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ORIGINAL ARTICLE

COVID-19 Infection and Gut Microbial Dysbiosis Among Filipinos with Type 2 Diabetes Mellitus

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Abstract

Background. Both type 2 diabetes mellitus (T2DM) and COVID-19 are associated with gut microbial alterations. It remains unclear whether COVID-19 causes further gut dysbiosis among individuals with T2DM.

Objective. This study aimed to characterize the gut microbiome of Filipinos with T2DM who had COVID-19.

Methodology. 101 Filipinos, aged 30–59, residing in the Greater Manila Area, were recruited into one of four groups: non-COVID/non-T2DM (A), COVID-recovered/non-T2DM (B), non-COVID/T2DM (C), and COVID-recovered/T2DM (D). Gut microbial composition was characterized through 16S rRNA gene profiling of stool samples using Illumina MiSeq-next-generation Sequencing. These sequences were subjected to mothur and PICRUSt2 for taxonomic and functional analyses.

Results. Gut microbial analysis revealed potential disease biomarkers, as *Roseburia* is more abundant among participants with COVID-19 history, while *Parabacteroides* is more abundant among participants with T2DM. Principal coordinate analysis (PCOA) revealed that participants with T2DM clustered together, while participants without T2DM displayed significantly different clustering.

Conclusion. These findings suggest that COVID-19 does not cause further gut dysbiosis among individuals with T2DM and that T2DM exerts a stronger influence on the gut microbiome compared to COVID-19. These findings are useful for clinicians to better understand the COVID-19 risk to T2DM.

Key words: gut microbiome, COVID-19, type 2 diabetes mellitus, 16S rDNA

INTRODUCTION

The gut microbiome impacts host physiology significantly. It is integral to host digestion and nutrition, polysaccharide breakdown, nutrient absorption, inflammatory responses, gut permeability and bile acid modification.[1] Diversity is integral to a healthy gut microbiome as it permits redundancy, defined as multiple communities of microbes performing similar functions. On the other hand, dysbiosis presents health risks to the host. This is defined as an imbalance in microbial populations and has been associated with a variety of diseases including malnutrition, inflammatory bowel diseases, neurological disorders, cancer, obesity and Type 2 Diabetes Mellitus (T2DM).[2] Specifically, among individuals with T2DM, gut microbial alterations previously documented include: (1) a decrease in relative abundance among *Bifidobacterium*, *Akkermansia* and *Faecalibacterium prausnitzii,* microbial communities either known to produce butyrate or up-regulate butyrate production, which is associated with insulin-resistance, and (2) an increase in relative abundance of *Dorea* which is associated with a reduction of the above-mentioned butyrate-producing bacteria.[2-4]

Gut microbial composition is also found to be associated with COVID-19 disease outcome and duration. Gut dysbiosis is suggested to be contributory to the course and severity of COVID-19 disease.[5] Gut dysbiosis is characterized by the following: (1) relative decrease in symbiotic microorganisms; (2) relative increase in potentially harmful microorganisms; and (3) overall reduction in microbial diversity.[6] In a recently concluded study among Filipinos with active COVID-19 infection of varying severity, it was shown that some gut microbial alterations are found for severe COVID-19 infections but not for asymptomatic and mild disease. It was shown that *Enterococcus* and *Streptococcus*, recognized to be opportunistic pathogens, are enriched among those who suffer from severe COVID-19 infections. This further suggests a possible contribution of the gut microbiome to the pathophysiology of severe COVID-19. This enrichment is also found to be accompanied by a reduction in *Bifidobacterium*, *Collinsella*, *Roseburia*,

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Dorea, *Megamonas* and *Coprococcus*, which have established immunomodulatory roles.[7] Furthermore, the composition of the gut microbiome also appears to be influenced by COVID-19 infection, and the alterations may also persist up to 6 months after infection.[8] Chen et al., monitored the gut microbial composition of participants longitudinally at three different time-points: acute (at the time of illness onset), convalescence (2 weeks after hospital discharge) and post-convalescence (6 months after discharge). They found that the gut microbial richness is reduced significantly at the acute phase among COVID-19 patients compared with uninfected controls. Interestingly, they also found that the microbiota richness seemingly increased but was not restored to normal levels 6-months after recovery.[8] While this study was done on a small sample size, it suggests that COVID-19 infection alters gut microbial composition 6 months after recovery. This time frame coincides with the duration of time at which post-COVID symptoms may still continue to persist.

The COVID-19 pandemic puts individuals with Type 2 Diabetes Mellitus patients at a greater risk than the general population. Epidemiologic analysis of clinical and demographic data done early in the pandemic reveals that individuals with Type 2 Diabetes Mellitus are about three-times as likely to die from COVID-19, relative to the general population.[9] The gut dysbiosis among individuals with Type 2 Diabetes Mellitus is suggested to play a role in this apparent increased risk of severe disease and mortality.[5] *Clostridiales* was previously found to be dominant among Filipinos with T2DM, and *Lactobacillus* and *Bifidobacterium* were found to be dominant among Filipinos with T2DM and obesity.[10] Currently, there is limited knowledge on whether COVID-19 infection adds further alterations to the already dysbiotic gut microbiome among individuals with T2DM.

COVID-19 appears to be associated with alterations on the gut microbiome after recovery. Individuals with T2DM known to be at risk for developing gut microbial dysbiosis might be at risk for developing further gut microbial alterations after COVID-19 infection. This study aimed to characterize how COVID-19 alters the gut microbiome among individuals with and without Type 2 Diabetes Mellitus, three to twelve months after recovery. Specifically, this study sought to (1) compare gut microbial composition of Filipinos with and without COVID-19 history among those with and without T2DM; (2) predict gut microbial function of Filipinos with and without COVID-19 history among those with and without T2DM and (3) determine an association between gut microbial composition with T2DM status and COVID-19 history.

