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Original Article **Open Access**

SARS-CoV-2 faecal negativity and gastrointestinal eubiosis in non-severe COVID-19 in Lagos, Nigeria

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Abstract:

Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), when disseminated to the gastrointestinal tract (GIT), can cause alterations in the composition and diversity of the gastrointestinal tract microbiota. However, there is a paucity of data linking SARS-CoV-2 fecal negativity with the GI microbial balance. This study investigated the association between the GI bacterial composition and clinically defined asymptomatic, mild/moderate COVID-19 fecal-negative individuals.
Methodology: This was a cross sectional, comparative study of GI bacterial composition in COVID-19 nasopharyngeal (NP) -positive (n=7) and negative (n=5) participants at the testing facility of the Nigerian Institute of Medical Research between 6th July and 8th August 2022. Faecal samples were collected from the 12 participants. The RNA extracted from the NP-positive samples was used for RT–qPCR detection of the nucleocapsid and open reading frame (*ORF*1ab) genes, while DNA from all the faecal samples was used for 16S rRNA metataxonomic analysis.
Results: The participants comprised males (n=4) and females (n=8), ages 17-74 years. The NP-positive participants reported no (n=2, 28.5%), mild (n=4, 57.1%) and moderate (n=1, 14.3%) clinical symptoms. The viral genes were undetected with uniform or rich bacterial species in the fecal samples. The most abundant bacterial phyla were the Firmicutes, Bacteroidota, and Proteobacteria while *Prevotella copri, Phocaeicola vulgatus*, and the immuno-modulatory, anti-inflammatory bacterium, *Faecalibacterium prausnitzii*, were the dominant species. There were no significant differences in alpha diversity, Pielou evenness (p=0.223) and Shannon richness index (p=0.062), or beta taxonomic diversity (PERMANOVA p=0.357) between NP-positive and negative participants.
Conclusion: This study did not reveal any differences in the gut bacterial community between asymptomatic and mild/moderate COVID-19 patients, and apparently healthy controls. The asymptomatic, mild, and moderate COVID-19 patients in this study maintained balanced gut bacterial diversity, with undetected viral gene in their stool, which may pose little or no threats to the public health in terms of SARS-CoV-2 shedding to the environment. This may however not be generalizable given the small sample size.

Keywords: Gastrointestinal, eubiosis, microbiome, SARS-CoV-2, fecal, RT–PCR.

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Négativité fécale du SRAS-CoV-2 et eubiose gastro-intestinale dans le cas du COVID-19 non sévère à Lagos, Nigeria

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Résumé:

Contexte: Le coronavirus 2 du syndrome respiratoire aigu sévère (SARS-CoV-2), lorsqu'il se dissémine dans le tractus gastro-intestinal (TGI), peut entraîner des altérations de la composition et de la diversité du microbiote intestinal. Cependant, les données liant la négativité fécale au SARS-CoV-2 à l'équilibre microbien gastro-intestinal sont rares. Cette étude a examiné l'association entre la composition bactérienne gastro-intestinale et les individus asymptomatiques, présentant une forme légère à modérée de COVID-19 et dont les selles sont négatives.
Méthodologie: Il s'agit d'une étude transversale comparative de la composition bactérienne gastro-intestinale chez des participants positifs (n=7) et négatifs (n=5) au test nasopharyngé (NP) de dépistage de la COVID-19, recrutés au centre de dépistage de l'Institut nigérian de recherche médicale entre le 6 juillet et le 8 août 2022. Des échantillons de selles ont été prélevés chez les 12 participants. L'ARN extrait des échantillons NP positifs a été utilisé pour la détection par RT-qPCR des gènes de la nucléocapside et du cadre de lecture ouvert (*ORF*1ab), tandis que l'ADN de tous les échantillons de selles a été utilisé pour l'analyse métataxonomique de l'ARNr 16S.
Résultats: Les participants étaient composés de 4 hommes et 8 femmes, âgés de 17 à 74 ans. Les participants NP positifs ont rapporté des symptômes cliniques absents (n=2, 28,5%), légers (n=4, 57,1%) et modérés (n=1, 14,3%). Les gènes viraux étaient indétectables, malgré une composition bactérienne homogène ou abondante dans les échantillons fécaux. Les phyla bactériens les plus abondants étaient les Firmicutes, les Bacteroidota et les Proteobacteria, tandis que *Prevotella copri*, *Phocaeicola vulgatus* et la bactérie immunomodulatrice et anti-inflammatoire *Faecalibacterium prausnitzii* étaient les espèces dominantes. Aucune différence significative n'a été observée concernant la diversité alpha, l'équitabilité de Pielou (p=0,223), l'indice de richesse de Shannon (p=0,062) ou la diversité taxonomique bêta (PERMANOVA p=0,357) entre les participants positifs et négatifs au test NP.
Conclusion: Cette étude n'a révélé aucune différence dans la composition du microbiote intestinal entre les patients asymptomatiques et ceux atteints d'une forme légère à modérée de COVID-19, et les sujets témoins apparemment sains. Les patients atteints de COVID-19 asymptomatiques, légères ou modérées inclus dans cette étude ont conservé une diversité bactérienne intestinale équilibrée, sans détection de gène viral dans leurs selles. Ceci suggère que le risque de propagation du SARS-CoV-2 dans l'environnement pourrait être faible, voire nul. Toutefois, ces résultats ne sont pas généralisables compte tenu de la petite taille de l'échantillon.

