Gut Microbiota Dysbiotic Pattern and its associated Factors in a Cameroonian Cohort with and without HIV infection

ABSTRACT

Aims: To compare the gut microbiota dysbiotic pattern between the HIV-negative individual and HIV-positive patients with /or without first-line ARV and cotrimoxazole prophylaxis treatment through culture-dependent technique. And additionally to access the associations of gut microbiota at the genus level with sociodemographic and clinical factors.
Study design: This was a cross-sectional study.
Place and Duration of Study: Participants were selected from the South West region at the Buea Regional Hospital UPEC unit. The study spanned from August 2018 to April 2019.
Methodology: We included 160 participants. Fecal and blood samples were collected from HIV-negative individuals (n=40), HIV-positive treatment naïve (n=40), HIV-positive + ARV (n=40) and HIV-positive + ARV + Cotrimoxazole prophylaxis (n=40). A self-structured questionnaire was administered to collect sociodemographic data. The stool samples were plated using three non-selective and ten selective media and colonies were identified using biochemical characterization methods. The CD4+ T cells (cells/mm^3) count were evaluated with BD FACSCount System. Data were analysed using SPSS version 21. Categorical variables were analysed using the Chi-square test and multinomial Logistic regression analysis was used to verify associations between variables.
Results: The HIV-treatment naïve individual fecal samples showed a significantly increased growth occurrence for *Candida* ($P < .001$) and *Fusobacteria* ($P < .001$); and a decreased growth occurrence for Enterobacteriaceae family ($P < .001$), *Staphylococcus* ($P < .001$), *Lactobacillus* ($P < .001$) and *Bifidobacteria* ($P < .001$) compared to those of HIV-negative individuals. HIV-positive individuals on ARV and Cotrimoxazole had their stool samples showing a significantly decreased growth occurrence for *Escherichia* ($P = .014$), *Salmonella* ($P = .002$) and *Staphylococcus* ($P = .04$) compared to HIV-positive patients on ARV only. Increased growth occurrence of particular gut microbiota among participants was more likely associated with age, origin, residence community, occupation, drink, diet, and CD4+T cell count.
Conclusion: Our findings uncover dysbiotic changes at the genus level in the gut through culture-dependent technique in an adult Cameroonian population. The study enriched our insight on the effect of ART and cotrimoxazole prophylaxis in promoting dysbiosis towards a positive outcome by lowering pathobionts levels. Additionally, we revealed associations of sociodemographic and clinical factors with the occurrence of particular gut microbiota, thus reiterating the need for more in-depth and longitudinal studies to corroborate our findings.

Key words: Culturable, Gut Microbiota, HIV, Antiretroviral, Cotrimoxazole, Dysbiosis, factors, Cameroon

1. INTRODUCTION

The human gastrointestinal (GI) tract harbours an intricate and dynamic population of microorganisms, described as the gut microbiota, which influences the healthy host nature amid resistance, homeostasis, and disease [1]. Various components add to the foundation of the human gut microbiota during development. Change in gut microbiota composition (dysbiosis) has shown to be associated with the pathogenesis of numerous inflammatory sicknesses, coronary illness, diabetes, and malignancy [2]. Gut microbiota dysbiosis is frequent among HIV infected individuals, and the alterations are present at all levels from phyla to species. Africa harbours about 95% of the global epidemic HIV , and most developing countries noted for low socio-economic status, high endemic parasitic infections, poor environmental conditions, and distinct dietary compositions may influence the composition and diversity of the gut microbiota [3,4]. Data on gut microbiome studies in Cameroon are scarce. Not many works outside the developed countries have investigated how alteration in gut microbiota impacts HIV disease outcome [5,6]. Expanding HIV Microbiota analysis into the populations most affected by HIV is an important future direction [7]. Prior studies have demonstrated higher relative abundance at the Phylum level for Actinobacteria and Proteobacteria among HIV patients compared to elite controllers [8]. Supporting the attribute of HIV-1 in driving dysbiosis is the finding that people on virally suppressive ART will, in general, have a microbiome shift nearer to that of uninfected controls as compared with untreated HIV people. ART has not related to the complete return of the microbiome in stool and rectal samples to normal level [9].

Using a culture-dependent approach to identify the dysbiotic pattern and the factors associated with it could guide interventions that are designed to modify the gut microbiota and thereby reducing inflammation-associated comorbidity. Analysis of fecal samples from individuals with dysbiosis is anticipated to enable characterization of the bacterial profile associated with different pathological conditions and improving therapeutic regimens. The ability to characterize the bacterial patterns both of normobiotic and dysbiotic patients may also help to evaluate the efficacy and further development of therapeutic approaches such as fecal microbiota transplantation (FMT), special diets, and use of probiotics [10, 11].

The pattern of gut microbiota has shown to differ with time. In the beginning, there is low diversity of the gut microbiota population, and the gastrointestinal tract is, for the most part, colonized with phyla Actinobacteria and Proteobacteria [12], meanwhile, there is expanded diversity and improved colonization rate amid the main long stretches of existence with the examples being peculiar to the newborn children [13]. Although grown-ups present an increasingly steady microbiota, dysbiosis occurs because of life events as a rule causing microbial community shifts [14]. Increase diversity of some members of the gut flora with altered levels from Clostridium difficile, Bacteroides fragilis, and lactobacilli [14], have been implicated with feeding methods. Studies have shown the effects of malnutrition in promoting youthful microbiota dysbiosis and reveal a high population of pathobionts like *Enterobacteriaceae* [15]. Works by De Filippo et al. [16] on the impact of diet in shaping gut microbiota have shown dietary intake among rural African population dominated with high starch substance, and plant polysaccharides have shown to demonstrate an upper microbiota abundance of Actinobacteria (10.1%) , and Bacteroidetes (57.7%) phyla.

Relative investigations of healthy people and factors like diabetes, population age, residence, physical disability, and neurocognitive state with gut microbiota level have shown dysbiosis of specific phyla among the gut microbiota [17]. With the present affiliations connected to dysbiosis at the dimension of the gut, HIV disease which has appeared to have serious harm to the intestinal mucosal compartment and depletion of mucosal immunity (CD4+ T cell) may have an extra impact in causing dysbiosis. Notwithstanding the adjustment of the host immune reaction to gut microorganisms amid HIV diseases with other puzzling elements including the way of life, diet, comorbidities, and treatment impacts with the different antiretroviral regimens have shown gut microbiota diversity reduction; moreover, Proteobacteria phyla, which contain the pathobionts species have presented higher recurrence among HIV infected people [18,19]. Although many early pilot studies reported HIV-associated changes in the enteric microbiome, both in composition and in diversity, more recent studies suggest that co-founding factors, such as sexual behaviour, may explain some of those original findings rather than HIV infection status per se. Therefore, there is a need to investigate other co-founding factors associated with dysbiosis during HIV infection. Despite the preponderance of new data gathered, firm conclusions on the exact nature of HIV-associated dysbiosis, including the impact of age, ethnicity, community residence, diet, occupation, ARV, and cotrimoxazole prophylaxis treatment in the populations most affected by HIV are warranted. Our study was aimed to capture the gut microbiota dysbiotic pattern among HIV-negative individuals, and HIV-positive patients with /or without first-line ARV and cotrimoxazole prophylaxis treatment through culture-dependent technique in an adult Cameroonian population, and to access the associations of gut microbiota at the genus level with sociodemographic and clinical factors.