METHODOLOGY

Study design

This cross-sectional study involves a descriptive and observational approach to compare gut microbiome profiles among individuals with and without Type 2 Diabetes Mellitus among those with and without COVID-19 history in the past three to twelve months. Demographic, anthropometric, diet and clinical data were collected upon enrollment of the participants to the study. All collected information was documented with an individual case report form and a food frequency questionnaire. Stool samples were collected from all the participants of the study.

The study was implemented in the Greater Manila Area. Participants of the study are 30 to 59 years old. One hundred and one individuals were recruited via convenience sampling. Recruitment was done primarily from responses from advertisements posted on social media, snowball referrals from participants as well as referrals from colleagues. After satisfying the eligibility criteria, twenty-four (24) were assigned to group A: without T2DM, without COVID-19 history. Twenty-five (25) were assigned to group B: without T2DM with COVID-19 history. Twenty-seven (27) were assigned to group C: with T2DM, without COVID-19 history. Twenty-five (25) were assigned to group D: with T2DM, with COVID-19 history.

Sample size computation for this study was based on a calculator using the Dirichlet-multinomial model for hypothesis testing and power calculation for taxonomic-based human microbiome data based on a study by La Rosa et al.[11] This considers prior knowledge of certain parameters such as the most abundant or least abundant operational taxonomic units (OTUs) and their relative change in abundance among the cases. Parameters considered in this sample-size computation were based on the work of Li et al. (2020), characterizing the gut microbiome profiles of T2DM patients in China.[3] It was shown that a sample size of n=24 participants at $\alpha \leq 0.01$ or $\alpha \leq 0.05$ would attain 95% statistical power (Figure 1). Thus, each study group, as well as the control group, was set at a minimum of twenty four (24) participants.

Participant eligibility criteria

All participants recruited are Filipino, resident of the Greater Manila Area and aged 30 to 59 years old. Additional inclusion criteria include: hemoglobin A_{1c} <6.5 % for sub-groups without T2DM; hemoglobin A_{1c} ≥6.5 % or physician-diagnosed T2DM for sub-groups with T2DM; never had a positive result for COVID-19 RT PCR test or antigen test for sub-groups without COVID-19 history; diagnosed with COVID-19 confirmed with proof of COVID-19 RT-PCR test result in the previous 3 to 12 months for sub-groups with COVID-19 history.[12]

While history of antibiotic intake during the past 180 days can affect the composition of the gut microbiome, excluding them would be difficult to achieve as some participants to be recruited conceivably have had to receive antibiotics for concomitant bacterial infections while being managed for COVID-19. Hence, the history of antibiotic intake was shortened to the past 60 days for participants

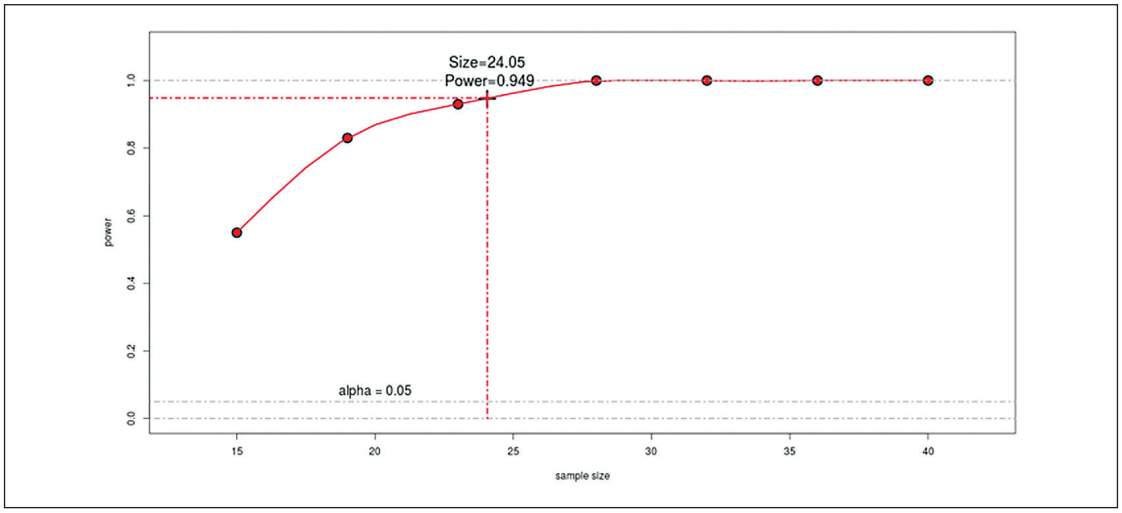

Figure 1. Curve showing statistical power per calculated sample size for stool gut microbiome studies.
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to be excluded from the study. A meta-analysis performed by Elvers et al., found that after cessation of common antibiotic therapy, gut bacteria recovers to their baseline within a few weeks.[13] Other considerations for exclusion were pregnancy, breastfeeding, history of gastrointestinal surgery and being physically unable to provide viable and uncontaminated stool samples. Further exclusion criteria include the use of the following medications: proton-pump inhibitors, H2-receptor antagonist, tricyclic antidepressants, narcotics, anticholinergics, laxatives and non-steroidal anti-inflammatory drugs in the past 30 days.

Sample collection

After consent has been successfully secured from the participant, the investigator facilitated sample collection in the same visit. Two specimens were collected: (1) blood and (2) stool. For the blood collection, the investigator used a 3-cc syringe to extract 1 to 2-cc of venous blood from the median cubital vein of the participant. The extracted blood was subjected to hemoglobin A_{1c} determination. For the stool collection, the investigator gave verbal and printed instructions, as well as a stool collection kit for the participant. Stool samples were kept in a 50-cc conical tube with DNA/RNA Shield (ZymoResearch™) viral inactivation buffer.

