



# Unique Community of Gut Bacterial Microbiome as Indicator for HIV Infection and Progression

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## **Authors' contributions**

This work was carried out in collaboration among all authors. Authors SEA, EAA, JNA and CNN managed the conceptualization and design of the study. Authors EAA, CNN and TBP supervised the study. Author MTP, edited and reviewed the manuscript, Author SEA provided resources. Authors SEA, EJE & MGM managed data curation and manuscript preparation. All authors validate and approved the manuscript.

## **Article Information**

DOI: 10.9734/IJTDH/2023/v44i91430

### **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/98479>

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## ABSTRACT

The human digestive tract harbors complex microbial communities within its epithelial cell lining. Disruption in enteric immunity will promote gut dysbiosis, which can successively induce chronic inflammation within the mucous membrane and periphery. Interpretation of the specific gut microbiome changes observed during HIV infection is warranted in populations most affected. This was a case-control and comparative study design carried out between June 2018 to September 2019. A total of 40 volunteer adult participants were recruited (15 HIV-negative and 25 HIV-positive) at the Buea Regional Hospital. Blood analysis was done for CD4<sup>+</sup> T cell count and HIV viral load. Fecal samples from all participants were analyzed using the 16S rRNA gene sequencing on the next-generation Illumina® MiSeq™ sequencer.

Biomarker Linear Discriminant Analysis (LDA) score from LEfSe analysis indicated that the specific gut microbiome, *Lachnoclostridium* sp32343-sp32393-sp32423 communities could serve as an indicator for HIV infection. Findings also showed that *Bacteroides vulgatus* (seq 11 & seq 42), *Megamonas funiformis* (seq 63), unclassified members of Prevotallaceae family sp14289 (seq 51), sp13942 (seq 4), and *Prevotella copri*-sp13942 (seq 5) could be used as gut microbiome biomarkers for increased HIV viral load and decreased CD4<sup>+</sup> T cell count. Meanwhile gut microbiome biomarkers for decreased HIV viral load and increased CD4<sup>+</sup> T cell count were identified as Succinivibrionaceae sp56244 (seq 47), *Eubacterium rectale* (seq 8), *Megamonas funiformis* (seq 1 and seq 14), *Prevotella copri* (seq 29, seq 34, and seq 12) and unclassified Prevotellaceae sp13927 (seq 17), sp13942 (seq 5). Specific gut microbiome communities of *Lachnoclostridium* sp32343-sp32393-sp32423 could be used as an indicator of HIV presence. Some gut bacteria microbiome can be utilized in the management of HIV disease progression.

**Keywords:** Gut; bacteria; microbiome; biomarkers; HIV; progression.

### Key Points:

Gut microbiome communities of *Lachnoclostridium* sp32343-sp32393-sp32423 can serve as indicator of HIV infection.

CD4<sup>+</sup> T cell count (500 – 1,500 cells/μL) during HIV infection are associated with increased presence of Succinivibrionaceae sp56244 (seq 47), *Eubacterium rectale* (seq 8), *Megamonas funiformis* (seq 1 and seq 14), *Prevotella copri* (seq 29, seq 34, and seq 12) and unclassified Prevotellaceae sp13927 (seq 17), sp13942 (seq 5).

CD4<sup>+</sup> T cell count (<500 cells/μL) during HIV infection are associated with the presence of *Bacteroides vulgatus* (seq 11 & seq 42), *Megamonas funiformis* (seq 63), unclassified members of Prevotallaceae family sp14289 (seq 51), sp13942 (seq 4), and *Prevotella copri*-sp13942 (seq 5).

## 1. INTRODUCTION

The collection of microorganisms permanently resident in the human gastrointestinal (GI) tract together with their genes is described as the gut microbiome. Intensive work from the Human Microbiome Project has shown that these microbial communities are intimately integrated with the human "self" and may lead to a new understanding of "self" [1]. With current knowledge and awareness of the different

habitats of microorganisms, the human body has been shown to harbor many commensals (non-pathogenic) and pathogenic microbial species (pathobionts) that have co-evolved with the human genome, adaptive immune system, and lifestyle [2]. Humans have evolved with these microorganisms, passed down from mother to child, for hundreds of millions of years, and studies are now uncovering the more significant and complex role they play in human health and disease than ever before acknowledged [3].

Today, scientists are educating the scientific world to think not about "bad" and "good" microorganisms, but rather about good and bad communities of microbial organisms (microbiome). The gut microbiota functions in collaboration with the host's defences and the immune system to protect against pathogen colonization and invasion [4]. It also performs metabolic functions, thereby providing several sources of essential nutrients and vitamins to the human system. Furthermore, they aid in the extraction of energy and nutrients, such as short-chain fatty acids (SCFA) and amino acids, from food [5]. Ultimately, the host depends on its intestinal microbiota for several vital functions, and thus specific intestinal microbiota may give the host health-specific advantages. Gut microbial dysbiosis has been shown to influence both positive and negative outcomes on human health [6]. It has been suggested that added advances in our understanding of the microbiome will hopefully give exciting prospects for exploiting and manipulating the microbiome to improve our health [7]. With these exciting prospects from microbiome studies, diagnosis of diseases and disease-causing pathogens will soon be made possible by understanding the microbiome of individuals, and their communities.