2. MATERIAL AND METHODS

2.1 Ethics statement

This study was approved by the FHS Institutional Review Board of the University of Buea, and informed consent was obtained from each participant through the signing of a consent form.

2.2 Study design and participants

This was a cross-sectional study, including 160 participants. Participants were categorised as HIV-negative individuals (n=40), HIV-positive treatment naïve (n=40), HIV-positive + ARV (n=40) and HIV-positive + ARV + Cotrimoxazole prophylaxis (n=40). Participants were selected from the South West region to take part in the survey. The study spanned from August 2018 to April 2019.

2.3 Data collection and processing

2.3.1 Data collection

Fecal samples were collected from HIV-negative individuals (n=40), HIV-positive treatment naïve (n=40), HIV-positive + ARV (n=40), and HIV-positive + ARV + Cotrimoxazole prophylaxis (n=40) at the Buea Regional Hospital UPEC unit. A self-structured questionnaire was administered to collect sociodemographic data on the age, gender, place of residence, and diet of participants.

2.3.2 Sample processing

Fresh fecal samples collected in stool containers from each of the 160 participants were transported following defecation and stored in airtight bags and ice-pack until conveyed to a bio-safety cabinet at the Medical Research and Bacteriology unit of the Faculty of Health Sciences, University of Buea. The samples were processed by inoculating aseptically into pre-prepared selective and non-selective media for culture. Venous blood (4 ml) was also collected and stored in 5 ml ethylene-diamine-tetra-acetate (EDTA) vacutainer tubes, and the whole blood samples were processed within five hours after collection.

2.3.3 Culturing and identification

The culture media were prepared aseptically following the manufacturer's instructions. The weight of the fecal samples was determined with the electronic weighing balance, and 1/10 dilution series were made for the samples under anaerobic and aerobic conditions. The samples were plated by using the non-selective and selective media, and plates incubated for 24hours at 37°C under different conditions (Table 1), after which the colonies were identified by using biochemical characterization methods.

Table 1 Non-selective and Selective Media with varied culture conditions for the growth of Culturable gut microbiota

No	Media	Growth conditions	Nature of Media
1	Brain Heart Infusion Agar	Aerobe, 37°C	Non-selective
2	Brain Heart Infusion + sheep blood 5% + vancomycin 10 µg/ml	Aerobe, 37°C	Selective
3	Brain Heart Infusion + sheep blood 5% + vancomycin 10 µg/ml	Anaerobe, 37°C	Selective
4	Brain Heart Infusion + sheep blood 5%,	Aerobic, 37°C	Non-selective
5	Brain Heart Infusion + sheep blood 5%,	Anaerobic, 37°C	Non-selective
6	Brain Heart Infusion + Vanco µg/l	Aerobic, 37°C	Selective
7	Brain Heart Infusion + gentamicin,	Anaerobic, 37°C	Selective
8	Brain Heart Infusion + Vanco µg/l + gentamicin	Anaerobic 37°C	Selective
9	MacConkey agar	Anaerobic 37°C	Selective
10	MacConkey agar	Aerobic 37^0C	Selective
11	Mannitol salt agar	Anaerobic 37°C	Selective
12	deMan Rogosa Sharpe	Anaerobic 37°C	Selective
13	Sabouraud dextrose agar	Anaerobic 35°C	Selective

2.3.4 Flow Cytometry

The CD4+ T cells (cells/mm3) count were evaluated in HIV-negative and HIV-positive individual with FACSCount System Beckton Dickinson.

2.4 Data analysis

Data collected were analysed by using the SPSS software version 21. Demographic data were calculated by
using descriptive statistics, while categorical variables were analysed by using the Chi-square test ($\chi 2$), $p<0.05$. Themultinomial Logistic regression analysis was used to verify associations between variables.

3. RESULTS

3.1 Sociodemographic and clinical characteristics

Table 2 illustratesthe characteristics of the study participants. All participants were Cameroonian originally from Southwest 71(44.4%),Northwest 60 (37.5), and West 29 (18.1%) of the country. All participants resided in the Southwest for at least two years, with a majority residing in Buea 37 (23.1%). Most of the study participants were workers with the majority having a pink collar job 57 (35.6%), and least white collar 17 (10.6%). An equal proportion of 40 (25%) each for HIV-negative, HIV-positive treatment naïve, HIV-positive on ARV, and HIV-positive on ARV+Cotrimoxazole. With regards to food and drinks, most of the study participants were currently on Energy + body-building+ protective foods 65 (40.6%), and non-alcoholic drinks 89 (55.6%). The immune status of our study participants showed a majority of 78 (48.8%) for CD4+ T cell count 201 – 350 cells/mm3, and least 17 (10.6%) for CD4+ T cell count < 200 cells/mm3.

Table 2 Demographic and clinical data

Characteristics	Variables	Frequency (%)
Gender	Male	69 (43.1)
	Female	91(56.9)
Age (years)	18 - 30	33 (20.6)
	31 - 40	36 (22.5)
	41 –50	38 (23.8)
	51 –60	27 (16.9)
	>60	26 (16.3)
Origin	southwest	71(44.4)
	northwest	60 (37.5)
	west	29 (18.1)
Area of residence in Southwest (last 2 years)	buea	37 (23.1)
	ekona	21 (13.1)
	limbe	16 (10.0)
	muea	23 (14.4)
	mutengene	19 (11.9)
	muyuka	21 (13.1)
	tiko	23 (14.4)
Occupation	white-collar worker	17 (10.6)
	pink-collar worker	57 (35.6)
	blue-collar worker	37 (23.1)
	unemployed	49 (30.6)
HIV-status	HIV-negative	40 (25.0)
	HIV-positive treatment naïve	40 (25.0)
	HIV-positive + ARV	40 (25.0)
	HIV-positive + ARV + cotrimoxazole	40 (25.0)
Food eaten (within 24 hours)	energy + body-building	35 (21.9)
	energy + protective	60 (37.5)
	energy + body-building+ protective	65 (40.6)
Drink consume (within 24 hours)	alcoholic	71 (44.4)
	non-alcoholic	89 (55.6)
CD4+ T cell count (cells/mm^3)	< 200	17 (10.6)
	201 – 350	78 (48.8)
	351 – 450	22 (13.8)
	>451	43 (26.9)

3.2 Cultured gut microbiota diversity

The most common gut microbiota identified in our study belongs to the *Phylum*Firmicutes (*Clostridium*,100%), which was followed by *Phylum* Bacteroidetes (*Bacteroides*, 77.5%),*Phylum* Proteobacteria (*Escherichia*, 63.1%) ,*Phylum* Ascomycota (*candida,* 61.9%), and *Phylum* Firmicutes (*Staphylococcus*, 58.8%) (Table 3). The least occurrence was *Enterococci,* 27.5% from the *Phylum* Firmicutes. Depiction in a chronological manner of cultured gut microbiota is shown in Figure 1.