DNA isolation

DNA was obtained from the 0.25 grams of stool samples acquired from the study participants through protocols and reagents described in a commercially available DNA Isolation Kit (QIAmp PowerFecal Pro DNA Kit). DNA concentration was determined using the Promega QuantusTM Fluorometer (France) using QuantiFluor(r) Dye Systems.

Next generation sequencing

DNA isolates with a concentration of more than 20 ng/mL were sent to Macrogen Inc. (Republic of Korea) for 16S rRNA gene V3-V4 amplicon sequencing. The DNA Library preparation and Illumina MiSeq-based next-generation sequencing was performed by Macrogen Inc. with the DNA library constructed using the Nextera XT DNA Library Preparation Kit. The fastq files derived from NGS were processed on a Galaxy server with the latest version of *mothur* (c. 1.48.0) for downstream analysis. Based on the protocol previously published by Schloss et al. (2020), the paired end reads of all 16S rRNA gene sequences were consolidated in a singular dataset.[14] This dataset was subjected to the bioinformatics pipeline illustrated in Figure 2.

Bioinformatics analysis

Downstream analysis was performed on MicrobiomeAnalyst (https://dev.microbiomeanalyst.ca/MicrobiomeAnalyst/home.xhtml, accessed December 2023), a web-based and R-based graphical interface platform enabling statistical analysis of the microbiome data. Alpha diversity analysis was estimated using the following indices: Observed OTUs, Shannon and Faith pd. Beta diversity, on the other hand, was determined using Principal Coordinate Analysis (PCoA). MicrobiomeAnalyst uses Microbiome Multivariable Associations with Linear Models (MaAsLin 2) to assess multivariable association of microbial community features with complex metadata in population-scale observational studies. Differentially abundant taxa were evaluated both in their raw counts, as well as their log-2 transformed counts. Kruskal-Wallis rank-sum test was performed to detect the significant differences between study groups with respect to these counts, a p-value of <0.05 as well as a False Discovery Rate (FDR) value of <0.05 was considered significant to minimize spurious correlations. Although log-2 transformation is useful in

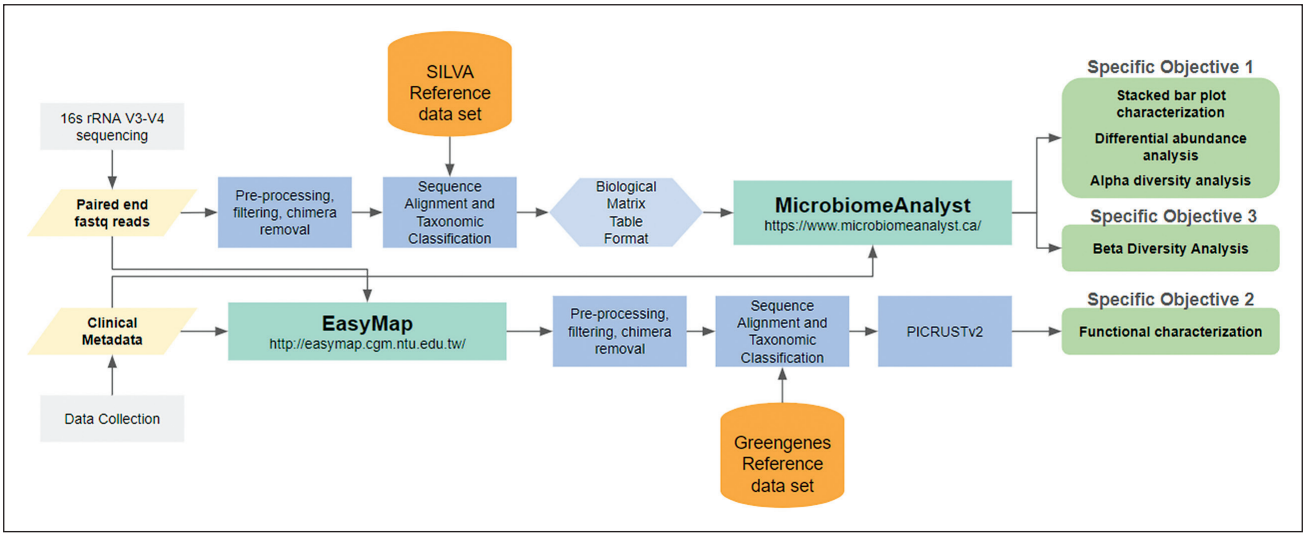

Figure 2. Bioinformatics pipeline used in this study.

This figure was created using Microsoft Powerpoint.

addressing variance, one limitation of this study is the lack of compositional transformation analysis. Functional inferences of the microbial communities were done using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) (v2.2.0-b). This was done on EasyMAP (https://easymap.cgm.ntu.edu.tw/, accessed December 2023), a web-based interface enabling 16S Microbiome analysis. In this analysis, the Greengenes V3-4 classifier was used as the reference database. This step integrates LEfSe and PICRUSt to predict functions of microbes that are significantly different between groups. PICRUSt2 predictions were based on the following gene families: Enzyme Classification numbers and Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs (KO) (v77.1) Alpha value for Kruskal-Wallis test was set at 0.05, while threshold on logarithmic LDA score for discriminative features was set at 2.