Mots-clés: Système gastro-intestinal, eubiose, microbiome, SARS-CoV-2, selles, RT-PCR.

Introduction:

The 2019 coronavirus disease (COVID-19) pandemic resulted in an unprecedented global crisis; a large number of disease-associated deaths occurred on different scales in countries across the world. This prompted research directed towards both pharmaceutical and non-pharmaceutical interventions, while testing was also encouraged to increase. The Nigerian Institute of Medical Research, Nigeria, initiated a modified drive-through testing platform where the largest number of patients were tested in the country (1,2). Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the aetiology of the disease (COVID-19), is transmitted through respiratory droplets, aerosols, and sometimes the faeco-oral route (3).

Despite the primary involvement of the upper respiratory tract, substantial viral infection has been reported in the gastrointestinal tract (GIT) of patients with moderate to severe COVID-19 and viral stool shedding even weeks after disease resolution (4-9). However, little or no viral stool clearance has been reported in patients with mild to moderate disease, which comprises up to 81% of those who contract COVID-19 (10,11).

The pathophysiology of SARS-CoV-2 and its link with the GIT have been well reported. After contact, the virus binds to angiotensin-converting enzyme 2 (ACE-2) receptors in the lungs. The viral spike glycoprotein attached to ACE-2 enables viral entry into cells, which leads to replication and dissemination to other parts of the body. The intestinal epithelium, which highly expresses ACE-2 receptors, can harbor SARS-CoV-2 after swallowing viral-laden sputum (gut-lung cross talk); this serves as a site of extrapulmonary viral infection, as evidenced by the presence of SARS-CoV-2 RNA in the feces of patients with COVID-19 (7,12-15).

The role of the gut microbiota in the development of many diseases, including immune-mediated, metabolic, neurodegenerative, and psychiatric diseases, is becoming increasingly clear (16-18). An imbalance in the intestinal microbial community/diversity that can be associated with disease is referred to as dysbiosis. The most typical characteristics are a depletion in the richness and diversity of the microbiota, a loss of probiotics/beneficial microbiota or an overgrowth of harmful ones.

Additionally, host-specific factors such as lifestyle habits, hygiene, infections, inflammation, high sugar intake, low fiber intake, and drugs can cause dysbiosis (19-21). Several human observational studies have reported dysbiosis of the gut microbiota composition in COVID-19 patients at the time of diagnosis and during the course of the disease, with decreased bacterial diversity observed in COVID-19 patients (13,22,23). These studies documented cases in populations in China, the USA, Japan,

Bangladesh, the UAE, Portugal, Germany, and Hungary. In Africa and Nigeria, there is a paucity of data connecting the gut microbiome with mild and asymptomatic COVID-19. Many other studies have linked the composition of the gut microbiota to severe COVID-19 and GI symptoms. Therefore, this study was designed to investigate the diversity of the gut microbiota and extent of viral shedding in patients with clinically defined asymptomatic, mild, or moderate COVID-19.