Table 3 Cultured microbiota diversity and frequency

NO	*Gut microbiota*	*Phylum*	*Growth frequency (%)*
1	*Clostridium*	Firmicutes	160 (100)
2	*Candida*	Ascomycota	99 (61.9)
3	*Escherichia coli*	Proteobacteria	101(63.1)
4	*Klebsiella*	Proteobacteria	69 (43.1)

5	*Proteus*	Proteobacteria	74 (46.3)
6	*Salmonella*	Proteobacteria	61 (38.1)
7	*Enterobacter*	Proteobacteria	62 (38.8)
8	*Staphylococcus*	Firmicutes	94 (58.8)
9	*Bacteroides*	Bacteroidetes	124 (77.5)
10	*Lactobacillus*	Firmicutes	64 (40.0)
11	*Bifidobacteria*	Actinobacter	64 (40.0)
12	*Fusobacterium*	Fusobacteria	77 (48.1)
13	*Enterococci*	Firmicutes	44 (27.5)

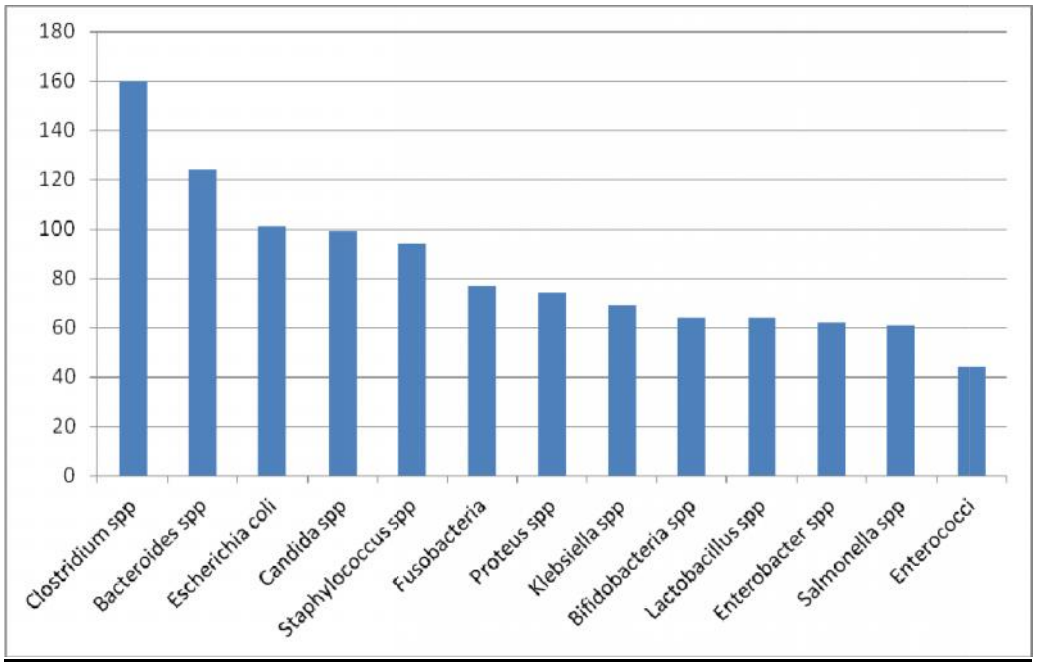

Figure 1: illustration of cultured gut microbiota frequency in a Cameroonian cohort with or without HIV infection

3.3 The dysbiotic pattern among HIV-seronegative individuals compared to HIV-positive individuals with or without treatment on ARV/or cotrimoxazole prophylaxis

Comparing the cultured gut microbiota occurrence revealed a significant increase in growth frequency of *Candida* (*P*< .001) and *Fusobacteria* (*P*< .001) among HIV-positive treatment-naive individuals when compared to HIV-negative individuals. While cultured microbiota belonging to the Enterobacteriaceae family, *Staphylococcus*, and *Bifidobacteria* demonstrated a significantly decreased growth frequency among HIV-positive treatment-naive individuals when compared to HIV-negative individuals (*P*< .001) (supplementary Table S1).

Further analysis to compare HIV-positive treatment naïve and HIV-positive on ARV showed that the increasing frequency of *Candida* and *Fusobacteria* among HIV-positive individuals on ARV was significantly lowered when compared to HIV-positive treatment naïve individuals (*P* = .005 and *P* = .004 respectively) (Supplementary Table S2).

The gut microbiota growth occurrence between HIV-positive individuals on ARV only, with those of individuals on ARV+cotrimoxazole prophylaxis demonstrated that there was a significant decrease in growth frequency in *Escherichia* (*P* = .014), *Salmonella*(*P* = .002) and *Staphylococcus* (*P* = .044) with ARV+cotrimoxazole prophylaxis as compared to HIV-positive individuals on ARV only. (Supplementary Table S3).

Lastly, analyzing HIV-positive treatment naïve and ARV+cotrimoxazole prophylaxis for gut microbiota growth frequency showed that *Escherichia* and *Fusobacterium* were significantly higher (P < .001 and *P* = .015 respectively) among HIV-positive treatment naïve as compared to those on ARV+Cotrimoxazole prophylaxis treatment. (Supplementary Table S4). The above-cultured gut microbiota dysbiotic variations are shown in Figure 2.

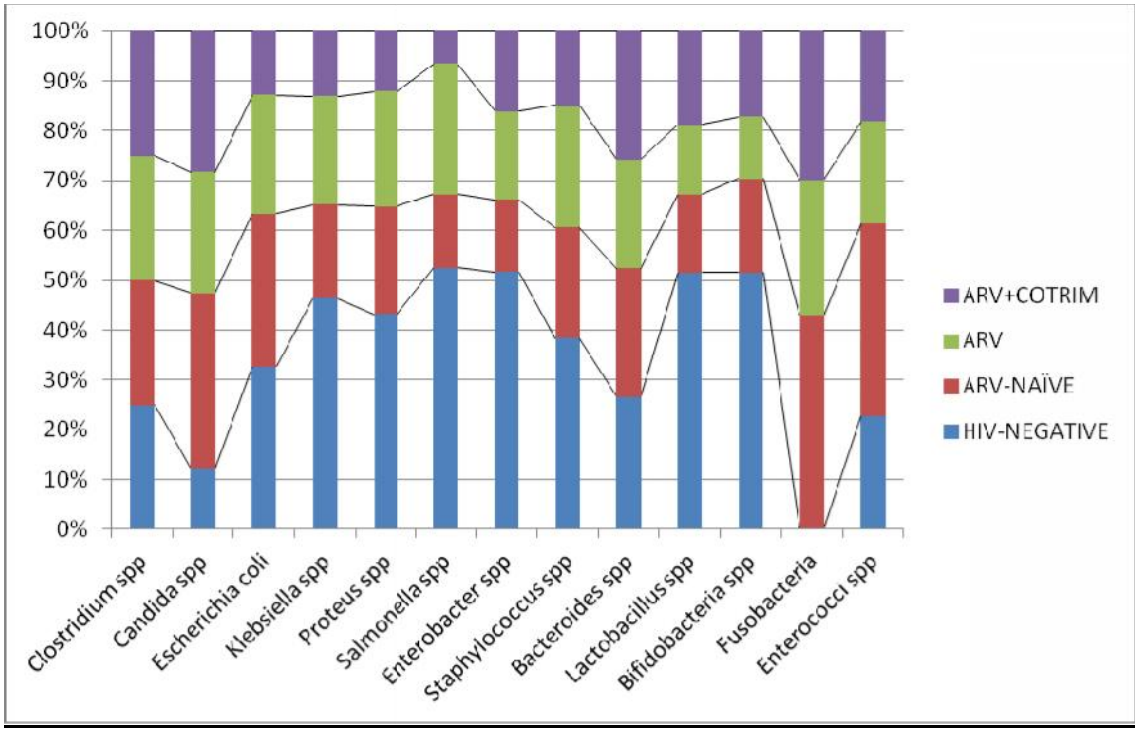

Figure 2: cultured gut microbiota growth frequency among HIV-seronegative individuals compared to HIV-positive individuals with or without treatment on ARV/or cotrimoxazole prophylaxis.

