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**Original Article**

# Fecal Microbiome Composition in Healthy Adults in Ghana

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**SUMMARY:** Recent studies have indicated an association between gut microbiome composition and various disorders, including infectious diseases. The composition of the microbiome differs among ethnicities and countries, possibly resulting in diversified interactions between host immunity and the gut microbiome. Characterization of baseline microbiome composition in healthy people is an essential step for better understanding of the biological interactions associated with individual populations. However, data on the gut/fecal microbiome have not been accumulated for individuals in West Africa. In the present study, we examined the fecal microbiome composition in healthy adults in Ghana. Toward this, 16S rRNA gene libraries were prepared using bacterial fractions derived from 55 Ghanaian adults, which were then subjected to next-generation sequencing. The fecal microbiome of the Ghanaian adults was dominated by *Firmicutes* (*Faecalibacterium, Subdoligranulum*, and *Ruminococcaceae* UCG-014), *Proteobacteria* (*Escherichia-Shigella* and *Klebsiella*), and *Bacteroidetes* (*Prevotella* 9 and *Bacteroides*), consistent with previous observations in African cohorts. Further, our analysis revealed differences in microbiome composition and a lower diversity of the fecal microbiome in the Ghanaian cohort compared with those reported in non-African countries. This is the first study to describe substantial fecal microbiome data obtained using high-throughput metagenomic tools on samples derived from a cohort in Ghana. The data may provide a valuable basis for determining the association between the fecal microbiome and progression of various diseases in West African populations.

## INTRODUCTION

The gut microbiota, which influences host immune, metabolic, and nutritional functions, is known to be involved in human health as well as in numerous other conditions (1–3). Cumulative studies have indicated an association between the gut microbiome composition and a variety of disorders, including infectious diseases (1–8). Characterization of the baseline microbiome composition in healthy individuals is essential for understanding the biological interactions between host factors and the microbiome in disease progression.

Gut microbiome composition, which is influenced by numerous factors, including dietary habits linked with socio-cultural practices and geographic provenance, varies globally (9,10), and individual populations are characterized by versatile interactions between host factors and the microbiome. For instance, an association between gut microbiome dysbiosis and disease progression in HIV-1-infected individuals has been reported (6,7). In addition, the influence of host genetics (HLA-B27 and HLA-DRB1) on gut microbial dysbiosis

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in ankylosing spondylitis and rheumatoid arthritis has been reported (11). Furthermore, the higher colorectal cancer risk and mucosal proliferation rates in African Americans than in native Africans are associated with dietary behavior (socio-economic status) influencing gut microbial composition (12). Thorough evaluation of the gut/fecal microbiome is thus important for better understanding of the pathogenesis of a variety of diseases in specific geographical regions.

Data on the gut microbiome have been accumulated primarily in Europe and the United States (US) (13,14). However, studies on the gut microbiome in sub-Saharan Africa are limited, and data on the gut/fecal microbiome have not been accumulated in West Africa. In the present study, we aimed at characterizing the fecal microbiome in healthy adults in Ghana, West Africa.

## MATERIALS AND METHODS

**Study population**: A total of 55 healthy Ghanaian adults above 18 years of age from 6 communities in the eastern region of Ghana (Akwadum, Jumapo, Koforidua, Oyoko, Suhum, and Tafo) were enrolled in our cross-sectional study. The participants were recruited during a community health screening exercise. Those who took antibiotics within 4 weeks prior to sample collection were not enrolled. This study was approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR; approval number: 096/16-1; dated May 3, 2017) and the Ethical Committee of the National Institute of Infectious Diseases (approval number: 685; dated June 16, 2016). Written informed consent for sample collection and subsequent analyses was obtained from all the participants.

**Preparation of bacterial fractions from fecal samples**: Stool samples were collected from all participants enrolled. The samples were transported to the NMIMR, processed within 24 h of sample collection, and stored at –80 °C until further processing. Bacterial pellets were prepared from the frozen fecal samples as described previously (15), with minor modifications. Briefly, 1 g of stool was washed 3 times with 3 mL of SM-plus buffer (100 mM NaCl, 50 mM Tris-HCl [pH 7.4], 8 mM $MgSO_4 \cdot 7H_2O$, 5 mM $CaCl_2 \cdot 2H_2O$, 0.01 % [w/v] gelatin) and centrifuged at 6,000 × *g* for 5 min. The pellets were then resuspended in 20 mL of SM-plus buffer and filtered through a 100-µm cell strainer (Corning, Corning, NY, USA). From this 20 mL of bacterial filtrate, 1 mL was used for DNA extraction.