More than 30 million people are reported to be affected worldwide with HIV and approximately two million new cases are reported each year [8]. In Cameroon, approximately 540 000 people were living with HIV in 2018, amongst whom, 52% had access to Anti-retroviral therapy (ART), 18,000 deaths, and 23,000 new HIV infections [9]. Untreated HIV-infected individuals demonstrate rapid and substantial CD4<sup>+</sup> T cell depletion and chronic immune system activation. Based on accumulating evidence, HIV-1 infection is tightly linked to the gastrointestinal tract which is a primary site of viral replication [10]. Studies have revealed significant depletion of CD4<sup>+</sup>T-cells occurring in the intestinal mucosa at the gut-associated lymphoid tissue (GALT) in the early phase of HIV infection. The depletion of CD4<sup>+</sup> T cells has resulted in damage and dysfunction of the gastrointestinal system. The negative effect of HIV pathogenesis is linked to the deterioration of gut homeostasis, leading to dysbiosis. Gut microbiota may be associated with improvement in the CD4<sup>+</sup> T cell count which continues to be an important prognostic indicator and predicts non-AIDS events and mortality in addition to AIDS-associated morbidity and mortality [11]. Identifying the gut microbiome during HIV infection presence and progression

may provide health-specific advantages for diagnosis and the disease prognosis. With advances in gut microbiota extraction and isolation techniques, much interest is currently observed from researchers and clinicians experts in wanting to utilize microbiome examination for the management of patients. Recently, it has been conceivable to characterize individual microbiota courses of action into community types that can be predictive of one another [12]. The high prevalence of diverse strains of HIV [13] in Cameroon might in result pressure on the gut microbiome, which might lead to the possibility of emerging new and unidentified strains of gut flora in the community. A previous study on Gram stain flora morphotype and distribution pattern reveals a decrease with the reduction of CD4<sup>+</sup>T cell count and vice versa in a Cameroonian population [14]. However, studies on the identification of unique gut microbiome species in the presence of HIV infection and the correlation with HIV prognosis are limited.

## 2. METHODS

### 2.1 Study Setting and Design

This was a case-control and comparative study design carried out between June 2018 to September 2019.

### 2.2 Study Participants

From a cohort of 320 individuals, 25 patients infected with HIV and 15 HIV-negative control individuals were purposively recruited following the specified criteria for this study (Table 1).

#### 2.2.1 Inclusion and exclusion criteria

surveyed our participants for demographic data covering socio-economic status, systems reviews, lifestyles (alcohol use and smoking), drug and antibiotic use, and diet composition in their meals. In addition, screening for HIV was performed to confirm their HIV status. The methods were carried out following the relevant guidelines and regulations standards. Of the cases, 6 were HIV treatment naïve, 12 participants were on HAART and 7 participants were HIV co-infected with tuberculosis. No participant presented comorbidity with infectious agents such as the hepatitis B and C virus, and none was experiencing diarrhoea at the time of faecal sampling or had taken antibiotics within the previous 30 days.

**Table 1. Characteristics of Fecal samples purposively selected for 16S rRNA sequencing**

| Codes  | Type of samples                                       | No. Extracted | Gender |
|--------|---|---------------|--------|
| N.01   | HIV- negative extracted stool samples                 | 5             | Male   |
| N.02   | HIV- negative extracted stool samples                 | 5             | Female |
| N.03   | HIV- negative extracted stool samples                 | 5             | Female |
| PT.04  | HIV-TB co-infected extracted stool samples            | 4             | Male   |
| PT.06  | HIV-TB co-infected extracted stool samples            | 3             | Female |
| P.05   | HIV- positive extracted stool samples                 | 4             | Male   |
| P.07   | HIV- positive extracted stool samples                 | 4             | Female |
| P.08   | HIV- positive extracted stool samples                 | 4             | Female |
| PN.09  | HIV- positive treatment-naive extracted stool samples | 3             | Male   |
| PN.010 | HIV- positive treatment-naive extracted stool samples | 3             | Female |

### 2.2.2 Stool sample collection

Each individual was requested for a stool sample. Study participants were given a sterile stool collection container with appropriate instructions on how to deposit the sample without any contamination from urine. The container was closed and brought out immediately (within 5 minutes). The stool samples were transported on an ice bath with an ice pack from the collection site (Buea Regional Hospital) to the Faculty of Health Sciences, Medical Research, and Bacteriology Laboratory (FHS-MRBL). The stool samples were aliquoted into 3 containers. A container of the shared stool sample was then coded and stored in a  $-80^{\circ}\text{C}$  freezer at the Infectious Disease Laboratory, Faculty of Health Sciences, the University of Buea for DNA extraction and 16S rRNA sequencing.

### 2.2.3 Blood sample collection

Venous blood (10 ml) was also collected by venepuncture from participants (patients and controls) into 2 separate 5 ml ethylene-diamine-tetra-acetate (EDTA) vacutainer tubes. One of the tubes was used for HIV Screening and  $\text{CD4}^{+}$  T cell count, while the other tube was used for Viral load testing.

### 2.2.4 HIV Screening

Briefly, HIV screening was done using the method described by Respass et al. [15]. The HIV determine test strip (Abbott Laboratories, Abbott Park, IL, USA) was labelled with the participant identification number. The protective foil cover of the strip was pulled off. Then 50  $\mu\text{l}$  of plasma was collected with a pipette and applied to the absorbent pad on the test strip. One drop of chase buffer was added to the specimen pad and the specimen was allowed on the bench to

run through the test strip. Reading and recording of the results were done after 15 minutes. Positive samples demonstrated two red lines, while negative samples had just one red line on the test strip.

