:944-955.e8.
16. Hegazy M, et al. Beyond probiotic legend: ESSAP gut microbiota health score to delineate SARS-COV-2 infection severity. Br J Nutr. 2022;127:1180–9.
17. Njenga MK, et al. Why is there low morbidity and mortality of COVID-19 in Africa? Am J Trop Med Hyg. 2020;103:564–9.
18. Lawal Y. Africa's low COVID-19 mortality rate: a paradox? Int J Infect Dis. 2021;102:118–22.
19. Kamulegeya R, et al. Biobanking: strengthening Uganda's rapid response to COVID-19 and other epidemics. Biopreserv Biobank. 2022;20:238–43.
20. Gohl DM, et al. Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. Nat Biotechnol. 2016;34:942–9.
21. Eren AM, Vineis JH, Morrison HG, Sogin ML. A filtering method to generate high quality short reads using Illumina paired-end technology. PLoS ONE. 2013;8:e66643.
22. Bolyen E, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–7.
23. Callahan BJ, et al. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3.
24. Katoh K, Rozewicki J, Yamada KD. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform. 2019;20:1160–6.
25. Kuraku S, Zmasek CM, Nishimura O, Katoh K. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Res. 2013;41:W22–8.
26. Quast C, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2012;41:D590–6.
27. Lin H, Peddada SD. Analysis of compositions of microbiomes with bias correction. Nat Commun. 2020;11:3514.
28. Lin H, Eggesbø M, Peddada SD. Linear and nonlinear correlation estimators unveil undescribed taxa interactions in microbiome data. Nat Commun. 2022;13:4946.
29. Mallick H, et al. Multivariable association discovery in population-scale meta-omics studies. PLoS Comput Biol. 2021;17: e1009442.
30. Wickham H. Ggplot2: elegant graphics for data analysis. Cham: Springer International Publishing; 2016. https://doi.org/10.1007/978-3-319-24277-4.
31. Gaio D, et al. Hackflex: low cost Illumina Nextera Flex sequencing library construction. Microb Genom. 2019. https://doi.org/10.1101/779215.
32. Kalantar KL, et al. IDseq—an open source cloud-based pipeline and analysis service for metagenomic pathogen detection and monitoring. GigaScience. 2020;9:111.
33. Franzosa EA, et al. Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat Microbiol. 2018;4:293–305.
34. Ananthakrishnan AN, et al. Gut microbiome function predicts response to anti-integrin biologic therapy in inflammatory bowel diseases. Cell Host Microbe. 2017;21:603-610.e3.
35. Fang X, et al. Gastrointestinal surgery for inflammatory bowel disease persistently lowers microbiome and metabolome diversity. Inflamm Bowel Dis. 2021;27:603–16.

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36. Mills RH, et al. Multi-omics analyses of the ulcerative colitis gut microbiome link *Bacteroides vulgatus* proteases with disease severity. Nat Microbiol. 2022;7:262–76.
37. Olendzki B, et al. Dietary manipulation of the gut microbiome in inflammatory bowel disease patients: pilot study. Gut Microbes. 2022;14:2046244.
38. Khorsand B, et al. Overrepresentation of *Enterobacteriaceae* and *Escherichia coli* is the major gut microbiome signature in Crohn's disease and ulcerative colitis; a comprehensive metagenomic analysis of IBDMDB datasets. Front Cell Infect Microbiol. 2022;12:1015890.
39. Ministry of Local Government, Republic of Uganda. https://molg.go.ug/cities/.
40. Hansen CHF, et al. Early life treatment with vancomycin propagates *Akkermansia muciniphila* and reduces diabetes incidence in the NOD mouse. Diabetologia. 2012;55:2285–94.
41. Qu S, et al. *Akkermansia muciniphila* alleviates dextran sulfate sodium (DSS)-induced acute colitis by NLRP3 activation. Microbiol Spectr. 2021;9:e00730-e821.
42. Yin H, Huang J, Guo X, Xia J, Hu M. *Romboutsia lituseburensis* JCM1404 supplementation ameliorated endothelial function via gut microbiota modulation and lipid metabolisms alterations in obese rats. FEMS Microbiol Lett. 2023;370:fnad016.
43. Le S-NH, et al. The impact of antibiotics on the gut microbiota of children recovering from watery diarrhoea. NPJ Antimicrob Resist. 2024;2:12.