**DNA extraction, amplification, and 16S rRNA gene sequencing**: DNA was extracted from the fecal sample-derived bacterial fractions as described previously (16). Subsequently, 16S rRNA gene libraries were prepared according to the 16S Metagenomic Sequencing Library Preparation Guide (Illumina, San Diego, CA, USA; Part # 15044223 Rev. B). Briefly, the hypervariable V3–V4 regions of the 16S rRNA gene were amplified using specific primers, i.e., forward, 5′-ACACGACGCTCTTCCGATCTCCTACGGGNGGCWGCAG-3′; and reverse, 5′-GACGTGTGCTCTTCCGATCTGACTACHVGGGTATCTAATCC-3′, including Illumina overhang adapter sequences (indicated by underlines) (17). Next, adapter ligation for the PCR amplicons was performed using NEB Next Multiplex Oligos for Illumina (Dual Index Primers Set 1; NEB Japan, Tokyo, Japan). Sequencing was performed at the NMIMR on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) using the MiSeq Reagent Kit v3 (600-cycle) with a 20 % PhiX (Illumina) spike-in.

**Sequence analyses**: Sequences were quality-filtered, denoised, and analyzed using the Quantitative Insights Into Microbial Ecology 2 pipeline (QIIME 2™ version 2019.4) (18). Briefly, paired-end reads were denoised into amplicon sequence variants using DADA2 (19). Taxonomy was assigned to the resulting amplicon sequence variants against the SILVA database (release 132) (20) and subsequently trimmed to the V3–V4 region of the 16S rRNA gene using a Naive Bayesian classifier (21).

**Statistical analyses**: GraphPad Prism version 7.04 and R were used for statistical analyses. Comparisons were performed using Wilcoxon rank sum test or Kruskal Wallis test with Benjamini, Krieger, and Yekutieli false discovery rate correction. A $p$ value below 0.05 was considered to indicate statistical significance.

## RESULTS

**Analysis of the fecal microbiome of healthy adults in Ghana**: A total of 55 adults from the eastern region of Ghana were enrolled in the present study. The median age of the participants was 45 years (interquartile range, 33–51), and 42 of them (76 %) were females. Stool samples were collected from all the participants, and 16S rRNA gene libraries were prepared using the bacterial fractions isolated from these samples, which were then subjected to next-generation sequencing.

The fecal microbiome composition in the healthy Ghanaian adults is shown in Fig. 1. Analysis of mean relative abundance showed that *Firmicutes* was dominant at the phylum level (> 50%), while *Ruminococcaceae* was dominant at the family level (> 33%) (Table 1). The 7 most abundant genera were *Faecalibacterium* (20%), *Subdoligranulum* (11%), *Escherichia-Shigella* (7%), *Prevotella* 9 (4%), *Ruminococcaceae* UCG-014 (3%), *Bacteroides* (3%), and *Klebsiella* (3%) (Table 1 and Fig. 2). *Faecalibacterium*, *Subdoligranulum*, and *Ruminococcaceae* UCG-014 belong to the family of *Ruminococcaceae* in the phylum of *Firmicutes*. *Escherichia-Shigella* and *Klebsiella* belong to the family of *Enterobacteriaceae* in the phylum of *Proteobacteria*. *Prevotella* 9 and *Bacteroides* are included in the phylum *Bacteroidetes*. Analysis of the Shannon indices (22) showed no significant difference in alpha diversity among the fecal microbiomes of females and males, indicating no significant impact of gender on the fecal microbiome diversity (Fig. 3).

**Comparison of fecal microbiome composition among cohorts from Ghana and non-African countries**: To compare the fecal microbiome composition of the cohort from Ghana with that of cohorts from non-African countries, we used data on the fecal microbiome collected in the US and Papua New Guinea (PNG), which were retrieved from the metagenomic analysis server MG-RAST (https://www.