3.4 Association between microbiota growth frequency and demographic factors

Findings of our study show that specific gut microbiota is associated with sociodemographic factors (sex, origin, residence, occupation, diet, and drinks) and clinical factors (HIV status and CD+T cell count) (Table 4).

3.4.1 Age group versus gut microbiota dysbiotic pattern

Enterobacteriaceae (*Enterobacter, Klebsiella*), Gram-positive bacilli (*Bifidobacteria, Lactobacillus*) and Gram-positive cocci (*Staphylococcus*) were more likely to display increased occurrence with younger age group 18 – 30 years as compared to the older age groups greater than 60 years old.However, non-*Enterobacteriaceae* Gram-negative bacilli (*Fusobacteria, Bacteroides*) and Enterobacteriaceae (*Salmonella*) were more likely to display decreased occurrence with age 18 – 30 years as compared to the older age groups greater than 60 years. (Supplementary Table S5, and S6).

3.4.2 ARV and Cotrimoxazole prophylaxis versus microbiota dysbiotic pattern

HIV-positive treatment naïve and those on ARV with or without Cotrimoxazole prophylaxis were more likely to display the decreased occurrence of Enterobacteriaceae (*Klebsiella, Salmonella*, *Enterobacter, Escherichia*), Gram-positive bacilli (*Lactobacillus, Bifidobacteria*), Gram-negative bacilli (*Fusobacteria*) and Gram-positive cocci (*Staphylococcus*) compared with HIV-negative individuals. Fusobacteria were more likely to display increase occurrence only with HIV-positive treatment naïve participants. Only Candida was more likely to display increased occurrence among HIV-positive treatment naïve and those on ARV with or without Cotrimoxazole prophylaxis. (Supplementary Tables S5, S6, and S7).

3.4.3 CD4+ T cells count versus microbiota dysbiotic pattern

Participants with low CD+T cell count (less than 350 cells/mm3) were more likely to display the increased occurrence of both Candida and *Fusobacteria*. Conversely, decreased occurrences of *Escherichia, Proteus, Bifidobacteria*, and *Staphylococcus* were more likely to be displayed with low CD+T cell count (less than 350 cells/mm3) as compared to CD+ T cell count (greater than 450 cells/mm3). (Supplementary Tables S5, S6, and S7).

3.4.4 Dietary and Alcohol intake versus gut microbiota dysbiotic pattern

Samples analyzed from participants that recently feed (last 24 hours) on a diet composed of primarily Energy + body-building+ protective were more likely to display increased occurrence with *Salmonella*, *Enterobacter, Klebsiella, Staphylococcus*, and *Enterococci*. While the sample analyzed from participants that recently consume alcohol drinks (last 24 hours) were more likely to display increased occurrence with *Escherichia, Enterobacter*, and *Proteus*. (Supplementary Tables S5, and S7).

3.4.5 Occupation versus gut Microbiota dysbiotic pattern

Decreased occurrences of *Klebsiella, Salmonella*, and *Proteus* were more likely to be displayed by participants with blue-collar work, while the increased occurrence of *Fusobacteria* was more likely to be shown with participants on both pink and blue collar work. (Supplementary Tables S5, and S6).

3.4.6 Community residence versus gut microbiota dysbiotic pattern

Participants in particular Community residence like Muea were more likely to display the decreased occurrence of *Klebsiella, Enterobacter, Proteus*, and *Bifidobacteria*, while those from Ekona and Muyuka were more likely to display the decreased occurrence of *Lactobacillus* and *Bifidobacteria*. Only *Fusobacteria* was more likely to display increase occurrence in almost all the communities. (Supplementary Tables S5, and S6).

Table 4: Factors associated with gut microbiota growth frequency (P-value significant at < 0.05)

GUT MICROBIOTA	SEX	AGE	ORIGIN	RESIDENCE	OCCUPATION	DIET	DRINKS	CD4+T CELL	HIV
Escherichia	-	-	**0.010**	-	-	-	**0.018**	**0.0001**	**0.003**
Klebsiella	-	**0.016**	-	**0.050**	**0.002**	-	-	**0.0001**	**0.0001**
Proteus	-	-	-	-	**0.0001**	-	**0.012**	**0.0001**	**0.0001**
Salmonella	-	-	-	-	**0.001**	-	-	**0.0001**	**0.0001**
Enterobacter	-	**0.002**	-	**0.006**	**0.001**	**0.027**	**0.033**	**0.0001**	**0.0001**
Bacteroides	-	-	-	-	-	-	-	-	-
Fusobacterium	-	**0.015**	-	**0.0001**	-	**0.002**	-	**0.0001**	**0.0001**
Bifidobacterium	-	**0.029**	**0.051**	**0.029**	**0.0001**	-	-	**0.0001**	**0.0001**
Lactobacillus	-	-	-	**0.046**	**0.0001**	-	-	**0.0001**	**0.0001**
Clostridium	-	-	-	-	-	-	-	-	-
Staphylococcus	-	-	-	**0.021**	-	**0.016**	-	**0.0001**	**0.0001**
ENTEROCOCCI	-	-	-	-	-	-	-	**0.0001**	-
Candida	-	-	-	-	-	**0.001**	-	**0.0001**	**0.0001**

4. DISCUSSION

Our study is the first to compare gut microbiota genus in HIV-positive adult patients with or without first-line antiretroviral and cotrimoxazole prophylaxis treatments with those of HIV-negative individuals, and the factors associated with their likely increased frequency in an urban and rural mixed population from Cameroon by using a culture-dependent approach.

Our study showed that with culture technique, the most dominated gut microbiota genus in HIV-positive patients (*Clostridium* from Firmicutes, and *Bacteroides*from *Phylum*Bacteroidetes) was similar to those from HIV-negative individuals, this is in line with Cheng et al. [20] who reported a microbiota profile with the most dominant taxa Firmicutes, Bacteroidetes, and Actinobacteria in both HIV-infected and uninfected individuals. Comprehensively, the growth occurrence at the taxa level of individual gut microbiota genus was lower with all HIV-positive patients irrespective of ARV and of cotrimoxazole, when compared with those of HIV negative individuals, suggesting the direct effect of HIV-infection in promoting dysbiosis.

In HIV-treatment naïve patients, significantly higher proportions of *Candida*and *fusobacteria*were noted, when compared with HIV-negative individuals. Consistent with prior reports, microbiota profile in HIV-positive individuals showed an increased abundance of bacteria *Fusobacterium*from the *Fusobacteriaceae* family [20], and opportunistic pathogens, including *Candida albicans* [21]. These results suggest that changes in gut microbiota composition, specifically the enrichment of *Fusobacterium* and *Candida* during HIV infection without treatment, can serve as markers of disease present and progression in our study population. The explanation for such overrepresentation of the dysbiotic pattern has been associated with the loss of effector CD4+ T cells during HIV infection. The

depletion of this effector T cells results in the inability of the immune system to mount an effective response to these enteropathogens, described as pathobionts, thereby resulting in their outgrowth [22]. Butyric acid-producing *Fusobacterium* has shown to have a pathogenic role during HIV infection with its higher abundance in the oral cavity, and HIV-associated periodontitis [23]. Studies with HIV negative individuals have associated increased gut *Fusobacterium* with inflammatory bowel disease [24], colorectal cancer[25], and acute appendicitis [26]. Thus, an overrepresentation of *Fusobacterium* among HIV infected individuals will lead to the re-activation of latently infected cells in GALT, thus promoting deterioration of the gut mucosal barrier [20]. Fusobacterium ability to directly cause cell death could lead to increased local and systemic immune activation, subsequently increasing bystander CD4 T-cell death [27]. This could explain some of the low CD4+T cell HIV infected individuals experience irrespective of suppressive ART [20].