Materials and method:

Study site:

This study was carried out on drive-in and walk-through COVID-19 testing facilities at the Nigerian Institute of Medical Research (NIMR) Lagos, Nigeria. The NIMR is a leading medical research institute located in the commercial capital (Lagos) of Nigeria. Lagos is the most populous city in Nigeria, with approximately 24 million inhabitants (https://lagosstate.gov.ng/about-lagos/). During the COVID-19 pandemic, the COVID-19 testing facilities in NIMR received more than 50,000 people, making it one of the largest in the country.

Ethical considerations:

The conduct of this study was approved (IRB/21/069) by the institutional review board of the Nigerian Institute of Medical Research (NIMR-IRB), while social approval was given by the Lagos State Ministry of Health. The written informed consent obtained from the participants after they understood the research and are locked up in a cabinet that is only accessible to the Principal Investigator. The electronic copies of all data (questionnaire and result sheets) are anonymized using unique codes and saved on a passworded computer.

Study design:

This was a cross-sectional comparative study of SARS-CoV-2-infected patients and apparently healthy Nigerians (negative control) in Lagos. For the purpose of this study, N refers to nasopharyngeal (NP) negative while P refers to nasopharyngeal (NP) positive participants.

Sample size calculation:

Using a two-sided t-test, a significance level (α) of 0.05, and assuming an effect size (Cohen's d) of 0.8 (a large effect size), the required sample size for 80% power was estimated to be 26 participants. However, our study included less than half of this required number, this implies a key limitation of our study, highlighting the need for future research with a larger sample size to validate our findings and improve the robustness of statistical inferences.

Data capture:

Sociodemographic data of participants, including hospital admission history, symptoms, presence/absence of comorbidities, and treatment/drug intake, were collected via a short questionnaire administered by the research assistant after consent form was signed.

Sample collection and nucleic acid extraction:

Stool samples were collected from the consenting participants visiting the NIMR walk-in and drive-through testing facilities following standard procedures. The selection of participants was not in a particular format but rather at convenience. They were recruited into the study as they visited the facilities, irrespective of their demographics. Stool samples were collected from 6th July 2022 till 8th August 2022 in an OMNIgene GUT microbiome device (DNA Genotech Inc., Ottawa, Canada) and stored in a freezer until processing.

Viral RNA extraction was performed on 10th August 2022 on all samples from participants who tested positive for nasopharyngeal RT–qPCR (NP) using Qiagen kits (QIAamp Viral RNA, Germany), while total DNA was extracted from all stool samples on 12th of August 2022 using ZymoBiomics DNA Miniprep metagenomics kits (Zymo Research, USA). All the nucleic acid extraction was done according to the manu facturer's instructions. The extracts were quantitated, and the purity was evaluated using a Nanodrop One/One spectrophotometer (Thermo Scientific, UK).

Gene amplification and metagenomic analysis:

The viral nucleocapsid (*N*) and open reading frame (*ORF*1ab) genes were detected on 10th of August 2022 by RT–qPCR (Applied Biosystems) using the SARS–CoV-2 Detection Assay (SCODA) workflow. Briefly, 5µl of extracted RNA was added to 20µl of Mastermix to make a 25µl reaction mixture with the viral synthetic genes and RNase-free water as positive and negative controls, respectively. The PCR conditions included a cycle of reverse transcription at 52°C for 10 min; inactivation at 95°C for 10 seconds; 40 cycles of denaturation at 95°C for 5 seconds; and annealing and extension at 56°C for 30 seconds. The cutoff cycle threshold for the target genes was 38.