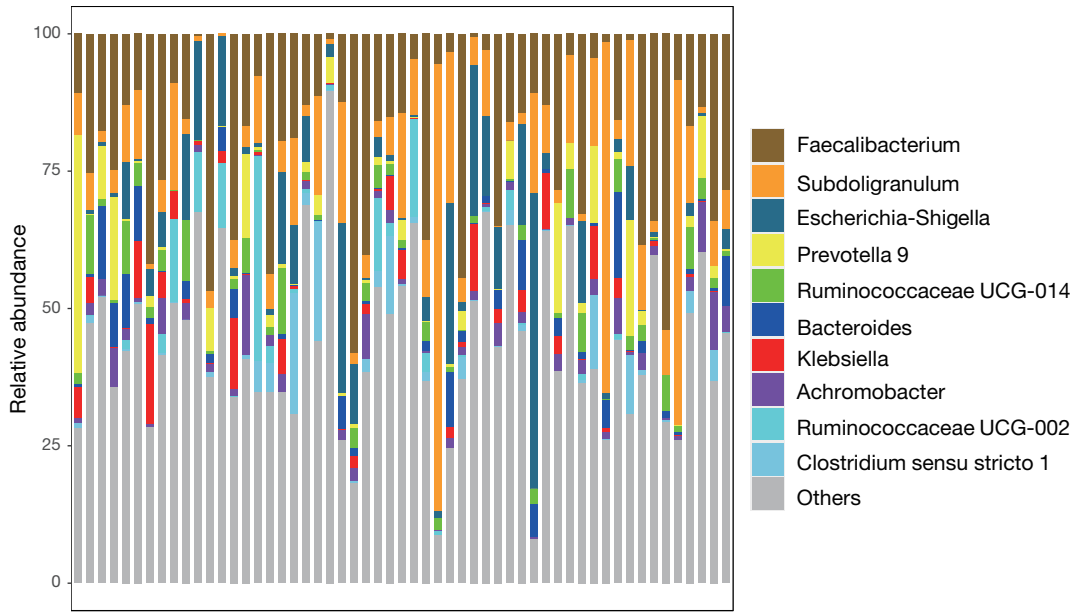


Fig. 1. Taxa bar plots showing top 10 abundant genera in fecal microbiome in the whole participants. Individual bars represent frequencies of the genera in fecal microbiome of individual healthy Ghanaians ($n$ = 55).

Table 1. Top 20 abundant genera in fecal microbiome of healthy Ghanaian adults

| Phylum | Class | Order | Family | Genus | Mean rel. abundance[1)] | PNG[2)] | US[2)] |
|---|---|---|---|---|---|---|---|
| *Firmicutes* | *Clostridia* | *Clostridiales* | *Ruminococcaceae* | *Faecalibacterium* | 20% | 2% | 6% |
| | | | | *Subdoligranulum* | 11% | 4% | 5% |
| | | | | *Ruminococcaceae UCG-014* | 3% | 0.4% | 0.1% |
| | | | | *Ruminococcaceae UCG-002* | 2% | 0.2% | 0.4% |
| | | | | *[Eubacterium] coprostanoligenes group* | 2% | 1% | 3% |
| | | | *Clostridiaceae 1* | *Clostridium sensu stricto 1* | 2% | 2% | 0.3% |
| | | | *Lachnospiraceae* | *Agathobacter* | 2% | 2% | 4% |
| | *Negativicutes* | *Selenomonadales* | *Veillonellaceae* | *Dialister* | 2% | < 0.1% | 0.2% |
| | | | | *Megamonas* | 2% | nd | < 0.1% |
| | *Bacilli* | *Lactobacillales* | *Streptococcaceae* | *Streptococcus* | 1% | 23% | 2% |
| *Proteobacteria* | *Gammaproteobacteria* | *Enterobacteriales* | *Enterobacteriaceae* | *Escherichia-Shigella* | 7% | 0.3% | < 0.1% |
| | | | | *Klebsiella* | 3% | < 0.1% | < 0.1% |
| | | | | *Enterobacteriaceae Unclassifed* | 2% | 1% | < 0.1% |
| | | *Betaproteobacteriales* | *Burkholderiaceae* | *Achromobacter* | 2% | nd | nd |
| | | *Xanthomonadales* | *Xanthomonadaceae* | *Stenotrophomonas* | 2% | < 0.1% | nd |
| *Bacteroidetes* | *Bacteroidia* | *Bacteroidales* | *Prevotellaceae* | *Prevotella 9* | 4% | 2% | 1% |
| | | | *Bacteroidaceae* | *Bacteroides* | 3% | 0.3% | 2% |
| *Actinobacteria* | *Coriobacteria* | *Coriobacteriales* | *Coriobacteriaceae* | *Senegalimassilia* | 2% | 0.3% | < 0.1% |
| | | | *Eggerthellaceae* | *Collinsella* | 1% | 6% | 5% |
| | *Actinobacteria* | *Bifidobacteriales* | *Bifidobacteriaceae* | *Bifidobacterium* | 1% | 2% | 7% |