### 2.2.5 $\text{CD4}^{+}$ T cell count

The  $\text{CD4}^{+}$  T cell count of the study participant was determined using the method of Fonsah et al. [16]. The test was run according to a standardized Flow cytometric machine (BD Biosciences FACSCount, New Jersey, USA) following the manufacturer's procedure. Briefly, the EDTA tube with the whole blood sample was mixed and 50  $\mu\text{L}$  of whole blood was pipetted into the reagent tube labelled with the corresponding participant's number. The tube was then capped, vortexed, and incubated for 30 minutes at room temperature ( $20^{\circ}\text{C}$ – $25^{\circ}\text{C}$ ) in the workstation. This was followed by uncapping each sample tube and pipetting 50  $\mu\text{L}$  of a fixative solution into each tube. The tubes were recapped and vortexed upright before uncapping to run the sample with the Flow cytometric machine. Reading and recording of the results were done after the machine software message was indicated. The interpretation was done as follows: normal range 500 – 1,500 cells/ $\mu\text{L}$ , while below 500 cells/ $\mu\text{L}$  was considered as low  $\text{CD4}^{+}$  T cell count.

## 2.3 Viral Load Measurement

HIV viral load was done using the method described by Neogi et al. [17]. The viral load of each sample was measured using the Abbott RealTime HIV-1 Qualitative (Abbott Molecular Inc, Des Plaines, IL, USA) assay, following the manufacturer's instructions. Dried blood spot strips were prepared from freshly drawn whole blood after storage at room temperature for up to 6 hours, by spotting 50 $\mu\text{L}$  of the whole blood onto

a Whatman 903 filter paper, with 3 spots per card. Briefly, filter papers were air-dried overnight at room temperature and stored at 4°C in a plastic sealed bag with a silica desiccant until they were processed. The dried blood spot viral load was measured as follows: two blood spots from the same was patient punched out using a sterile puncher and placed into 1.7 ml of Lysis buffer provided with the Abbott sample preparation system (m2000sp) in 50 ml sealed conical tubes. The tubes were intermittently mixed while incubating at room temperature for 2 hours. RNA was extracted manually from the lysate according to the standard HIV-1 RNA 1.0 ml extraction protocol using the Abbott RNA sample preparation system. The viral load was measured from the extracted RNA using the "m2000 DBS HIV-1 RNA 'open-mode' protocol" (Abbott Molecular Inc, Des Plaines, IL, USA). Reading and recording of the viral load values were stratified into three levels: (i) VL 2.17 to 3 log<sub>10</sub> copies/ml (corresponding to 1000 copies/ml), (ii) VL >3 to 3.7 log<sub>10</sub>copies/ml, (1000- about 5000 copies/ml), and (iii) VL >3.7 log<sub>10</sub> copies/ml (corresponding to approximately 5000 copies/ml).

## 2.4 Extraction and Purification of Microbiota DNA from the Stool Sample

The extraction of bacterial DNA procedure was done using the method described by Shantelle et al. [18] with the ZymoResearch DNA MiniPrep Extraction kit (Zymo, Irvine, CA, USA). The Stored (-80 °c) faecal sample (200mg) was purposively selected (Table 1) and added to a ZR Bashing bead lysis tube. Then 750 µL of ZymoBIOMICS lysis solution was added to the tube and cap tightly. The tube was then secured in a bead beater fitted with a 2 mL tube holder assembly and it was processed at maximum speed for 20 minutes. The beaten ZR Bashing Bead lysis tube was then centrifuged at ≥ 10,000 × g for 1 minute. The supernatant (400 µL) was transferred to the Zymo-Spin III-F filter in a collection tube and centrifuged at 8,000 × g for 1 minute. Then the used Zymo-Spin III-F filter was discarded. Binding preparation was done by adding 1,200 µL of ZymoBIOMICS DNA binding buffer to the filtrate in the collection tube and the tube was mixed thoroughly. From the binding step, the mixture of 800uL was transferred to a Zymo-spin IIC-Z column in a collection tube and centrifuged at 10,000 × g for 1 minute. The flow-through from the collection tube was discarded and the aforementioned step was repeated.

The Zymo-spin IIC-Z column containing the settled contents was then inserted into a new collection tube. Wash buffer 1 (400 µL) from ZymoBIOMICS DNA, was then added to the Zymo-spin IIC-Z column in the new collection tube and centrifuged at 10,000 × g for 1 minute. Then the flow-through was discarded. Wash buffer 2 (700 µL) from ZymoBIOMICS DNA, was then added to the Zymo-spin IIC-Z column in the new collection tube and centrifuged at 10,000 × g for 1 minute. The flow-through was then discarded. ZymoBIOMICS DNA wash buffer 2 (200 µL) was then added to the Zymo-spin IIC-Z column containing the settled contents in the new collection tube and centrifuged at 10,000 × g for 1 minute. The Zymo-spin IIC-Z column was transferred to a clean 1.5mL micro-centrifuge tube and 100 µL of ZymoBIOMICS DNase/RNase free water was added directly to the column matrix and incubation was done for 1 minute. Then centrifugation was carried out at 10,000 × g for 1 minute to elute the DNA. Zymo-spin III-HRC filter was placed in a new collection tube and 600 µL of ZymoBIOMICS HRC prep solution was added and centrifuged at 8,000 × g for 3 minutes. After centrifugation, the eluted DNA was then transferred to the prepared Zymo-spin III-HRC filter in a clean 1.5 mL micro-centrifuge tube, which was followed by centrifugation at 16,000 × g for 3 minutes. The extracted DNA was purified with Clean and Concentrator-25 columns (Zymo, Irvine, CA, USA) as per the manufacturer's directives. Isolated DNA was stored at -80 °C until analyzed.