HIV treatment naïve patients also demonstrated lower proportions with the *Enterobacteriaceae* family, *Staphylococcus, Lactobacillus,* and*Bifidobacterium,* when compared with HIV-negative individuals. Reduction in the phylum Firmicutes was also shown among HIV infected individuals not yet on ART [28], which was in line with our study. Also, reports on lower counts of *Lactobacillus* [29] and *Bifidobacterium* were shown in the stool of HIV-treatment naïve individuals [30]. Previous works have linked the depletion of *Bifidobacterium* and *Lactobacillus* during HIV infection and their effects in gut barrier destruction and poor immune function in the GALT [31]. Our study suggests that there is an association between depletion of protective *Lactobacillus* and bifidobacteria, and enrichment of pathobionts fusobacteriaand *Candida*. This data promotes and encourages the implementation of Lactobacillus as probiotics corroborating with the previous report on their protective role in lowering microbial translocation and keeping a better immunological state [32,33].

In HIV patients on antiretroviral, there was a significantly lower growth occurrence for *Candida* and fusobacteriaand an overrepresentation growth frequency among some members of the phylum Proteobacteria particularly the *Enterobacteriaceae* family when compared with HIV-treatment naïve patients. Similar findings were observed from Gonzalez-Hemandez et al. [30] work, who found a significant increase in Proteobacteria. In the examination by Dinh et al. [34], Proteobacteria and a few subtaxa, including *Enterobacteriaceae*, which contains many regular pathogens, were overrepresented in HIV-positive people and were related to immune activation. This relationship between HIV infection and expanded abundance of Proteobacteria, especially in mucosal examples, might be more critical than the *Prevotella*/*Bacteroides* shifts given the propensity of Proteobacteria to translocate in the non-human primate model [35]. *Enterobacteriaceae* family members have been associated with inflammation [36]. These microorganisms may contribute to the gastrointestinal disease and chronic immune activation observed in HIV patients.

Contrarily, another study reported a decrease Proteobacteria after utilization of ART [8]. The contrast might be related to demographic factors like lifestyle and microbiota baseline features. Also, microbiota differences among HIV-infected on ARV might be directly influenced by HIV infection, duration, and kind of ART treatment regimen. Confirmation of the latter claim was demonstrated with ritonavir-boosted protease inhibitors linked with increased occurrence of non-infectious diarrhea [33]. Interestingly, our results show that the first line ARV administration was linked with suppression of pathobionts *Candida*and fusobacteriaand increased occurrence of members of the Enterobacteriaceae family containing other pathobionts taxa and suggesting that the current first-line ARV used in our study population needs to be giving with other prophylaxis treatment that could control the pathobionts growth levels.

HIV patients on Antiretroviral plus cotrimoxazole prophylaxis treatment showed a significantly lower growth occurrence for fusobacteria*, Escherichia, Salmonella,* and *Staphylococcus* as compared to HIV-positive patients on antiretroviral only. Contrarily to our findings, studies by Monaco et al. [7] demonstrate no difference in phylogenetic diversity among individuals on cotrimoxazole. Although limited works have supported the claims, ARV may have a direct impact on gut microbiota, which explains the persistent microbiota alterations between patients on long term ARV treatment vs. HIV-negative individuals [9]. And cotrimoxazole given as prophylaxis might have some unintended effect on gut microbiota. Putting together the resulting pattern of dysbiosis with individuals on combine administration of antiretroviral and cotrimoxazole prophylaxis,suggest a reduction of pathobionts from different *Phylum*. This might explain the clinical benefits of low microbial translocation among HIV infected patients on Antiretroviral and cotrimoxazole prophylaxis.

Our study revealed that the alterations in the gut microbiota might likely be influenced by age, immune status (CD+T cell count), occupation, dietary habits, community resident, origin, and drinking habits. Our results corroborate previous observations that demonstrate significant associations between gut microbiome with socioeconomic factors, age, geography, and diet [4,12,16]. Associationwith specific phylum were displayed with children from Burkina Faso in Africa, showing how their food appears to

enrich the microbiota composition [16]. Most African diet is composed basically with carbohydrates and plants showing enterotype with low *Bacteroides/Prevotella* ratio [4,38] as compared with high *Bacteroides/Prevotella* ratio in western diet heavily characterized with high protein and saturated fat [39]. Also, in line with our results, community resident appeared to influence microbiome composition as this was demonstrated with enterotype, being similar among people dwelling in the same site [37]. A few natural elements have been associated in shaping the microbiota, including topographical area and living courses of action (urban or rural) [12,40]. In the elderly population, a significant relationship has been identified between diversity and living arrangements, such as community-dwelling or long-term residential care [40].

The occupation was also shown to influence gut microbiota from other works [41] significantly. A typical experimental study showed that exposed macaques in close contact with humans, compared to a less exposed population, demonstrated the beta-diversity differential impact that shows dysbiotic pattern unstable gut microbiota composition [41], which may be tied to human contact in an urban environment.

With regards to CD4+ T cells alteration with microbiota, specific genera of microbial cells have also been reported to influence CD4+ T cells population. In a prior study, apoptotic death of CD4+T cells was increased in HIV-infected LPMC cultures following exposure to microbiota (pathobiont bacteria). Bacteroides fragilis, a prominent anaerobic commensal, is thought to inhibit CD4 differentiation into Th17 and increase differentiation into Tregs in mouse gut [42]. Gut microbiota may be associated with improvement in the CD4 count, which continues to be an important prognostic indicator and predicts non-AIDS events and mortality in addition to AIDS-associated morbidity and mortality[43,44]. As with previously noted microbiome associations, findings have not always been confirmed, and, importantly, note the taxonomic level at which the association was found. The abundance of the genus Bacteroides in stool and colonic biopsies was associated with lower peripheral CD4 recovery, whereas *Lactobacillales* abundance was associated with a higher peripheral CD4 percentage [45]. Lactobacillus appears to inhibit IDO1 and is selectively depleted in SIV-infected macaques, so this may be a mechanism by which *Lactobacillus* spp. prevent CD4 activation and depletion [46].

Relative investigations of healthy people and factors like age with gut microbiota level have shown dysbiosis of specific phyla among the gut microbiota [16,17]. Also, associations between genetic variation in host coding sequence and abundance of specific microbial taxa were noted in Blekham et al. [47]. Host genetic SNPs and LCT gene have shown to correlate with an abundance of *Bifidobacterium* in the GI tract [47].

The kind of drink was also found to corroborate our findings, in which alcohol consumption was shown to influence microbiota composition [48]. Despite the species-specific changes note, there is a trend for an increase in pro-inflammatory bacteria following exposure to alcohol [49]. Alcoholics have a lower abundance of bacteria from the phylum Bacteroidetes and butyrate-producing bacteria (generally believed to be anti-inflammatory) and greater bacteria from the *Phylum*Proteobacteria (generally considered to be pro-inflammatory [48,50].