[1)]: Mean relative genera abundance in our cohort in Ghana.
[2)]: Mean relative genera abundance in a cohort in Papua New Guinea (PNG) or United States (US) (MG-RAST accession number: mgp10381). nd, not described.

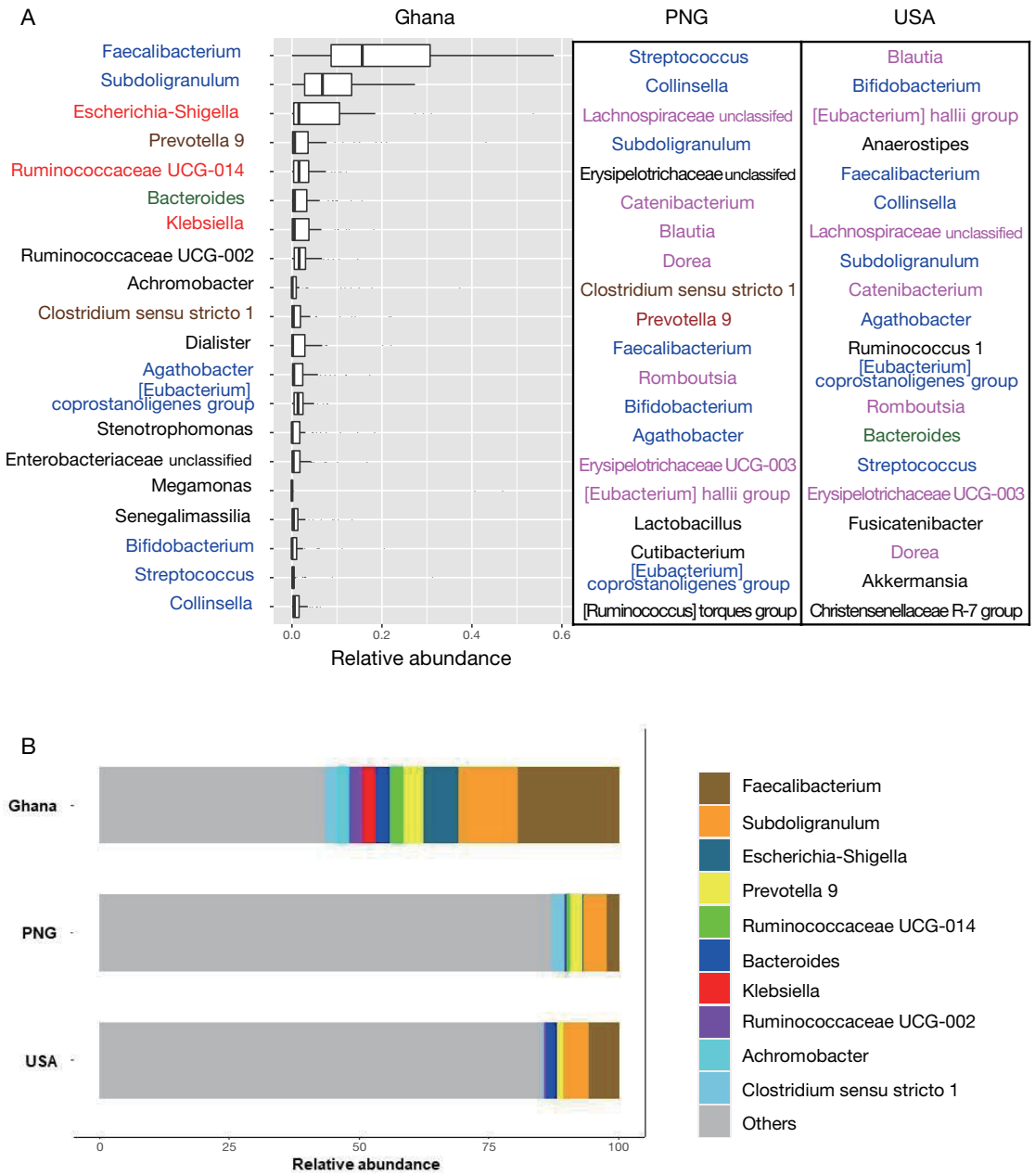


Fig. 2. Abundant genera in fecal microbiome of Ghanaians, Papua New Guineans (PNG), and US people. (A) Top 20 abundant genera found in fecal microbiome of cohorts in Ghana, PNG, and US. The top 7 abundant genera in our Ghana cohort are shown by bold. Genera included in top 20 abundant genera in all the 3 cohorts are shown by blue. Genera included in top 20 only in Ghana but not in PNG or US are shown by red. Genera included in top 20 in both PNG and US but not in Ghana are shown by pink. Genera included in top 20 in Ghana and PNG but not in US are shown by brown. The genus included in top 20 in Ghana and US but not in PNG is shown by green. (B) Comparison of relative abundance of fecal microbiome among Ghana, PNG, and US. Relative abundance of the top 10 genera in Ghana is shown.

mg-rast.org/) (accession number: mgp10381) (23).