Amplification of the V4 region was carried out using the 16S V4 updated primer sequence 515F/806R, forward barcoded: FWD: GTGYCAGCMGCCGCGGTAA; REV: GGACTACNV GGGTWTCTAAT (24,25), in a 25µl reaction mixture containing 0.8X PCR master mix (Platinum Hot Start PCR Master Mix (2x)), 0.2 µM of each

primer, 1µl of template DNA, and PCR grade water (Sigma). The thermocycling condition included an initial denaturation at 94°C for 3 min, followed by 35 amplification cycles at 94°C for 45 s, 50°C for 60 s, and 72°C for 90 s. This was followed by a final extension cycle at 72°C for 10 min. All the samples were amplified in triplicate and pooled into single 75µl volumes.

Amplicons were tested on a Bioanalyzer to confirm the presence of the PCR product. The amplicons were also run on a 1.5% agarose gel, and a single band of approximately 360 bp was used to confirm successful amplification. Subsequently, the amplicons were quantified with the Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher/Invitrogen) following the manufacturer's instructions.

The pooled amplicons were cleaned using the MoBio UltraClean PCR Clean-Up Kit following the manufacturer's instructions. The purity and concentration of the PCR products were subsequently measured. Samples with an A260/A280 ratio between 1.8 and 2.0 were aliquoted and used for library preparation and metagenomics sequencing (Laragen, Inc., USA).

Metagenomic sequencing:

The pure library was pooled and denatured into single strand, further diluted to concentration suitable for loading on the P1 flow cell of the Illumina NextSeq 2000 sequencing instrument with 15% PhiX control spike-in. The raw data (FASTQ files) were analyzed and annotated on One Codex database involving k-mer based classification, artifact filtering and species-level abundance estimation.

Taxonomic classification and data analysis:

Alpha diversity of the microbial community in the two groups of participants (positive-P, negative-N) was assessed by Pielou and Shannon indices using Kruskal–Walli's test of significance. The Pielou diversity index was used to determine the uniformity of the microbial community, while the Shannon index was used to determine the richness of the microbial community.

Beta taxonomic diversity was assessed with the Bray–Curtis' diversity index using permutational multivariate analysis of variance (PERMANOVA) for the test of significance and principal component analysis calculator (26) was used to determine variation in the microbiota of the participants. The taxonomic classification was performed using the Greengenes2 database trained for hypervariable 4 of the 16S rRNA (gg_2022_10_backbone.v4.nb).

Results:

The participants comprised males (n=4) and females (n=8), ages 17-74years. The NP-positive participants reported no (n=2, 28.5%), mild (n=4, 57.1%) and moderate (n=1, 14.3%) clinical symptoms. Of the 12 participants whose stool samples were analyzed, 7 presented with various degrees of symptoms; asymptomatic (n=2), mild (n=4) or moderate (n=1), while the others (n=5) were negative for NP-related COVID-19 from RT–PCR assay (Table 1).

Table 1: Symptomatology and demographics of COVID-19 study participants

Study ID	Symptoms	Sex	Age (years)
16-P	Asymptomatic	F	74
17-P	Asymptomatic	F	37
12-P	Mild	F	27
31-P	Mild	F	27
4-P	Mild	F	17
ME-P	Mild	F	58
3-P	Moderate	F	44
7-N	Negative	M	38
8-N	Negative	M	31
13-N	Negative	F	36
20-N	Negative	M	28
24-N	Negative	M	22