Several limitations were noted in our study. It was a cross-sectional study, so the changes captured only reflect the current state. Longitudinal studies are warranted to confirm the dysbiotic patterns. It is also possible that the increased growth occurrence associated with some demographic factors may be transient. This study was conducted in the Southwest region with participants recruited in Buea , which is characterized with a low-temperature gradient (average annual temperature of 18.6 ^{0}c) as compared to other parts of the country and the world implying the results might not be generalized in different settings. Our results were based only on culture-dependent technique, which could not capture all the gut microbiota and, moreover, we limited our comparison based on growth occurrence. Thus, we suggest future work to include sequencing approaches that will capture the majority of the gut microbiota alpha and beta diversity to better associate the underlying factors linking to dysbiosis in the Cameroonian population with or without HIV infection.

5. CONCLUSION

Our findings uncover dysbiotic changes at the genus level in the gut through culture-dependent technique in the adult Cameroonian population. The study enriched our insight on the effect of ART and cotrimoxazole prophylaxis in promoting dysbiosis towards a positive outcome by lowering pathobionts levels in immunocompromised individuals. Additionally, we revealed associations of sociodemographic and clinical factors with the occurrence of particular gut microbiota, thus, reiterating the need for more in-depth and longitudinal studies to corroborate our findings.

CONSENT

All authors declare that written informed consent was obtained from the participants for publication of this work. And as per international standard or university standard, patient's written consent has been preserved by the author(s).

ETHICAL APPROVAL

All authors hereby declare that the study has been examined and approved by the Faculty of Health Sciences Institutional Review Board, University of Buea, Cameroon. And have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

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APPENDIX

SUPPLEMENTARY MATERIALS

S_1: Dysbiotic pattern comparison between HIV-seronegative individuals and HIV-positives treatment naïve individuals

Gut Microbiota	*Growth frequency (%)*	*HIV-Negative (%)*	*HIV-Positive treatment naïve (%)*	*P-value*
Clostridium	80 (100)	40 (50.0)	40 (50.0)	-
Candida	47 (58.8)	12 (15.0)	35 (43.8)	0.0001
Escherichia	64 (80.0)	33 (41.3)	31 (38.8)	0.576
Klebsiella	45 (56.3)	32 (40.0)	13 (16.3)	0.0001
Proteus	48 (60.0)	32 (40.0)	16 (20.0)	0.0001
Salmonella	41 (51.3)	32 (40.0)	9 (11.3)	0.0001
Enterobacter	41 (51.3)	32 (40.0)	9 (11.3)	0.0001
Staphylococcus	57 (71.3)	36 (45.0)	21(26.3)	0.0001
Bacteroides	65 (81.3)	33 (41.3)	32 (40.0)	0.775
Lactobacillus	43 (53.8)	33 (41.3)	10 (12.5)	0.0001
Bifidobacteria	45 (56.3)	33 (41.3)	12 (15.0)	0.0001
Fusobacteria	33 (41.3)	0	33 (41.3)	0.0001
Enterococci	27 (33.8)	10 (12.5)	17 (21.3)	0.098

S_2:**Dysbiotic pattern comparison between HIV-positives treatment naïve and HIV-positive individuals on antiretroviral only**

Gut Microbiota	*Growth frequency (%)*	*HIV-Positive treatment naïve(%)*	*HIV-Positive + ARV (%)*	*p-value*
Clostridium	80 (100)	40 (50.0)	40 (50.0)	-
Candida	59 (73.8)	35 (43.8)	24 (30.0)	0.005
Escherichia	55 (68.8)	31 (38.8)	24 (30.0)	0.091
Klebsiella	28 (35.0)	13 (16.2)	15 (18.8)	0.639
Proteus	33 (41.3)	16 (20.0)	17 (21.3)	0.820
Salmonella	25 (31.3)	9 (11.3)	16 (20.0)	0.091
Enterobacter	20 (25.0)	9 (11.2)	11 (18.8)	0.606
Staphylococcus	44 (55.0)	21(26.3)	23 (28.8)	0.653
Bacteroides	59 (73.8)	32 (40.0)	27 (33.8)	0.204
Lactobacillus	19 (23.8)	10 (12.5)	9 (11.3)	0.793
Bifidobacteria	20 (25.0)	12 (15.0)	8 (10.0)	0.302
Fusobacteria	54 (67.5)	33 (41.3)	21(26.3)	0.004
Enterococci spp	26 (32.5)	17 (21.3)	9 (11.3)	0.056

S_3: Dysbiotic pattern comparison between HIV-positives individuals on ARV with HIV-positive individuals on antiretroviral plus Cotrimoxazole prophylaxis

Gut Microbiota	*Growth Frequency (%)*	*HIV-Positive +ARV (%)*	*HIV-Positive +ARV + cotrimoxazole (%)*	*p-value*
Clostridium	80 (100)	40 (50.0)	40 (50.0)	-
Candida	52 (65.0)	24 (30.0)	28 (35.0)	0.348
Escherichia	37 (46.3)	24 (30.0)	13 (16.3)	0.014
Klebsiella	24 (30.0)	15 (18.8)	9 (11.3)	0.143
Proteus	26 (32.5)	17 (21.3)	9 (11.3)	0.056
Salmonella	20 (25.0)	16 (20.0)	4 (5.0)	0.002
Enterobacter	21 (26.3)	11 (13.8)	10 (12.5)	0.799
Staphylococcus	37 (46.3)	23 (28.8)	14 (17.5)	0.044
Bacteroides	59 (73.8)	27 (33.8)	32 (40.0)	0.204
Lactobacillus	21 (26.3)	9 (11.3)	12 (15.0)	0.446
Bifidobacteria	19 (23.8)	8 (10.0)	11 (13.8)	0.431
Fusobacteria	44 (55.0)	21 (26.3)	23 (28.8)	0.653
Enterococci	17 (21.3)	9 (11.3)	8 (10.0)	0.785

S_4: Dysbiotic pattern comparison between HIV-positives treatment naives individuals with HIV-positive individuals on antiretroviral plus Cotrimoxazole prophylaxis

Gut Microbiota	*Growth Frequency (%)*	*HIV-Positive +ARV + cotrimoxazole (%)*	*HIV-Positive treatment naïve (%)*	*P-value*
Clostridium	80 (100)	40 (50.0)	40 (50.0)	-
Candida	63 (78.8)	28 (35.0)	35 (43.8)	0.056
Escherichia	44 (55.0)	13 (16.2)	31 (38.8)	0.0001

Klebsiella	22 (27.5)	9 (11.2)	13 (16.3)	0.317
Proteus	25 (31.3)	9 (11.3)	16 (20.0)	0.091
Salmonella	13 (16.3)	4 (5.0)	9 (11.3)	0.130
Enterobacter	19 (23.8)	10 (12.5)	9 (11.3)	0.793
Staphylococcus	35 (43.8)	14 (17.5)	21 (26.3)	0.115
Bacteroides	64 (80.0)	32 (40.0)	32 (40.0)	1.000
Lactobacillus	22 (27.5)	12 (15.0)	10 (12.5)	0.617
Bifidobacteria	23 (28.8)	11 (13.8)	12 (15.0)	0.805
Fusobacteria	56 (70.0)	23 (28.8)	33 (41.3)	0.015
Enterococci	25 (31.3)	8 (10.0)	17 (21.3)	0.030