Statistical analysis

Bioinformatics analysis incorporating MaAsLin 2 was conducted for the microbiome outcomes of this study. Traditional parametric tests were used for the comparison of dietary consumption of participants. All collected data except for dietary consumption were incorporated in the bioinformatics analysis. To assess for statistical differences in the collected clinical, anthropometric, demographic and dietary data, the following statistical tests were used: (1) Chi-square or Fischer's exact test for nominal variables; (2) ANOVA or t-test for continuous variables. For the dietary consumption of participants, ANOVA was subsequently followed-up by FDR test using two-stage linear step-up procedure of Benjamini, Krieger and Yekutiel. Microbiome These calculations were performed on GraphPad Prism 8.0.1. To assess for statistical differences in relative abundance of identified taxa, Kruskal-wallis rank sum test and Linear Discriminant Analysis (LDA) were performed. To calculate the statistical significance in observed relationships in the principal coordinate analysis (PCOA), PERMANOVA was performed. These calculations were performed on MicrobiomeAnalyst and EasyMap.

Research ethics oversight

The protocol of this study was submitted to the University of the Philippines Manila Research Ethics Board (UPMREB) for evaluation (UPMREB 2022-0046-01). Participant recruitment, data and stool specimen collection commenced only after the approval of the UPMREB Review Panel was secured. All principles of Bioethics in the conduct of scientific research, as well as national laws and regulations, were upheld throughout the study.

RESULTS

Characterization of the study participants

The clinical, sociodemographic and anthropometric data collected from each participant is summarized below (Table 1). ANOVA or unpaired t-test were performed to detect significant differences for continuous variables while Chi-square or Fischer's exact test were performed to detect significant differences for categorical variables.

Dietary composition of participants

Participants across all study groups have similar diets in terms of overall calories, carbohydrates, fats and dietary fibers consumed per day. However, the non-COVID/non-T2DM group (A) was reported to consume significantly more protein compared to the rest of the groups (Figure 3). Table 2 summarizes the p-values from ANOVA and the q-values from FDR testing in the statistical analyses of dietary consumption.

Table 1. Clinical, sociodemographic and anthropometric characteristics of participants

	A No COVID / No T2DM	B COVID / No T2DM	C No COVID / T2DM	D COVID / T2DM	*P*-values
N	24	25	27	25	
Sex (M/F)	(11/13)	(12/13)	(10/17)	(14/11)	0.5923
mean age (years) ± sd	39.29 ± 8.65	34.44 ± 4.39	48.363 ± 8.51	43.76 ± 8.51	<0.0001
mean weight (kg) ± sd	65.06 ± 12.29	70.20 ± 14.69	69.93 ± 21.00	76.52 ± 16.61	0.1241
mean BMI (kg/m2) ± sd	25.35 ± 4.18	26.35 ± 4.18	26.75 ± 7.61	28.81 ± 6.08	0.1990
mean HbA1c (%) ± sd	5.600 ± 0.34	5.496 ± 0.31	8.248 ± 1.79	7.808 ± 2.05	<0.0001
Proportion of fully vaccinated (%)	95.83%	100%	96.30%	96%	0.7948
mean days since COVID infection ± sd	-	191.2 ± 66.84	-	234.7 ± 87.99	0.0549
Number of individuals with following COVID severity classification at the time of COVID infection					
Asymptomatic	-	6	-	0	
Mild	-	9	-	0	
Moderate	-	10	-	24	
Severe	-	0	-	1	
Critical	-	0	-	0	
Proportion with the following comorbidities (%)					
Hypertension	12.5%	20%	51.85%	56%	0.0012
CKD	-	-	-	4%	0.3809
Bronchial asthma	8.33%	8%	-	4%	0.2519
COPD	4.17%	4%	-	-	0.5385
CVD	-	4%	3.7%	8%	0.5463
Thyroid disease	4.17%	-	-	-	0.3560
Hyperuricemia	-	4%	7.41%	4%	0.6077
Hyperlipidemia	-	4%	18.52%	16%	0.0750
Allergic rhinitis	-	4%	-	-	0.3809
PCOS	-	4%	-	-	0.3809
PTB history	-	-	3.7%	-	0.3809
Liver disease	-	-	3.7%	-	0.3809
Proportion taking the following anti-diabetes medications (%)					
Metformin	-	-	55.56%	64%	0.5822
Thiazolidinedione	-	-	3.7%	-	0.9999
Sulfonylurea	-	-	22.22%	16%	0.7289
Insulin	-	-	11.11%	12%	0.9999
SGLT2 Inhibitor	-	-	11.11%	16%	0.6983
GLP1 Analogue	-	-	-	4%	0.9999
DPP4 Inhibitor	-	-	14.81%	20%	0.7224

P-values were determined using one-way ANOVA for continuous variables, and Chi-square or Fischer's exact test for categorical variables.

Table 2. ANOVA and FDR testing results for dietary consumption of participants

	Total Calories	Carbohydrate	Fat	Protein	Dietary Fiber
ANOVA (p-values)	0.1148	0.5526	0.6106	0.0053*	0.0954
FDR test (q-values)					
A vs B	0.1462	0.7109	0.6413	0.0041	0.1009
A vs C	0.1638	0.7109	0.6413	0.0118	0.3740
A vs D	0.1462	0.7109	0.6413	0.0041	0.1971
B vs C	0.9240	0.9970	0.9996	0.4004	0.4274
B vs D	0.9240	0.9970	0.9996	0.4368	0.6435
C vs D	0.9240	0.9970	0.9996	0.4057	0.6435

P-values were determined using one-way ANOVA for continuous variables, *q*-values were determined using FDR approach using two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli.