## 2.5 Library Preparation (16S V3-V4), Pooling and Post-Library QC, and Illumina MiSeq® Sequencing(2x300)

### 2.5.1 Targeted library preparation

With the *Quick-16S*<sup>™</sup> NGS Library Prep Kit (Zymo Research, Irvine, CA), the DNA samples were prepared for targeted sequencing. The primers were custom-designed by Zymo Research to provide the best coverage of the 16S gene while maintaining high sensitivity. The primer set used was *Quick-16S*<sup>™</sup> Primer Set V3-V4 (Zymo Research, Irvine, CA). An innovative library preparation process was used for preparing the sequencing library. Wherein, PCR reactions were performed in real-time PCR machines to control cycles and therefore limit PCR chimera formation. The final PCR products were then quantified with qPCR fluorescence readings and pooled together on the bases of

equal molarity. With the Select-a-Size DNA Clean & Concentrator™ (Zymo Research, Irvine, CA), the final pooled library was cleaned up and then quantified with TapeStation® (Agilent Technologies, Santa Clara, CA) and Qubit® (Thermo Fisher Scientific, Waltham, WA).

### 2.5.2 Control samples

Positive control for each targeted library preparation was obtained from the ZymoBIOMICS® Microbial Community DNA Standard (Zymo Research, Irvine, CA). Negative controls (i.e. blank extraction control, blank library preparation control) were included in each run to assess the level of bioburden carried by the wet-lab process.

#### 2.5.2.1 Sequencing

The final library was sequenced on Illumina® MiSeq™ with a v3 reagent kit (600 cycles) by the Zymo Research Corporation in the USA. The sequencing was performed with >10% PhiX spike-in.

## 2.6 Bioinformatics Analysis

Unique amplicon sequences were inferred from raw reads using the Dada2 pipeline [19]. Chimeric sequences were also removed with the Dada2 pipeline. With the Uclust from Qiime v.1.9.1, the taxonomy assignment was done. The Zymo Research Database was used to assign Taxonomy with, a 16S database that is internally designed and curated, as a reference. Composition visualization, alpha-diversity, and beta-diversity analyses were performed with Qiime v.1.9.1 [20]. The taxonomy that has significant abundance among different groups was identified by LEfSe [21] using default settings. Other analyses such as heatmaps, Taxa2SV Deomposer, and PCoA plots were performed with internal scripts.

## 3. RESULTS

### 3.1 Demographic and Clinical Characteristics

The characteristics of the study participants purposively selected from the cohort are shown in Table 2. A total of 15 HIV-negative individuals and 25 HIV-infected persons were enrolled in the study. The HIV-infected cases were further

stratified based on their treatment status. Most of the study participants were females 28 (70%). The age ranges were equally represented. With regards to food and drinks consumed, all participants that were purposively selected were on Energy + body-building+ protective foods (100%), and non-alcoholic drinks (100%).

The mean HIV viral load was <40.0(40.0 - 711.5) RNA, copies/mL, while the mean CD4+T-cell counts was 800.0(454.0 - 950.0) cells/mm<sup>3</sup> and 450.0 (350.0- 800.0) cells/mm<sup>3</sup> for HIV-negative and HIV-infected respectively.

### 3.2 Specific Core Microbiome Signatures of HIV-negative and Positive Participants

To identify the specific bacterial taxa associated with HIV infection, a comparison was done between the composition of faecal microbiota observed in HIV-negative individuals and HIV patients using the linear discriminant analysis effect size (LEfSe). A cladogram that represented the structure of the faecal microbiota and the predominant bacteria in the HIV-negative individuals and HIV-positive patients is shown in Fig. 1 and the significant difference is illustrated in Fig. 2. LEfSe analysis revealed 27 discriminating features (LDA score Log<sub>10</sub>, Fig. 3), for HIV- negative and 2 discriminating features for HIV-positive individuals. Members of *Bifobacteriaceae*, *Actinobacteria*, *Streptococcaceae*, and *Clostridiaceae* were enriched in the HIV-negative samples, whereas *Lachnospiraceae* were enriched in HIV patients.

### 3.3 Associations between Specific Gut Microbial Communities and CD4<sup>+</sup> T Cell Count Levels

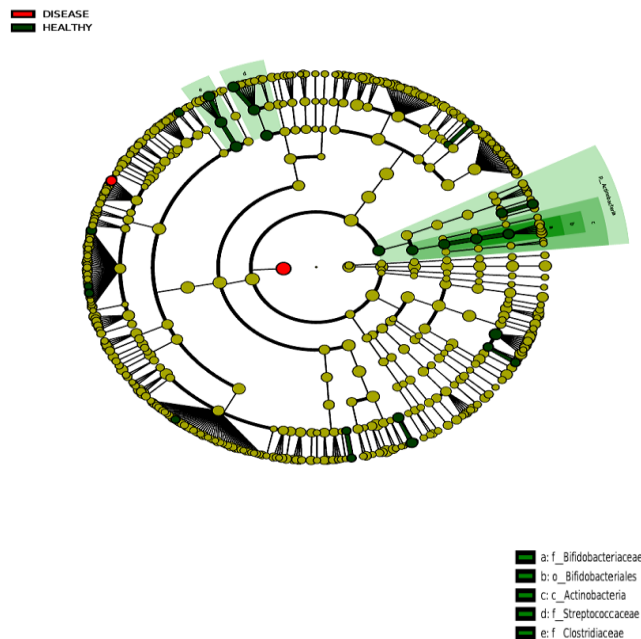
Findings from this study showed that the gut microbial community occurring among HIV patients could be used for follow-up and patient management. Findings demonstrated a compositional structure of gut microbial communities through LDA score correlating with particular CD4<sup>+</sup> T cell counts.