M = Male; F = Female; ID = Identification

Table 2: Bray–Curtis distance matrix of the samples

Sample	**Species similarity**											
	12-P	**13-N**	**16-P**	**17-P**	**20-N**	**24-N**	**3-P**	**31-P**	**4-P**	**7-N**	**8-N**	**ME-P**
12-P	0	0.8814	0.7842	0.8395	0.8379	0.742	0.7435	0.7087	0.8831	0.8072	0.7548	0.7589
13-N	0.8814	0	0.7893	0.9595	0.9579	0.8998	0.7269	0.7736	0.7436	0.889	0.9013	0.8953
16-P	0.7842	0.7893	0	0.8107	0.909	0.758	0.7041	0.7171	0.7861	0.8272	0.8586	0.8195
17-P	0.8395	0.9595	0.8107	0	0.5215	0.7554	0.7463	0.8003	0.9177	0.6325	0.8562	0.859
20-N	0.8379	0.9579	0.909	0.5215	0	0.8501	0.7759	0.8734	0.9554	0.4443	0.8	0.8729
24-N	0.742	0.8998	0.758	0.7554	0.8501	0	0.8317	0.6636	0.881	0.8201	0.8353	0.5227
3-P	0.7435	0.7269	0.7041	0.7463	0.7759	0.8317	0	0.7101	0.6378	0.6371	0.9075	0.8414
31-P	0.7087	0.7736	0.7171	0.8003	0.8734	0.6636	0.7101	0	0.6573	0.7651	0.873	0.7548
4-P	0.8831	0.7436	0.7861	0.9177	0.9554	0.881	0.6378	0.6573	0	0.8304	0.9357	0.9324
7-N	0.8072	0.889	0.8272	0.6325	0.4443	0.8201	0.6371	0.7651	0.8304	0	0.8234	0.8317
8-N	0.7548	0.9013	0.8586	0.8562	0.8	0.8353	0.9075	0.873	0.9357	0.8234	0	0.8712
ME-P	0.7589	0.8953	0.8195	0.859	0.8729	0.5227	0.8414	0.7548	0.9324	0.8317	0.8712	0

Values from 0 to 1 indicate identical composition to distinct species, where 0 represents similarity. The higher the figure, the greater the distance and dissimilarity

SARS-CoV-2 RNA was not detected in any stool sample by RT–qPCR. Sequencing of the 16S rRNA v4 amplicon yielded 747,917 sequences for all the analyzed samples. After quality control, 585,356 sequences belonging to 806 features were used for further analysis. The Pielou diversity did not significantly differ between the P and N groups (Kruska-Wallis p= 0.22). Similarly, the Shannon index was not significantly different between the two groups (Kruska-Wallis p=0.062).

The Bray–Curtis beta diversity was also not significantly different between the two groups (PERMANOVA p=0.357) and this is depicted in Table 2. However, the bacterial community of the microbiota of some of the participants who tested positive and negative for COVID-19 showed close clustering (3-P, 4-P, 16-P, and 13-N; 7-N, 20-N, and 17-P) and high divergence, as in the case of sample 8-N, as depicted in Fig 1. Sample ME-P, which was positive, had a bacterial community that clustered with and overlapped that of sample 24-N, which was negative for COVID-19 (Fig 2).

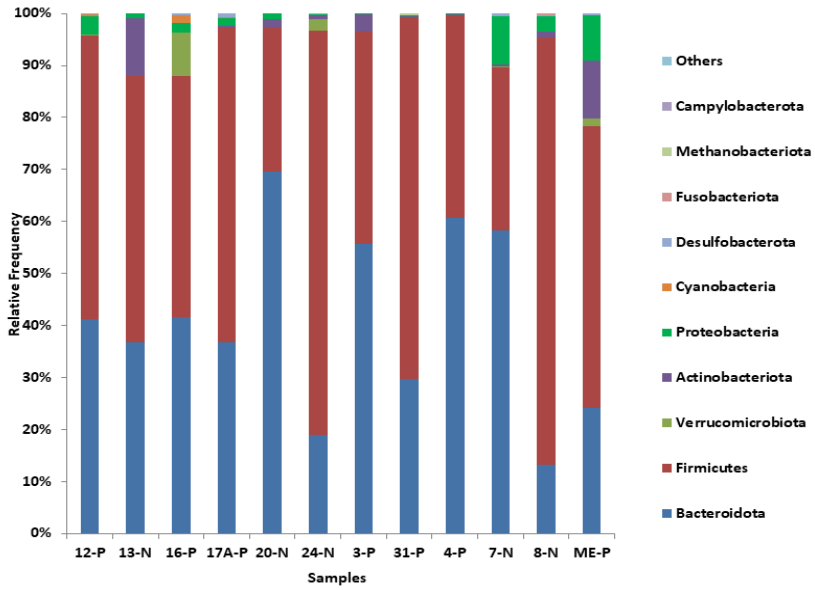

Fig 1: Relative abundance of bacterial phyla in the gut microbiota of COVID-19 positive and negative participants