Identification of microbial biomarkers

Identification of microbial biomarkers from all groups through linear discriminant analysis with effect size (LEfSe) revealed *Parabacteroides* (LDA score 4.6) to be significantly more abundant for the T2DM groups for both with and without history of COVID-19 (groups C and D) (Figure 4). Furthermore, *Roseburia* (LDA score 5.38) was revealed to be significantly more abundant for the COVID-19 recovered groups for both with and without T2DM (groups B and D) (Figure 4).

Alpha and beta diversity analyses

The alpha diversity observed across all study groups is not significantly different (Figure 5A). Beta diversity analysis, however, revealed statistically significant clustering (p -value <0.05) demonstrated by the Principal Coordinate Analysis plot (Figure 5B). The non-COVID/ non-T2DM group (A) had clustering overlap with the COVID-recovered/non-T2DM group (B).

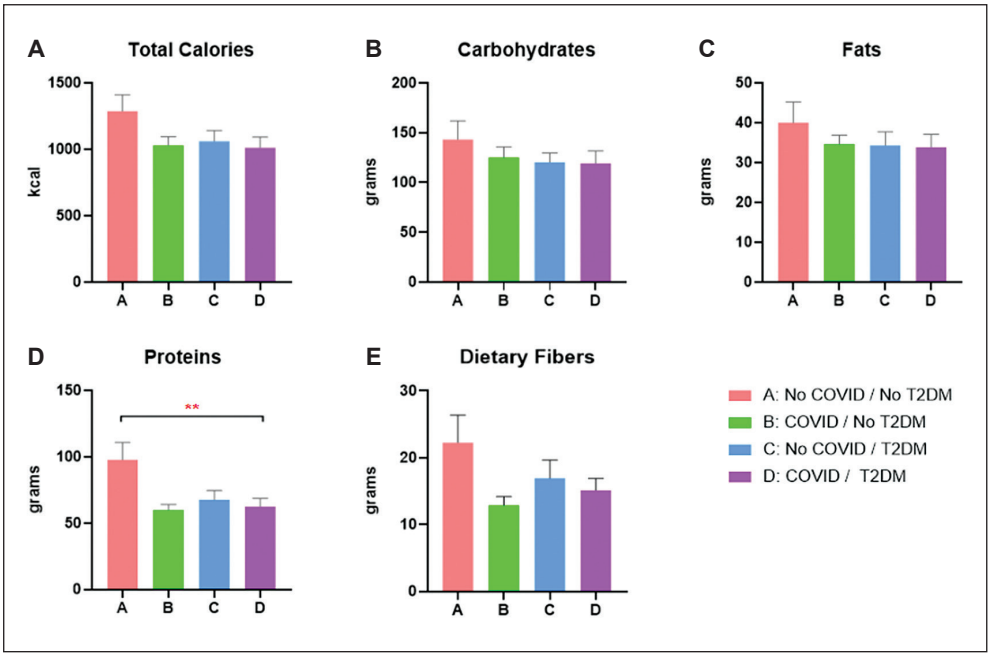

Figure 3. Dietary composition of the study participants. The bars represent the mean dietary intake per group while the error bars represent standard error of the mean. ANOVA was used to calculate significant differences between the groups, total calories *p*-value: 0.1148, carbohydrates *p*-value: 0.5526, fats *p*-value: 0.6106, proteins *p*-value: 0.0053 and dietary fibers *p*-value: 0.0965.

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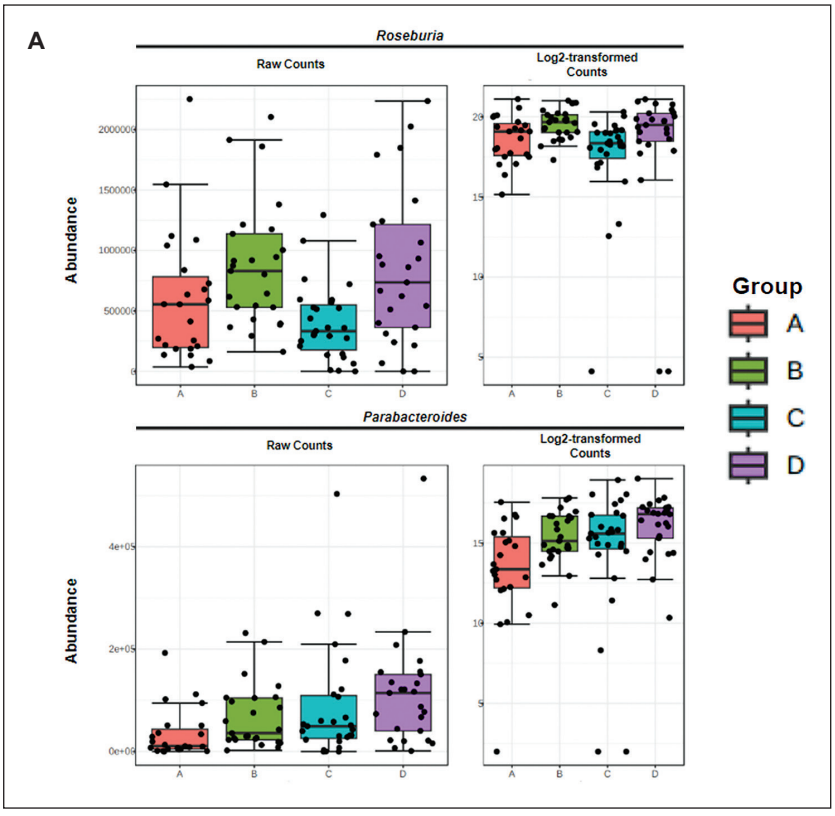