Observation from the study participants revealed that HIV patients with CD4<sup>+</sup> T cell count greater than 1000 cells/μL had two major family predominating: *Succinivibrionaceae* (seq17:f\_\_Succinivibrionaceae;g\_\_NA;s\_\_sp562 44 (seq 17, seq 19, & seq73) and *Prevotellaceae* (*Prevotella copri* (seq 31, seq 45, & seq 70),

*f\_\_Prevotellaceae;g\_\_NA;s\_\_sp13931(seq 67), Romboutsia ilealis (seq37) and Eubacterium rectal (seq 8))* (Fig. 4). The findings also showed that, HIV patients with CD4<sup>+</sup> T cell count between 500 – 1000 cells/ $\mu$ L have a compositional structure consisting of mostly members of the Prevotellaceae family including: *f\_\_Prevotellaceae;g\_\_NA;s\_\_sp13942(seq2),f\_\_Prevotellaceae;g\_\_NA;s\_\_sp13927 (seq 7), Prevotella copri (seq 4 and seq 9) and Enterobacteriaceae, Escherichia-Shigella coli (seq14)* were more apparent (Fig. 5).

**Table 2. Demographic and clinical characteristics of the survey study participants**

| Characteristics  | Variables                           | Frequency (%)        |
|--|-------------------------------------|----------------------|
| Gender   | Male                                | 12 (30)              |
|  | Female                              | 28 (70)              |
| Age (years)  | <21                                 |                      |
|  | 21 – 30 rearrange                   | 10 (25)              |
|  | 31 - 40                             | 10 (25)              |
|  | 41 –50                              | 10 (25)              |
|  | 51 –60                              | 10 (25)              |
| HIV-status   | HIV-negative                        | 15 (37.5)            |
|  | HIV-positive treatment naïve        | 6 (15)               |
|  | HIV-positive + HAART                | 6 (15)               |
|  | HIV-positive + HAART+ Cotrimoxazole | 6 (15)               |
|  | HIV-positive+HAART+ATB              | 7 (17.5)             |
| Food eaten (within 72 hours)                           | Energy + body-building+ protective  | 40 (100)             |
|  | Non-alcoholic                       | 40 (100)             |
| Drink consume (within 72 hours)                        |                                     |                      |
| HIV-1 Viral load RNA,copies/mL, median (IQR)           | HIV positive                        | <40.0(40.0 - 711.5)  |
| CD4+T-cellcounts, median (IQR) Current, cells/ $\mu$ L | HIV negative                        | 800.0(454.0 - 950.0) |
|  | HIV positive                        | 450.0 (350.0- 800.0) |