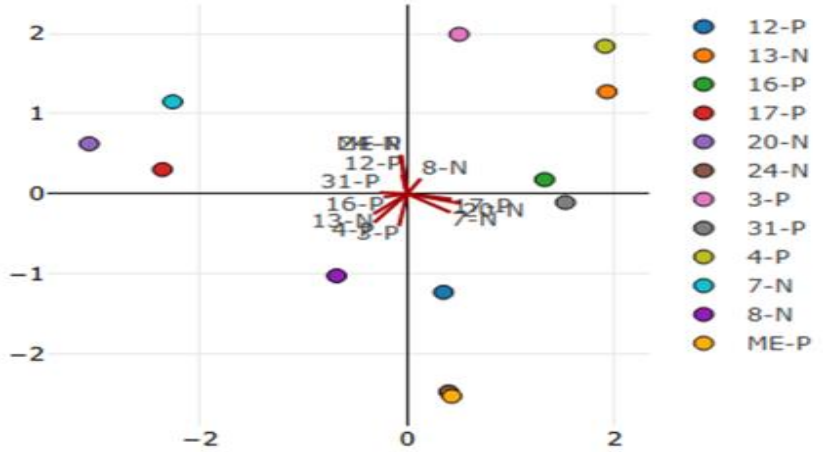

Fig 2: Principal Component Analysis (PCA) of the bacterial species in the gut microbiota of COVID-19 positive and negative participants.

Ten major phyla were identified among others in the 12 samples. Firmicutes and Bacteriodota were the most abundant phyla in the samples, with relative abundances of 82.2%, 54.6%, 46.3%, and 27.7% (Firmicutes) and 13.2%, 41.2%, and 41.7% 69.6% (Bacteriodota) in samples 8 N, 12-P, 16-P, and 20-N, respectively, as shown in Fig 1. At the genus level, four genera, namely, *Faecalibacterium*, *Phocaeicola*, *Prevotella*, and *Bacteroides*, were the most dominant. The genus *Phocaeicola* was more dominant in samples 16-P and 4-P, with relative abundances of 15.9% and 19.2%, respectively. *Faecalibacterium* was more dominant in sample 8-N, with a relative abundance of 47.7%.

Akkermansia was only present in samples 12-P, 16-P, 24-N, 7-N, and ME-P, with relative abundances of 0.03%, 8.0%, 2.2%, 0.1% and 1.2%, respectively (Fig 3). *Prevotella copri*, *Phocaeicola vulgatus,* and *Faecalibacterium prausnitzii* were the most dominant among the other species identified. Although *Faecalibacterium prausnitzii* was the dominant species identified, this bacterium was not detected in the 13 N subgroup. Similarly, *Prevotella copri* was absent in 12-P, 13-N, and 16-P. Among the other identified dominant species, *Bacteroides stercoris* (23.1%) and *Gemmiger qucibialis* (16.7%) were more dominant in sample 4 (Fig 4).

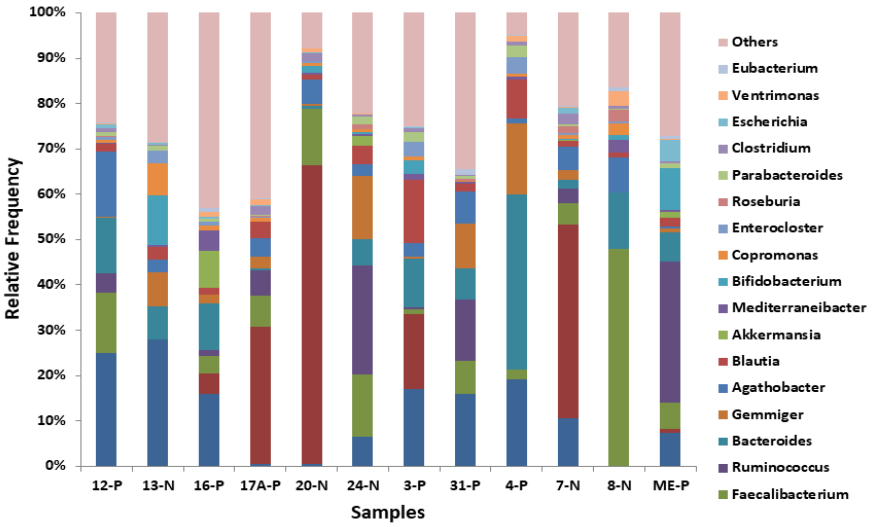

Fig 3: Relative abundance of bacteria genera in the gut microbiota of COVID-19 positive and negative participants.