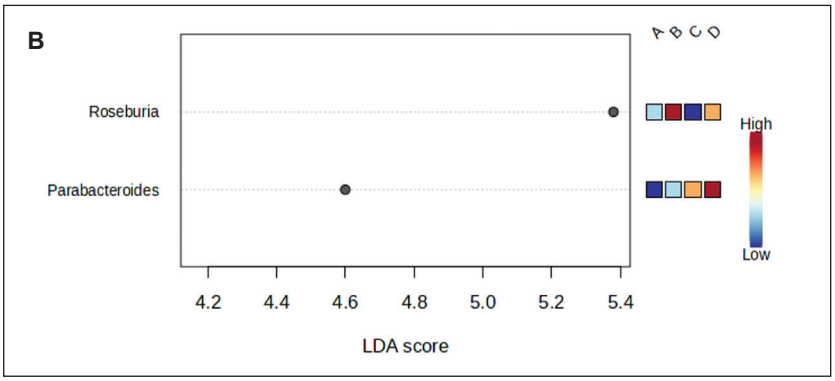

Figure 4. (A) Differentially abundant OTUs across all four groups. *Roseburia*, *p*-value: 0.0014513, FDR: 0.058891. *Parabacteroides*, *p*-value: 0.0017369, FDR: 0.058782. Kruskal-Wallis rank sum test was performed to evaluate differentially abundant taxa. **(B)** Dot plot showing Linear Discriminant Analysis Effect Size (LEfSe) to evaluate differentially abundant taxa across all four groups. *Roseburia* LDA score 5.38, *Parabacteroides* LDA score 4.6. Kruskal-wallis rank sum test followed by LDA to evaluate relevance or effect size of differential abundant taxa.

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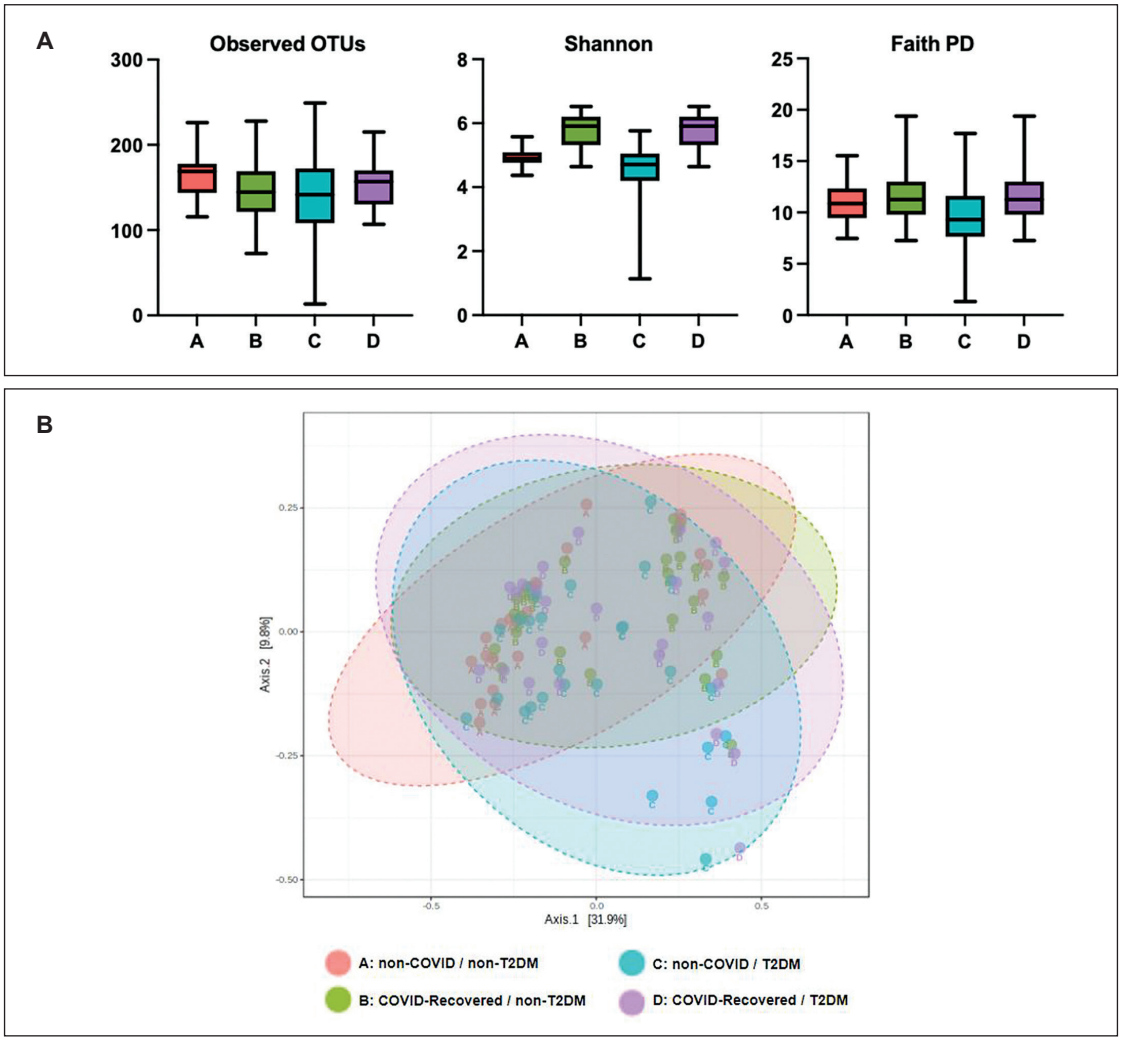

Figure 5. (A) Alpha diversity indices of the fecal microbiota among study groups. All four study groups show no significant differences in terms of their alpha diversity indices. Non-COVID/non-T2DM (A), COVID-recovered/non-T2DM (B), non-COVID/T2DM (C) and COVID-recovered/T2DM (D). **(B)** Principal coordinate analysis to differentiate among study groups. PERMANOVA analysis revealed *p*-value: 0.009, R-squared: 0.05403 and F-value: 1.8277. Non-COVID/non-T2DM (A), COVID-recovered/non-T2DM (B), non-COVID/T2DM (C) and COVID-recovered/T2DM (D).