44. Rashidi A, et al. Gut microbiota response to antibiotics is personalized and depends on baseline microbiota. Microbiome. 2021;9:211.
45. Frank DN, et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci. 2007;104:13780–5.
46. Al Khatib HA, et al. Profiling of intestinal microbiota in patients infected with respiratory influenza A and B viruses. Pathogens. 2021;10:761.
47. Li Z, et al. Differences in alpha diversity of gut microbiota in neurological diseases. Front Neurosci. 2022;16: 879318.
48. Choileáin SN, et al. CXCR3+ T cells in multiple sclerosis correlate with reduced diversity of the gut microbiome. J Transl Autoimmun. 2020;3: 100032.
49. Braun T, et al. Diet-omics in the study of urban and rural crohn disease evolution (SOURCE) cohort. Nat Commun. 2024;15:3764.
50. Cani PD, De Vos WM. Next-generation beneficial microbes: the case of *Akkermansia muciniphila*. Front Microbiol. 2017;8:1765.
51. Routy B, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science. 2018;359:91–7.
52. Qu S, et al. Excessive consumption of mucin by over-colonized *Akkermansia muciniphila* promotes intestinal barrier damage during malignant intestinal environment. Front Microbiol. 2023;14:1111911.
53. Keane JM, et al. *Akkermansia muciniphila* reduces susceptibility to *Listeria monocytogenes* infection in mice fed a high-fat diet. Gut Microbes. 2023;15:2229948.
54. Krawczyk B, Wityk P, Gałęcka M, Michalik M. The Many Faces of *Enterococcus spp*—Commensal. Probiotic and Opportunistic Pathogen Microorganisms. 2021;9:1900.
55. Dubin K, Pamer EG. Enterococci and their interactions with the intestinal microbiome. Microbiol Spectr. 2017;5.
56. Gardiner BJ, et al. Clinical and microbiological characteristics of *Eggerthella lenta* Bacteremia. J Clin Microbiol. 2015;53:626–35.
57. Alexander M, et al. Human gut bacterial metabolism drives Th17 activation and colitis. Cell Host Microbe. 2022;30:17-30.e9.
58. Balakrishnan B, et al. *Eggerthella lenta* augments preclinical autoantibody production and metabolic shift mimicking senescence in arthritis. Sci Adv. 2023;9:eadg1129.
59. Cao J, et al. Integrated gut virome and bacteriome dynamics in COVID-19 patients. Gut Microbes. 2021;13:1887722.
60. Zhao Y, et al. Clonal expansion and activation of tissue-resident memory-like T$_H$17 cells expressing GM-CSF in the lungs of patients with severe COVID-19. Sci Immunol. 2021;6:eabf6692.
61. Raucci F, et al. Interleukin-17A (IL-17A), a key molecule of innate and adaptive immunity, and its potential involvement in COVID-19-related thrombotic and vascular mechanisms. Autoimmun Rev. 2020;19: 102572.
62. Guy-Grand D, et al. Origin, trafficking, and intraepithelial fate of gut-tropic T cells. J Exp Med. 2013;210:1839–54.
63. Lindeboom RGH, et al. Human SARS-CoV-2 challenge uncovers local and systemic response dynamics. Nature. 2024. https://doi.org/10.1038/s41586-024-07575-x.
64. Park MD. Macrophages: a Trojan horse in COVID-19? Nat Rev Immunol. 2020;20:351–351.
65. Montazersaheb S, et al. COVID-19 infection: an overview on cytokine storm and related interventions. Virol J. 2022;19:92.
66. Mussabay K, et al. Gut microbiome and cytokine profiles in post-COVID syndrome. Viruses. 2024;16:722.
67. Rezende RM, et al. Gamma-delta T cells modulate the microbiota and fecal micro-RNAs to maintain mucosal tolerance. Microbiome. 2023;11:32.
68. Schirmer M, et al. Linking the human gut microbiome to inflammatory cytokine production capacity. Cell. 2016;167:1125-1136.e8.
69. Xue F, He Z, Zhuang D-Z, Lin F. The influence of gut microbiota on circulating inflammatory cytokines and host: a Mendelian randomization study with meta-analysis. Life Sci. 2023;332: 122105.

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