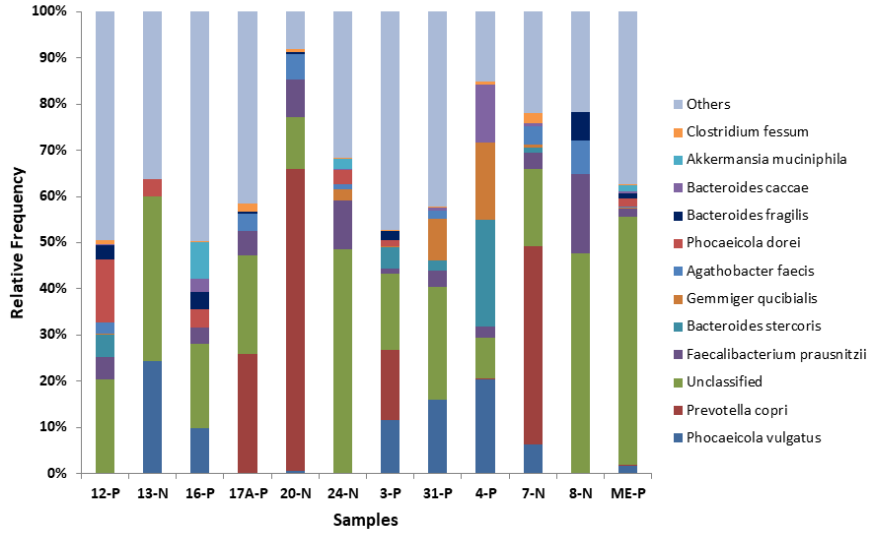

Fig 4: Relative abundance of bacteria species in the gut microbiota of COVID-19 positive and negative participants.

Discussion:

The asymptomatic, mild and moderate COVID-19 patients in this study maintained balanced gut bacterial diversity, with undetected viral genes in their stool. This poses no threats to the public health in terms of SARS-CoV-2 shedding to the environment, although this may not be generalizable given the small sample size. The small sample size is attributed to the transient nature of the disease as at the time the samples were being collected. The visit to the testing platform was already waning and as such, we set out to recruit as many as would give written informed consent without minding the sex, age group. The symptoms were extracted from their self-completed online questionnaire and as confirmed after clinical examination at the site by medical doctors. Many of the participants just wanted to confirm their status and were not ready to provide any stool samples while a few others who collected the sample containers gave the excuse of distance from the facility.

There have been conflicting reports on intestinal microbial diversity in patients with COVID-19; a lower intestinal diversity has been reported in these patients than in healthy controls in China and Bangladesh respectively (27,28), while a different pattern was demonstrated in another study in a Christian hospital, Hong Kong (29). It has also been reported in studies from China that higher intestinal microbial richness is observed in asymptomatic patients than in symptomatic patients and during disease progression (30-32). Although we were unable to collect severe or hospitalized cases in the present study, intestinal microbial evenness (p=0.22) and richness (p=0.06) were not associated with asymptomatic, mild, or moderate cases compared to COVID-19 negative control. In contrast, Kim et al., (33) reported intestinal dysbiosis after infection in patients with asymptomatic or mild COVID-19 in Korea.

The classification of asymptomatic or mild COVID-19 was performed according to the severity criteria of the Korea Centers for Disease Control and Prevention (http://ncov.mohw.go.ks/en/); age younger than 60 years, no underlying chronic diseases, an alert mentality, a body temperature less than 37.5°C, and no radiological evidence of pneumonia. In our study, the World Health Organization (WHO) severity criteria, which are slightly different, were used (34). These findings may be attributed to the fact that SARS-CoV-2 has not crossed the gut-lung axis of infected participants at the time of sample collection or because host immunity has taken care of it. This finding supports the report that severe cases are associated with a disruption of gut microbial diversity/richness. This is corroborated by the lack of detection of viral RNA in the stool samples of all NP-positive study participants.