This figure was generated using Microbiome Analyst.

Functional characterization of the gut microbiome

PICRUSt2 analysis between the non-COVID/non-T2DM group (A) and the COVID-recovered/non-T2DM group (B) revealed several differentially active pathways (Figure 6A). Glycan biosynthesis and metabolism was found to be more active for the gut microbiome of non-COVID/non-T2DM group (A), while amino acid metabolism, membrane transport and environmental adaptation were found to be more active for the gut microbiome of COVID-recovered/T2DM group (B).

PICRUSt2 analysis between the non-COVID/T2DM group (C) and the COVID-recovered/T2DM group (D) revealed several differentially active pathways (Figure 6B). Mismatch repair, folate biosynthesis and propanoate metabolism were predicted to be more active for the gut microbiome of group C, while bacterial chemotaxis and glycerophospholipid metabolism were found to be more active for the gut microbiome of group D.

DISCUSSION

Diet

Diet is a strong modifier of the gut microbiome composition. It is involved with the host immune system directly via bacterial cellular molecules or indirectly via the production of soluble bioactive compounds, such as SCFAs.[15-17] Furthermore, it has been shown that short-term alteration of diet would reproducibly alter the human gut microbial composition.[18] The only macronutrient found to be significantly different for dietary consumption is protein. This may contribute to the observed differentially abundant taxa, as protein consumption is positively associated with overall microbial diversity, and depending

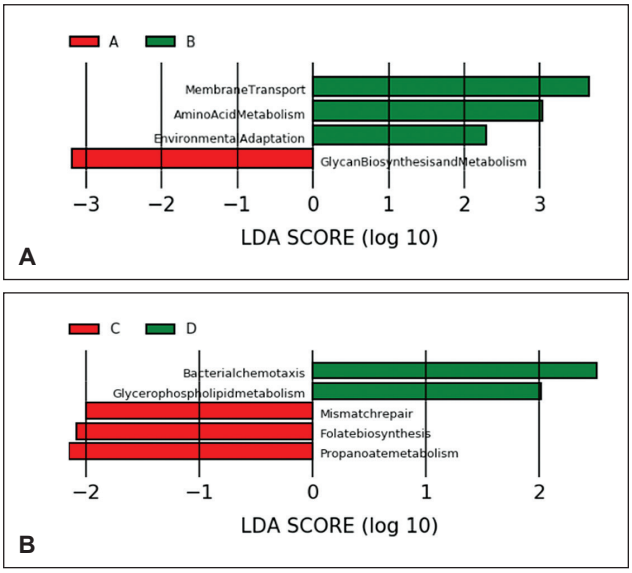

Figure 6. (A) PICRUSt2 results at KEGG metabolic pathway analysis level 2 for study groups A and B. Linear discriminant analysis (LDA) combined with effect size measurements (LEfSe) showing predicted functions for the gut microbiome of study groups A and B. **(B)** PICRUSt results at KEGG metabolic pathway analysis level 3 for study groups C and D. Linear discriminant analysis (LDA) combined with effect size measurements (LEfSe) showing predicted functions for the gut microbiome of study groups C and D. A *p*-value of <0.05 and 2.0 or higher LDA score were considered significant in Kruskal–Wallis.

This figure was generated using EasyMAP.

on the source of protein, some microbial taxa may be enhanced or reduced.[19]

Age

This study purposely used an age range of 30 to 59 in the effort to control the effect of age on the gut microbiome. The rationale for this range is that the T2DM population starts to increase in the 30s.[10] Furthermore, the adult-type gut microbiome remains stable and only starts to decay after the 60s.[10] While there is significant differences in age across the study groups (Table 1), all participants included are in the same age category, minimizing the effect of age on the results.

Identified potential biomarkers

Parabacteroides belongs to the phylum Bacteroides, and it has been described to break down branched amino acids. Previous studies have also characterized the genus *Parabacteroides* as inversely correlated with insulin resistance, and was shown to be relatively reduced among T2DM individuals when compared with non-T2DM controls.[20,21] Given these, the fact that *Parabacteroides* was found to be significantly enriched for the non-COVID/T2DM (C) and COVID-recovered/T2DM (D) is intriguing. This may be explained by the fact that metformin intake is associated with enrichment of *Parabacteroides*.[22] In addition to this, metformin intake has also been reported to be associated with enrichment of *Akkermansia, Bacteroides, Phascolarctobacterium* and *Coriobacterium* and reduction of *Bifidobacterium*, *Clostridium* and *Dorea*.[22-24] Taking these observations into account, COVID-19 may still have some influence over the gut microbial composition especially for the COVID-recovered/non-T2DM group (B). It can be deduced, however, that COVID-19 may not have pushed the T2DM group D into further dysbiosis.

Roseburia belongs to the phylum Firmicutes. It is described to be short-chain fatty acid producers impacting colonic motility, immunity and anti-inflammation.[25,26] Both *Roseburia* and *Parabacteroides* were previously identified as biomarkers for COVID-19 severity. De Jesus and Dalmacio found that both *Roseburia* and *Lachnospiraceae* to be reduced among individuals with active severe COVID-19 and is relatively enriched for individuals with only active asymptomatic, mild and moderate COVID-19.[7] It is important to note that almost all of the participants recruited in this study had asymptomatic, mild and moderate COVID-19. The fact that *Roseburia* and *Lachnospiraceae* are found to be enriched among the COVID-recovered/non-T2DM (B) and COVID-recovered/T2DM (D) may have been a protective factor during the COVID-19 infection, protecting these individuals from severe COVID-19.