Comparison of the 20 most abundant genera in the fecal microbiome revealed large differences in their composition among the cohorts from Ghana, PNG, and the US (Table 1 and Fig. 2). *Faecalibacterium*, *Subdoligranulum*, *Agathobacter*, *[Eubacterium] coprostanoligenes* group, *Bifidobacterium*, *Streptococcus*, and *Collinsella* were included in the top 20 genera in all the 3 cohorts from the 3 respective countries. In particular, *Faecalibacterium* and *Subdoligranulum* were relatively abundant in all the 3 cohorts. *Ruminococcaceae* UCG-014 (*Ruminococcaceae* family) as well as *Escherichia-Shigella* and *Klebsiella* (*Enterobacteriaceae* family) were included in the 7 most abundant genera in our Ghanaian cohort but were not among the top 20 in the PNG and US cohorts. Conversely, *Lachnospiraceae* (unclassified), *Catenibacterium*, *Blautia*, *Dorea*, *Romboutsia*, *Erysipelotrichaceae* UCG-003, and the *[Eubacterium] hallii* group were included in the top 20 in both the PNG and US cohorts but were not in the top 20 in our Ghanaian cohort. With regard to the remaining

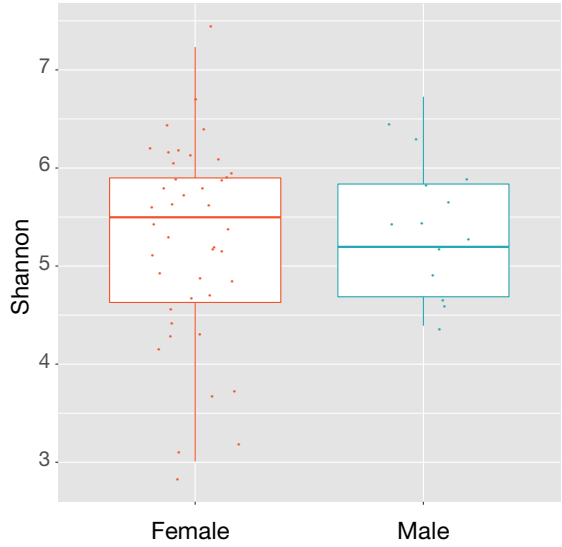


Fig. 3. Comparison of alpha diversity of fecal microbiome between females and males. Shannon diversity of fecal microbiome was compared between females and males in our Ghana cohort. No significant difference was observed by Wilcoxon rank sum test.