**Fig. 1. Cladogram depicting Gut Microbiota as possible Biomarkers for HIV-positive and HIV-negative status**

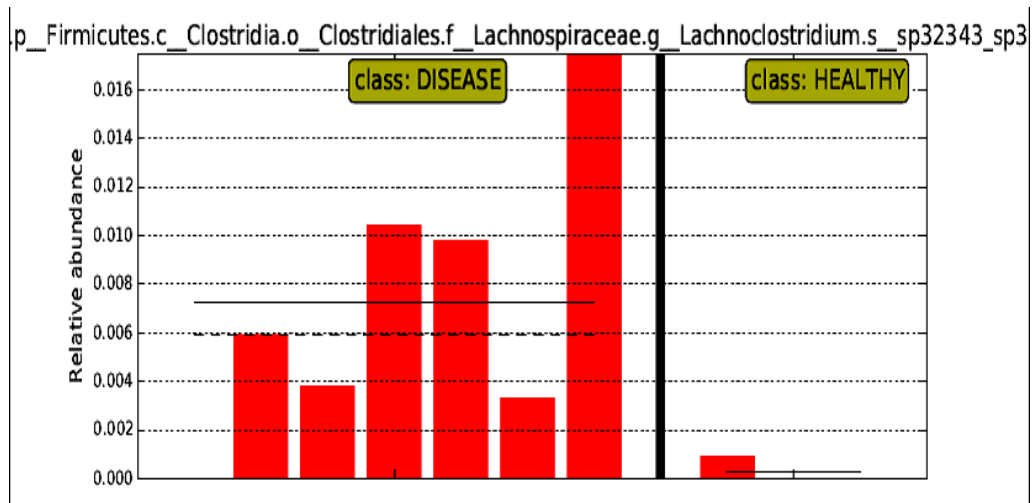


Fig. 2. Barplots for HIV-positive biomarker

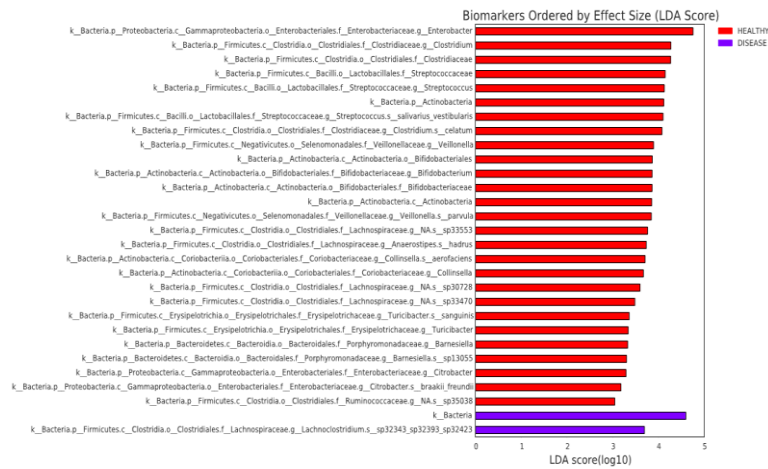


Fig. 3. LDA score for gut microbiome biomarkers for HIV disease present or absent