The viability of the nucleic acid (viral RNA and metagenomic DNA) in the stool collection device (OMNIgene GUT) was preserved, as it was stored frozen at -80°C. This needs to be mentioned given the 'unstable' nature of RNA which is delicate to handle compared to DNA. Also, the device is formulated to preserve nucleic acid (DNA at room temperature and RNA at ultra-low temperature of -80°C).

Taxonomically, bacteria are classified according to seven levels; kingdom, phylum, class, order, family, genus, and species. However, only a few phyla are represented, and they account for more than 160 species (35). The phyla Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia are dominant in the healthy gut, with Firmicutes and Bacteroidetes representing 90% of the gut microbiota (36). This was also observed in our study, in which more than 90% of the samples were from one of the 2 phyla. Additionally, in our study, Campylobacterota, Methanobacteriota, Desulfobacterota, and Cyanobacteria were present in some of the samples, while the others were almost invisible. Within the Firmicutes in this study, the obligately anaerobic, Gram positive, spore-forming *Clostridium fessum* was only present in the 7- N, 4-P, 17A-P, and 12-P. This bacterium was isolated for the first time in 2021 by Seo et al., (37) from the faeces of a healthy Korean subject.

The balance in microbial diversity in the gut of the study participants is in tandem with the findings of some reports (29,38-41), although some were in mice (40,41), the microbial species that negatively correlate with COVID-19 severity, such as the anti-inflammatory agent, immune modulatory bacteria, *Faecalibacterium prausnitzii*, *Bifidobacterium bifidum*, *Bifidobacterium adolescentis* and *Eubacterium rectale,* were richly abundant, as reported by other studies (29,38). Notably, all participants were predominantly infected with *Prevotella copri* in the intestinal tract, regardless of the status of COVID-19. This bacterium, prevalent in non-Westernized populations, has been shown to act as a 'double edged sword' in health and disease (39-41).

This study has some limitations. Although the small sample size (n=12) is a limitation, which was due to the transient nature of the pandemic as at the time of this study, our data are similar to those of Yeoh et al., (29), Kim et al., (33), and Mesoraca et al., (42). The

study however offers preliminary evidence that may inform future investigations into the relationship between SARS-CoV-2 faecal negativity and gastrointestinal eubiosis in non-severe COVID-19 cases. Also, the sex disparity observed in this study was due to the fact that more females were reporting for testing than the males. There seems to be discordant reports on the association of sex, age and geographic origin with gut microbiota in two studies (43, 44), conducted among donors from different regions including France, Denmark, Germany, Italy, Netherlands, UK and Sweden, although these studies employed fluorescence in-situ hybridization and flow cytometry in their microbiota analysis.

Conclusion:

The gut microbial diversity in asymptomatic, mild or moderate COVID-19 individuals in this study was not disrupted with beneficial bacteria such as *Prevotella copri*, *Phocaeicola vulgatus,* and *Faecalibacterium prausnitzii* being predominant. The viral RNA was undetected in their stool samples and therefore would not be able to shed the pathogen to the environment through the stool.

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Contributions of authors:

TAB conceptualized the study; TAB, MAF, TYR, and BAI designed the study; TAB, MAF, TYR, AbA, OYS, OSA, JOS, NA, TWF, CJI, BAI, SIS, and BLS were involved in sample collection, processing and data analysis; and TAB, MAF, TYR, AbA, AjA, JIY, GA, OYS, OSA, BAI, SIS, and BLS contributed to the writing of the manuscript. All the authors approved the final manuscript submitted for publication.

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Conflict of interests:

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Data availability:

The datasets used and/or analyzed during the study are available from the corresponding author (TAB) on reasonable request.

Disclaimer:

The views expressed in this article are the authors' and not an official position of the institution or funder. The authors are responsible for the article results, findings, and content.

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