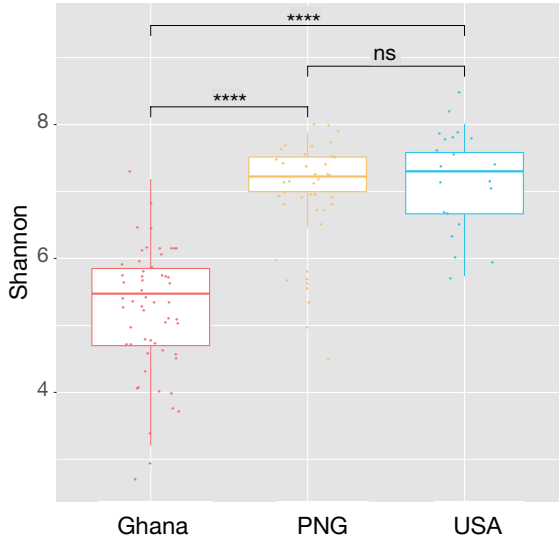


Fig. 4. Comparison of alpha diversity of fecal microbiome among Ghana, PNG, and US cohorts. Shannon diversity of fecal microbiome was compared among Ghana, PNG, and US cohorts. Our Ghana cohort showed significantly lower alpha diversity compared to PNG and US ($p < 0.001$ [****] by Kruskal Wallis test with Benjamini, Krieger and Yekutieli FDR correction).

genera assigned to the top 7 in the Ghanaian cohort, *Prevotella* 9 was found among the top 20 in the PNG cohort but not in the US cohort, whereas *Bacteroides* featured in the top 20 in the US cohort but not in the PNG cohort.

Analysis of the Shannon indices indicated that the alpha diversity of the fecal microbiome in the Ghanaian cohort was significantly lower than that in the PNG and US cohorts, although no significant differences were observed between data for the PNG and US cohorts (Fig. 4).

## DISCUSSION

The gut microbiota composition is influenced by various factors, such as dietary behavior linked with socio-cultural practices (9,10,12,24). It is imperative to contextually describe the microbiota in relation to the pathogenesis of many diseases considering these factors. It is thus important to obtain data on the microbiome in individual populations. This study presents data on fecal microbiome composition in healthy Ghanaian adults.

In general, the gut microbiome of sub-Saharan populations is dominated by genera belonging to the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (9,10,24). In particular, the dominance of *Prevotella* in African cohorts in contrast to that in non-African populations has been reported (10,24–27). This could be attributed to a high-fiber carbohydrate diet, which is also consumed by the Ghanaian population (10,24,26). Consistent with these previous observations in African cohorts, the fecal microbiome of the Ghanaian adults in this study was dominated by *Firmicutes* (*Faecalibacterium*, *Subdoligranulum*, and *Ruminococcaceae* UCG-014), *Proteobacteria* (*Escherichia-Shigella* and *Klebsiella*), and *Bacteroidetes* (*Prevotella* 9 and *Bacteroides*). Notably, *Escherichia-Shigella* and *Klebsiella* that were included in the top 7 genera in our cohort did not feature in the top 20 genera in the PNG and US cohorts. The fecal microbial signatures of our cohort suggest a pattern of dietary habit that is reflected in the gut microbiome with transition from rural to industrialized areas (28). This is consistent with the socio-economic characteristics of our cohort consisting of peri-urban communities.

Comparisons among the Ghanaian, US, and PNG cohorts revealed that the fecal microbiome composition of individuals from Ghana was largely different from that in individuals from the US and PNG, i.e., non-African countries. Remarkably, the fecal microbiome of the Ghanaians showed significantly lower alpha diversity than that reported for the US and PNG. These data could be important for the determination of an association between fecal microbiome composition and progression of various diseases in West African populations.

In summary, this is the first study to describe the fecal microbiome in Ghanaian adults using high-throughput metagenomic tools. We present valuable data on the enteric microbiome in a cohort in West Africa, where such data have not yet been systematically accumulated. Our study thus contributes to the understanding of the interactions between the host and enteric microbiota in a population-specific manner.

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**Conflict of interest** None to declare.

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