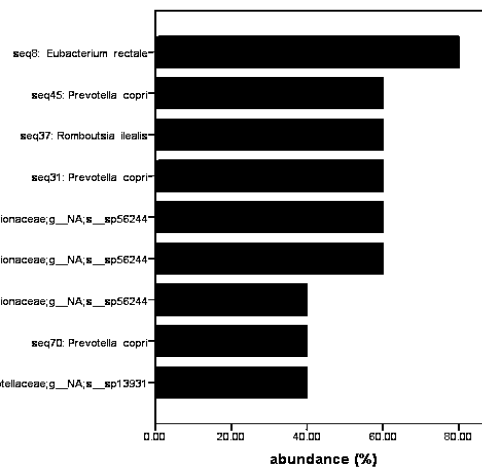


Fig. 4. Gut microbiome OTU abundance with CD4 >1000 cells/μL

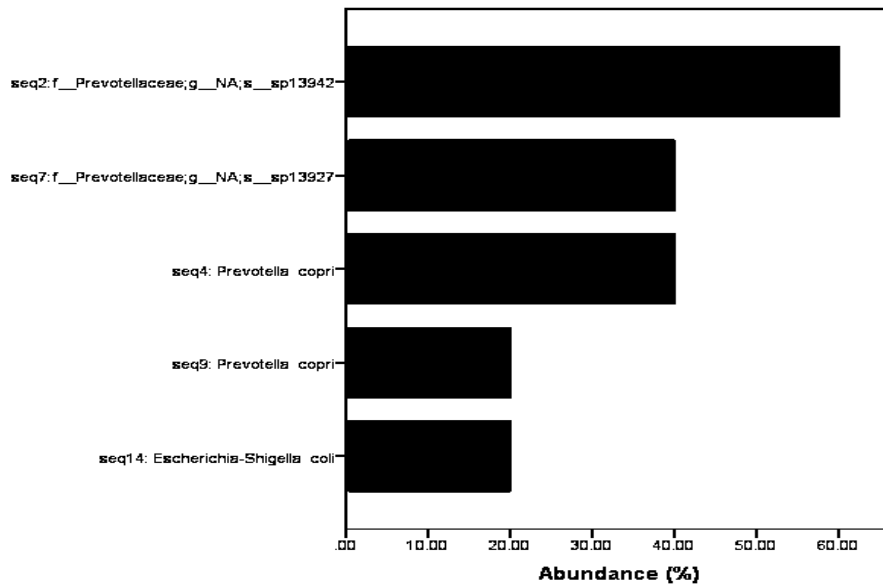


Fig. 5. Gut microbiome OTU abundance with CD4 500-1000 cells/μL

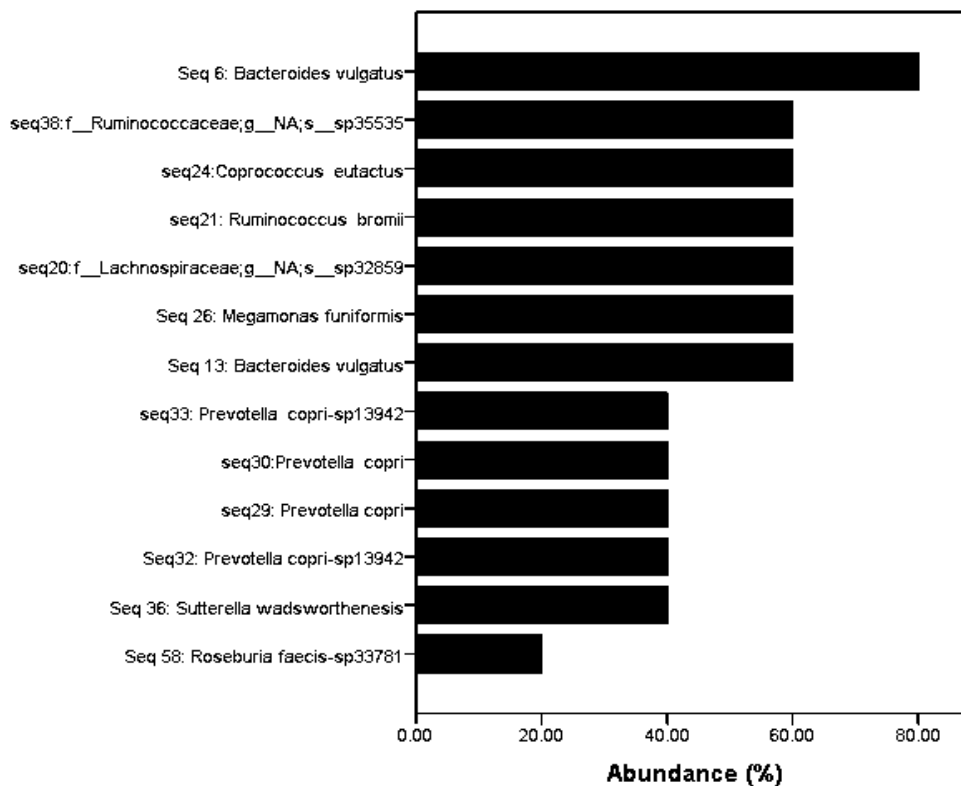


Fig. 6. Gut microbiome OTU abundance with CD4 < 350 cells/μL

The gut microbiome community associated with HIV patients with CD4<sup>+</sup> T cell counts of < 350 cells/μL includes: *Bacteroides vulgatus* (Seq 6 and Seq 13), *Megamonas funiformis* (Seq 26),

*Prevotella copri-sp13942* (Seq 32), *Sutterella wadsworthensis* (Seq 36), *f\_\_Lachnospiraceae;g\_\_NA;s\_\_sp32859* (seq 20), *Ruminococcus bromii* (seq 21),

*Coprococcus eutactus*(seq 24), *f\_\_Ruminococcaceae;g\_\_NA;s\_\_sp35535* (seq 38), *Prevotella copri-sp13942* (seq 33), seq 29: *Prevotella copri* (seq 29 & seq 30), and *Roseburia faecis-sp33781*(Seq 58) (Fig. 6).

### 3.4 Associations between Specific Gut Microbial Communities and HIV Viral Load

Some gut microbiome communities in this study showed significant associations with HIV viral load levels. Higher copies of viral load were associated with the following: *Bacteroides vulgatus* (seq 11 and seq 42), *Megamonas funiformis* (seq 63), *unidentified members of Prevotellaceae family sp14289* (seq 51), *sp13942* (seq 4), and *Prevotella copri-sp13942* (seq5). In contrast, lower copies of HIV viral load were associated with high OTU abundance of *Succinivibrionaceae sp56244* (seq 47), *Eubacterium rectale* (seq8), *Megamonas funiformis*(seq 1 and seq 14), *Prevotella copri* (seq 29, seq 34, and seq 12) and *unidentified Prevotellaceae sp13927* (seq 17), *sp13942* (seq 5).

## 4. DISCUSSION AND CONCLUSION

Findings from this study showed that specific gut microbial communities occurring among HIV patients may be used as biomarkers for HIV presence and HIV/AIDS disease progression. The study results highlighted a compositional structure of gut microbial communities through LDA score which served as biomarkers for HIV presence. The possible biomarker for HIV disease presence included *Lachnospiraceae* *sp32343-sp32393-sp32423*, originating from class clostridia and family *Lachnospiraceae*. Similar work has been observed from a sparse Partial Least Square-Discriminant Analysis and Random Forest identified by Homero et al. [22] which observed a greater abundance of *Lachnospiraceae*-OTU69, in HIV-infected individuals. Contrary to our study, genera within the *Erysipelotrichaceae* family have consistently been reported as part of the bacterial signature associated with microbiota dysbiosis in HIV-infected individuals [23 – 25].

Other gut microbiome communities for possible biomarkers for HIV disease progression had different dynamics concerning CD4<sup>+</sup> T cell count and viral load as observed in this study. Gut microbiome community associated with HIV

patients with CD4<sup>+</sup> T counts of < 350 cells/ul included: *Bacteroides vulgatus* (Seq 6 and Seq 13), *Megamonas funiformis* (Seq 26), *Prevotella copri-sp13942* (Seq 32), *Sutterella wadsworthensis* (Seq 36), *f\_\_Lachnospiraceae; g\_\_NA;s\_\_sp32859* (seq 20), *Ruminococcus bromii* (seq21), *Coprococcus eutactus* (seq24), *f\_\_Ruminococcaceae; g\_\_NA;s\_\_sp35535* (seq38), *Prevotella copri-sp13942* (seq33), seq29: *Prevotella copri* (seq 29 & seq30), and *Roseburia faecis-sp33781*(Seq 58). Our findings are in line with Hiippala et al. [26] who observed that, *Sutterella wadsworthensis* is predominant in immune-compromised individuals.

Regarding HIV patients with CD4<sup>+</sup> T cell counts between 500 – 1000 cells/ μL, a compositional structure consisting of mostly members of the *Prevotellaceae* family including *f\_\_Prevotellaceae;g\_\_NA;s\_\_sp13942* (seq 2), *f\_\_Prevotellaceae;g\_\_NA;s\_\_sp13927* (seq 7), *Prevotella copri* (seq 4 and seq 9) and *Enterobacteriaceae Escherichia-Shigella coli* (seq14) were more apparent. HIV-Patients with CD4<sup>+</sup>T cell counts greater than 1000 cells/ μL had two major families predominating *Succinivibrionaceae* and *Prevotellaceae* which include: *f\_\_Succinivibrionaceae; g\_\_NA; s\_\_sp56244* (seq 17, seq 19, & seq73), *Prevotella copri* (seq 31, seq45, & seq 70), *f\_\_Prevotellaceae;g\_\_NA;s\_\_sp13931*(seq 67), seq37: *Romboutsia ilealis* (seq37) and *Eubacterium rectal* (seq 8). Our work had similar findings to that of Nowak et al. [27] and Serrano-Villar et al. [28] in which the *Succinivibrionaceae* family significantly increase with increased CD4<sup>+</sup> T cell counts.