Alpha and beta diversity

Alpha diversity indices across all four groups were not found to be significantly different (Figure 5A). While a different alpha diversity is suggestive of an altered gut microbial profile, a similar alpha diversity does not always reflect a similar gut microbial profile. Similar alpha diversity suggests similar species diversity within the study groups.

Beta diversity, on the other hand, aims to characterize how similar or different groups or communities are. The Principal Coordinate Analysis plot (Figure 5B) revealed significant clustering between the non-T2DM groups and the T2DM groups. This supports the notion that the COVID-recovered/non-T2DM group (B) have a gut microbial structure that resembles that of the non-COVID/non-T2DM group (A), and that these two groups have considerable non-overlapping areas with the two T2DM groups C and D. This reflects the different gut microbial structure present for the two T2DM groups. This observation is consistent with the widely known gut microbial differences attributed to T2DM. While subtle differences may still be appreciated, it is evident that between the two variables, T2DM has a stronger influence on the gut microbiome compared with a history of COVID-19 infection. Findings from this study do not support the hypothesis that COVID-19 causes further dysbiosis among individuals with T2DM. This observation should take into consideration that most of the participants of this study only had mild disease of COVID-19 and at the time of recruitment, none of the participants exhibited Post-Acute COVID-19 syndrome. There is literature to support that having a more severe

clinical course of COVID-19 or the presence of post-acute-COVID-19 syndrome symptoms would potentially have a pronounced influence on the gut microbial structure that may persist months after the acute COVID-19 infection.[27-29]

Gut microbial function

Metabolic pathways predicted to be differentially active between the gut microbiome of groups A and B, are illustrated in Figure 6A. Glycans are building blocks of mucins, which are the main structural component of mucus and play a critical role in the interaction between microbes and epithelial surfaces.[30] Certain members of the gut microbiome interact with the host by metabolizing mucins, activating immunity by mucin degradation.[31] Likewise, amino acid metabolism, found to be more active for the gut microbiome of the COVID-recovered/non-T2DM group (B) can have either positive or negative effects on the host.[32] Some members of the gut microbiome are recognized as amino-acid fermenters, including *Clostridium*, *Bacillus-Lactobacillus-Streptococcus* groups and *Proteobacteria*. These bacteria are likely to contribute to protein digestion and subsequent absorption in the GI tract.

Metabolic pathways predicted to be differentially active between the gut microbiome of groups C and D, are illustrated in Figure 6B. Notably, glycerophospholipid metabolism is more active for the gut microbiome of the COVID-recovered/T2DM group (D). This pathway has been previously associated with depression, by modulating the gut-brain axis.[33,34] This is an interesting finding considering that there are reports linking Post-COVID-19 with depression.[35] On the other hand, folate synthesis and propanoate metabolism are found to be more active for the gut microbiome of the non-COVID/T2DM group (C). Folate is important for several metabolic processes including one-carbon transfer, methylation metabolism and the biosynthesis of thymidylate, purines and several amino acids.[36] Bacterial folate has previously been suggested as a potential nutrition source for the host.[37] The gut microbiota ferments dietary non-digestible carbohydrates into short-chain fatty acids (SCFA), examples of which are propionate, butyrate and acetate.[38] SCFAs improve gut health through several local effects, including maintenance of intestinal barrier integrity, mucus production, and protection against inflammation.

While PICRUST analysis provides valuable insights into gut microbial function, it should be emphasized that this approach has limitations. These results must be treated as predictions and would benefit from further tests for validation.

CONCLUSION

This study aimed to characterize how COVID-19 is associated with the gut microbiome among Filipino adults with and without T2DM. *Parabacteroides* and *Roseburia* have been identified as potential biomarkers. *Parabacteroides* is found to be significantly more abundant for the T2DM groups for both with and without history of COVID-19. Furthemore, *Roseburia* is found to be differentially enriched for those with COVID-19 history, for both the T2DM and the non-T2DM groups. This may be generalized only for those who recovered from asymptomatic, mild or moderate COVID-19, and for those who do not suffer from Post-Acute COVID-19. This suggests a possible protective role of *Roseburia* for COVID-19 recovered individuals who encountered only mild COVID-19 diseases. Between COVID-19 and T2DM, principal coordinate analysis reveals that T2DM exerts a stronger influence on the gut microbiome as shown at close clustering together found for the non-COVID/T2DM (C) and COVID-recovered/T2DM (D) group. The findings from this study does not support that COVID-19 causes further gut microbial dysbiosis among individuals with T2DM.

Gut microbiome functional analysis reveals several differentially active pathways. For the non-T2DM groups, glycan biosynthesis and metabolism are found to be more active for the non-COVID/non-T2DM group (A), while amino acid metabolism, membrane transport and environmental adaptation are found to be more active for the COVID-recovered/T2DM group (B). For the T2DM groups, glycerophospholipid metabolism is more active for the COVID-recovered/T2DM group (D). On the other hand, folate synthesis and propanoate metabolism are found to be more active for the non-COVID/T2DM group (C). While these findings are insightful, PICRUST results are predictive in nature, and further studies may be pursued to validate them.

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Statement of Authorship
All authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
ADV: Conceptualization, Validation, Formal Analysis, Investigation, Resources, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **LMD:** Conceptualization, Methodology, Validation, Formal Analysis, Resources, Writing – review and editing, Supervision.

Data Availability Statement
Datasets are not publicly available because participants in the study did not give written consent for their data to be shared.

Author Disclosure
The authors declare no conflict of interest.

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