Variables	Enterobacteriaceae						
	Klebsiella spp (Ref. no-growth)		Escherichia coli (Ref. no-growth)		Salmonella spp (Ref. no-growth)		Enterobacte (Ref. no-gro
	Growth occurrence	*p*	Growth occurrence	*p*	Growth occurrence	*p*	Growth occ
Age (years)							
18 – 30	4.344(1.450 – 13.016)	.009	1.956 (0.656 – 5.827)	.229	3.684(1.216 – 11.155)	.021	6.667(2.080
31 - 40	1.068 (0.371- 3.070)	.903	1.667 (0.582 – 4.776)	.342	1.194 (0.390 – 3.659)	.756	1.111 (0.34
41 –50	1.232 (0.437 – 3.476)	.694	1.008 (0.367 – 2.768)	.987	1.583 (0.533 – 4.703)	.408	1.944 (0.63
51 –60	0.944 (0.303 – 2.945)	.922	0.917 (0.309 – 2.718)	.875	1.597 (0.497 – 5.127)	.432	2.292 (0.69
>60 = 0							
Origin							
Southwest	2.552 (0.999 – 6.519)	.050	0.758 (0.281 – 2.042)	.583	1.921 (0.750 – 4.920)	.174	2.436 (0.92
Northwest	2.007 (0.768 – 5.249)	.155	0.298 (0.111 – 0.801)	.016	1.632 (0.621 – 4.289)	.321	2.095 (0.77
West =0							
HIV-Status							
HIV-Treat naïve	0.120 (0.043 – 0.333)	.0001	0.731 (0.243 – 2.201)	.577	0.073 (0.025 – 0.212)	.0001	0.073 (0.02
HIV + ARV	0.073 (0.025 – 0.212)	.0001	0.318 (0.113 – 0.893)	.030	0.167 (0.061 – 0.453)	.0001	0.083 (0.02
HIV + ARV + Cot	0.150 (0.055 – 0.410)	.0001	0.102 (0.036 – 0.292)	.0001	0.028 (0.008 – 0.101)	.0001	0.095 (0.03
HIV-Negative = 0							
Food eaten (24 hours)							
Energy + protective	2.528 (1.015 – 6.293)	.046	0.730 (0.307 – 1.737)	.477	1.673 (0.665 – 4.206)	.274	1.444 (0.57
Energy + body-building+ protective	2.801 (1.138 – 6.894)	.025	1.020 (0.429 – 2.426)	.965	2.476 (1.005 – 6.099)	.049	2.979 (1.21
Energy + body-building =0							
Occupation							
Pink-collar worker	0.559 (0.188- 1.664)	.296	1.644 (0.549 – 4.924)	.374	0.378 (0.125 – 1.145)	.085	0.478 (0.15
Blue-collar worker	0.245 (0.071 – 0.841)	.025	1.641 (0.511 – 5.273)	.406	0.207 (0.059 – 0.730)	.014	0.310 (0.09
Unemployed	1.404 (0.461 – 4.269)	.550	1.531 (0.502 – 4.671)	.454	1.185 (0.391 – 3.588)	.764	1.631 (0.53
White-collar= 0							
Drink consume(within 24 hours)							
Alcoholic	0.846 (0.450 – 1.590)	.603	2.234 (1.142 – 4.371)	.019	0.576 (0.299 – 1.108)	.098	0.492 (0.25
Non-alcoholic = 0							

Resident community (last 2 years)							
Buea	1.128 (0.393 – 3.236)	.822	0.652 (0.206 – 2.057)	.465	0.779 (0.275 – 2.211)	.639	1.506 (0.52
Ekona	0.473 (0.142 – 1.582)	.224	0.882 (0.234 – 3.328)	.853	0.286 (0.078 – 1.046)	.286	0.286 (0.07
Limbe	0.462 (0.125 – 1.703)	.246	0.454 (0.117 – 1.763)	.254	0.550 (0.150 – 2.021)	.368	0.417 (0.10
Muea	0.162 (0.042 – 0.629)	.009	0.385 (0.112 – 1.329)	.131	0.324 (0.094 – 1.117)	.324	0.193 (0.05
Mutengene	0.559 (0.164 – 1.911)	.354	0.485 (0.132 – 1.785)	.485	0.535 (0.155 – 1.848)	.323	0.535 (0.15
Muyuka	0.473 (0.142 – 1.582)	.224	0.574 (0.159 – 2.066)	.574	0.564 (0.170 – 1.877)	.351	0.367 (0.10
Tiko = 0							
CD4+ T cell count (cells/mm^3)							
< 200	0.093 (0.025 – 0.351)	.0001	0.635 (0.177 – 2.276)	.486	0.019 (0.002 – 0.161)	.0001	0.126 (0.03
201 – 350	0.176 (0.076 – 0.408)	.0001	0.233 (0.099 – 0.550)	.001	0.132 (0.056 – 0.311)	.0001	0.117 (0.04
351 – 450	0.051 (0.012 – 0.207)	.0001	1.588 (0.382 – 6.611)	.525	0.051 (0.012 – 0.207)	.0001	0.032 (0.00
>451 = 0							

S_6: Associations between sociodermographic and clinical factors with gut microbiota from Enterobacteriaceae family (Odds Ratios with 95% Confidence Intervals)

Variables	Gram-negative rods (non-Enterobacteriaceae)						Gram-poitive rods
	Fusobacteria (Ref. no-growth)		Bacteroides (Ref. no-growth)		Lactobacillus (Ref. no-growth)		Bifidobacteri (Ref. no-gro
	Growth occurence	*p*	Growth occurrence	*p*	Growth occurrence	*p*	Growth occu
Age (years)							
18 – 30	0.169 (0.054 – 0.527)	.002	0.222 (0.054 – 0.914)	.037	4.524 (1.441 – 14.203)	.010	3.306 (1.128
31 - 40	0.741 (0.261 – 2.107)	.574	0.535 (0.152 – 1.885)	.535	1.667 (0.530 – 5.241)	.382	0.726 (0.245
41 –50	0.529 (0.189 – 1.480)	.225	0.506 (0.149 – 1.714)	.274	2.174 (0.709 – 6.666)	.174	0.982 (0.344
51 –60	0.424 (0.140 – 1.283)	.129	0.465 (0.122 – 1.771)	.262	2.667 (0.814 – 8.738)	.105	1.299 (0.426
>60 = 0							
Origin							
Southwest	0.447 (0.184 – 1.084)	.075	0.240 (0.067 – 0.858)	.028	1.534 (0.613 – 3.843)	.361	0.460 (0.191
Northwest con	0.572 (0.231 – 1.413)	.226	0.326 (0.090 – 1.180)	.088	1.699 (0.665 – 4.341)	.268	0.327 (0.131
West = 0							
HIV-Status							
HIV-Treat naïve	$2.1E^8$ ($7.7E^8$ – $60.5E^8$)	.0001	0.848 (0.275 – 2.613)	.775	0.071 (0.024 -0.209)	.0001	0.091 (0.032
HIV + ARV	$5.0E^8$($5.0E^8$ – $50.9E^8$)	.0001	0.848 (0.275 – 2.613)	.775	0.091 (0.032 – 0.262)	.0001	0.080 (0.028
HIV + ARV + cotrimoxazole	$6.2E^8$($2.5E^8$ – $15.0E^8$)	.0001	0.441 (0.154 – 1.259)	.126	0.062 (0.020 – 0.186)	.0001	0.053 (0.017
HIV-Negative = 0							
Food eaten (24 hours)							
Energy + protective	1.631(0.700 – 3.800)	.257	2.087 (0.814 – 5.353)	.126	0.888 (0.367 – 2.152)	.793	0.888 (0.367
Energy + body-building+ protective	0.451(0.194 – 1.047)	.064	2.304 (0.902 – 5.887)	.081	1.977 (0.844 – 4.627)	.116	1.977 (0.844
Energy + body-building = 0							
Occupation							
Pink-collar worker	6.013 (1.730 – 20.896)	.004	0.945 (0.265 – 3.374)	.931	0.317 (0.104 – 0.973)	.045	0.478 (0.158
Blue-collar worker	6.771 (1.818 – 25.224)	.005	1.115 (0.284 – 4.376)	.876	0.245 (0.071 – 0.841)	.025	0.310 (0.090
Unemployed	0.927 (0.255 – 3.474)	.927	1.200 (0.321 – 4.485)	.786	1.673 (0.546 – 5.125)	.367	1.937 (0.635
White-collar worker = 0							
Drink consume (within 24 hours)							
Alcoholic	1.636 (0.873 – 3.066)	.125	0.867 (0.409 – 1.838)	.867	0.775 (0.409 – 1.471)	.436	1.160 (0.613
Non-alcoholic = 0							
Resident community (last 2 years)							
Buea	12.353 (2.524 – 60.454)	.002	1.586 (0.485 – 5.181)	.445	0.490 (0.170 – 1.414)	.187	0.333 (0.111
Ekona	33.600 (5.757 – 196.100)	.0001	8.750 (0.973 – 78.653)	.053	0.201 (0.054 – 0.743)	.016	0.137 (0.036
Limbe	6.300 (1.075 – 36.936)	.041	1.896 (0.407 – 8.824)	.415	0.643 (0.177 – 2.333)	.502	0.199 (0.050
Muea	24.000 (4.381 – 131.472)	.0001	1.000 (0.285 – 3.512)	1.00	0.179 (0.049 – 0.653)	.009	0.154 (0.043
Mutengene	3.750 (0.636 – 22.099)	.144	0.948 (0.255 – 3.525)	.948	0.714 (0.209 – 2.443)	.592	0.394 (0.111
Muyuka	14.000 (2.587 – 75.749)	.002	1.859 (0.456 – 7.581)	.387	0.257 (0.073 – 0.910)	.035	0.219 (0.061
Tiko = 0							