Summarily our findings were inconsistent with the work of Lu et al. [29] which observed that the abundance of *Rumino-coccaceae*, *Succinivibrionaceae*, *Bacteroidaceae* was decreased whereas that of *Enterobacteriaceae*, *Fuso-bacteriaceae*, *Veillonellaceae* and *Prevotellaceae* were increased in the CD4 count < 200 cells/μL group compared to the CD4 count > 200 cells/μL group. The difference in the findings might be a result of specificity in our results in terms of genus and species levels comparison. Overall, such dissimilarities could be due to variations in gut microbiota steady-state before infection-induced destabilization, disease status, environmental factors, environmental interaction, and additionally the effective usage of cotrimoxazole prophylaxis among the HIV positive study participants in the country.

Gut microbiome biomarkers with HIV viral suppression were indicated with OTU abundance. Higher copies of viral load were associated with *Bacteroides vulgatus* (seq 11 and seq 42), *Megamonas funiformis* (seq 63), unidentified members of Prevotallaceae family sp14289 (seq51), sp13942 (seq4), and *Prevotella copri*-sp13942 (seq 5). In contrast, lower copies of HIV viral load were associated with high OTU abundance of *Succinivibrionaceae* sp56244 (seq 47), *Eubacterium rectale* (seq8), *Megamonas funiformis*(seq1 & seq14), *Prevotella copri* (seq 29, seq 34, and seq 12) and unidentified *Prevotellaceae* sp13927 (seq 17), sp13942 (seq 5). Works reporting variation of HIV viral load levels with specific gut microbiome are scarce. (until there is no reference to quote???) However findings from our study suggest that the microbial fecal community of HIV-infected patients could be used for possible follow-up and management of HIV patients.

Conclusively, the gut microbiome community *Lachnoclostridium* sp32343-sp32393-sp32423 could serve as a signature for HIV infection. Also, 12 gut microbiome communities, could serve as possible core gut microbiomes for HIV-negative status. Specifically, CD4<sup>+</sup> T cell count (500 – 1,500 cells/ $\mu$ L) during HIV infection are associated with increased presence of *Succinivibrionaceae* sp56244 (seq 47), *Eubacterium rectale* (seq 8), *Megamonas funiformis* (seq 1 and seq 14), *Prevotella copri* (seq 29, seq 34, and seq 12) and unclassified *Prevotellaceae* sp13927 (seq 17), sp13942 (seq 5).

CD4<sup>+</sup> T cell count (<500 cells/ $\mu$ L) during HIV infection are associated with the presence of *Bacteroides vulgatus* (seq 11 & seq 42), *Megamonas funiformis* (seq 63), unclassified members of Prevotallaceae family sp14289 (seq 51), sp13942 (seq 4), and *Prevotella copri*-sp13942 (seq 5).

Altogether, we have detected the presence of a bacterial signature that can differentiate HIV-infected individuals from non-infected individuals. It is worth noting that a community previously overlooked showed a different profile of gut microbiota in HIV-positive individuals. The *Lachnoclostridium* sp32343-sp32393-sp32423 signature, suggests the need for experimental studies probing intestinal microbiota mechanisms in the context of HIV infection. More so, carrying out further studies on metabolomics to

understand the functionality of these gut microbiomes we identified with CD4<sup>+</sup> T cell variation and HIV viral load, might throw more light on the gut microbiome's role in forcefully stabilizing the gut-system during dysbiosis.

## DISCLAIMER

This paper is an extended version of a preprinted document by the same author.

The preprint document is available at this link: <https://www.medrxiv.org/content/medrxiv/early/2022/05/26/2022.05.24.22275521.full.pdf>

## DATA AVAILABILITY

The raw sequencing data was zipped and can be accessed at: <https://epiquest.s3.amazonaws.com/epiquest-zr2768/CMCDFQUJAMKPZKAFWSY3FJLBJDR-TBYVE/rawdata/zr2768.rawdata.190904.zip>

## CONSENT AND ETHICAL APPROVAL

Ethical clearance was obtained from the Institutional Review Board of the Faculty of Health Sciences of the University of Buea, Cameroon (Reference N<sup>o</sup>: 2018/826-06/UB/SG/IRB/FHS). All individuals included in the study signed an informed consent form.

## ACKNOWLEDGEMENT

We are thankful to the Department of Medical Laboratory Science, University of Buea (Cameroon), for providing an instrumental facility at the Medical Research and Bacteriology Laboratory (FHS-MRBL) and the Infectious Disease Laboratory, Faculty of Health Sciences, University of Buea for DNA extraction. We are also grateful to the Buea Regional Hospital and UPEC unit for their facilities and services provided. We also acknowledge the ZYMO Research Cooperation USA (Zymo Research, Irvine, CA), for their services rendered in the Library Preparation (16S V3-V4), Pooling, and Post-Library QC, and Illumina MiSeq<sup>®</sup> Sequencing(2x300) and also for Bioinformatics analysis.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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