CD4+ T cell count (cells/mm^3)							
< 200	100.800 (10.722 -947.637)	.0001	1.458 (0.271 – 7.848)	.660	0.070 (0.018 – 0.274)	.0001	0.081 (0.021
201 – 350	58.545 (7.667 – 447.082)	.0001	0.537 (0.207 – 1.390)	.200	0.083 (0.033 – 0.207)	.0001	0.067 (0.027
351 – 450	252.000(24.529 – 2588.8)	.0001	0.486 (0.140 – 1.689)	.486	0.054 (0.014 – 0.204)	.0001	0.241 (0.078
>451 = 0							

S_7: Associations between sociodermographic and clinical factors with Gram -positive and Gram -negative rods (non-Enterobacteriacea (Odds Ratios with 95% Confidence Intervals)

S_8: Associations between sociodermographic and clinical factors with Gram –positive cocci and fungi (non-Enterobacteriacea (Odds Ratios with 95% Confidence Intervals)

Variables	Fungi		Gram-positive cocci			
	Candida (Ref. no-growth)		Staphylococcus (Ref. no-growth)		Enterococci (Ref. no-growth)	
	Growth occurrence	*p*	Growth occurrence	*p*	Growth occurrence	*p*

Age (years)						
18 – 30	0.461 (0.161 – 1.315)	.147	4.261(1.400- 12.972)	.011	0.844(0.272 – 2.616)	.769
31 - 40	1.625 (0.554 – 4.762)	.376	2.143(0.767 – 5.983)	.146	1.607 (0.554 – 4.659)	.382
41 –50	1.354 (0.476 – 3.851)	.570	1.143(0.767 – 5.983)	.310	0.698 (0.228 – 2.139)	.529
51 –60	1.063 (0.350 – 3.227)	.915	1.705(0.575 – 5.055)	.336	0.281 (0.065 – 1.212)	.089
>60 = 0						
Origin						
Southwest	0.548 (0.220 – 1.369)	.198	0.552(0.215 – 1.415)	.216	1.506 (0.562 – 4.033)	.415
Northwest	0.900 (0.347 – 2.333)	.828	0.407(0.156 – 1.062)	.066	0.957(0.338 – 2.706)	.933
West = 0						
HIV-Status						
HIV-Treat naïve	16.333(5.143 – 51.872)	.0001	0.123(0.037 – 0.410)	.001	2.217 (0.856 – 5.742)	.101
HIV + ARV	3.500 (1.386 – 8.835)	.008	0.150(0.045 – 0.503)	.0001	0.750 (0.261 – 2.153)	.593
HIV + ARV + Cotrimoxazole	5.444 (2.092 – 14.168)	.001	0.060(0.018 – 0.203)	.0001	0.871 (0.311 – 2.442)	.793
HIV-Negative = 0						
Food eaten (24 hours)						
Energy + protective	3.368 (1.345 – 8.434)	.010	1.059(0.460 – 2.438)	.893	0.784 (0.327 – 1.882)	.586
Energy + body-building+ protective	0.817 (0.358 – 1.861)	.630	2.765(1.173 – 6.514)	.020	0.383 (0.151 – 0.970)	.043
Energy + body-building = 0						
Occupation						
Pink-collar worker	4.000 (1.290 – 12.402)	.016	1.400(0.461 – 4.256)	.553	2.927(0.600 – 14.274)	.184
Blue-collar worker	5.179 (1.494 – 17.953)	.010	0.379(0.117 – 1.232)	.107	3.600 (0.707 – 18.338)	.123
Unemployed	1.071 (0.350 – 3.282)	.904	1.444(0.464 – 4.494)	.526	3.000 (0.606 – 14.864)	.178
White-collar = 0						
Drink consume (within 24 hours)						
Alcoholic	1.737 (0.903 – 3.343)	.098	0.836(0.444 – 1.575)	.580	1.762 (0.875 – 3.546)	.113
Non-alcoholic = 0						
Resident community (last 2 years)						
Buea	0.729 (0.256 -2.075)	.729	0.518(0.166 – 1.617)	.257	0.840 (0.232 – 3.045)	.791
Ekona	1.923 (0.548 – 6.748)	.307	0.217(0.060 – 0.782)	.020	2.700 (0.725 – 10.055)	.139
Limbe	2.306 (0.569 – 9.359)	.242	1.059(0.245 – 4.583)	.939	2.800 (0.691 – 11.344)	.149
Muea	2.769 (0.763 – 10.049)	.121	0.188(0.053 – 0.668)	.010	0.758 (0.175 – 3.278)	.711
Mutengene	0.692 (0.204 – 2.347)	.555	0.988(0.248 – 3.935)	.987	1.662 (0.416 – 6.636)	.472
Muyuka	1.538 (0.452 – 5.242)	.491	0.574(0.159 – 2.066)	.395	1.440 (0.366 – 5.669)	.602
Tiko = 0						
CD4+ T cell count (cells/mm^3)						
< 200	10.769 (2.638 – 43.959)	.001	0.072(0.018 – 0.281)	.0001	2.036 (0.623 – 6.655)	.239
201 – 350	6.374 (2.807 – 14.474)	.0001	0.128(0.046 – 0.360)	.0001	0.858 (0.362 – 2.036)	.729
351 – 450	4.615 (1.511 – 14.097)	.007	0.145(0.041 – 0.513)	.003	1.790 (0.587 – 5.464)	.306
>451 = 0						