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## Rectal microbiota among HIV-uninfected, untreated HIV, and treated HIV-infected men who have sex with men (MSM) in Nigeria

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### Abstract

**Objective**—Untreated advanced HIV infection alters the gut microbiota, but it is unclear whether antiretroviral therapy (ART) reverses these changes. We compared the composition of the rectal microbiota among three groups of men who have sex with men (MSM): HIV-uninfected, untreated HIV, and ART-treated HIV-infected.

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**Contributors:** WB and MC designed the TRUST study. RN, WB, and MC conceived the analysis for the manuscript. Data collection and management was facilitated by RN, WD, JR, BM. RN conducted the data analysis with input from SMB, JR, HL, and BM. RN drafted the manuscript and SMB, JR, BM, TC, SB, HL, MC, and WB provided critical review and editing. All authors have seen and approved the paper; the corresponding author had full access to the data and had final responsibility for the decision to submit for publication.

**Design**—A cross-sectional study was conducted among 130 MSM (55 HIV-uninfected, 41 untreated HIV, 34ART-treated HIV) in Abuja, Nigeria.

**Methods**—Bacterial 16S rRNA genes were amplified from rectal swabs, sequenced and clustered into *Genera*-level operational taxonomic units. Alpha diversity was quantified using the Shannon Index and compared among groups using the Kruskal-Wallis test; Associations with other scale variables were quantified using Spearman's Rank Correlation ( $R_s$ ). The relative abundance of the top 15 taxa was compared according to HIV infection/treatment status using the Wilcoxon-Rank sum test.

**Results**—HIV treated MSM had a decrease in a commensal phylum, *Bacteroidetes* ( $p<0.01$ ). Alpha diversity was positively correlated with viral loads ( $R_s=0.32$ ,  $p<0.01$ ). Statistically significant shifts in relative abundance of rectal microbiota for the HIV treated group included a decrease in the most abundant bacteria, *Prevotella* ( $p=0.02$ ) and an increase in pathogenic bacteria, *Peptoniphilus* ( $p=0.04$ ), *Finegoldia* ( $p=0.01$ ), *Anaerococcus* ( $p=0.03$ ), and *Campylobacter* ( $p=0.03$ ) as compared to the other groups.

**Conclusions**—Untreated HIV infection does not significantly alter the rectal microbiota, whereas prior treatment is associated with a shift towards a more pathogenic pattern of microbiota. Treatment with an antibiotic, co-trimoxazole, in conjunction with ART may have contributed to this shift.

### Keywords

antiretroviral therapy; co-trimoxazole; *Prevotella*; alpha diversity; relative abundance

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### Introduction

The rectal microbiota may be important in driving mucosal immune responses that defend against infectious pathogens and regulate local inflammation during receptive anal intercourse [1]. HIV has been implicated in reducing rectal microbiota diversity, shifting the composition of commensals, enhancing microbial translocation, and disrupting local immunity [2-8]. While several studies have focused on the upper gastrointestinal tract microbiota, virtually no data are available from sub-Saharan Africa and only a few studies [9, 10] have begun to characterize the rectal microbiota.

The rectal microbiota is dominated by 5 phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Fusobacteria*, ranked in order of abundance. Early studies suggested a decline in species richness and increase in a more pathogenic phyla, *Fusobacteria*, among individuals with advanced HIV infection who are not on antiretroviral therapy (ART) [9, 10]. *Fusobacteria* harbor virulence factors that potentially disrupt local immunity [11-14], which may contribute to the pathogenesis of diseases such as inflammatory bowel disease and gastrointestinal malignancies [15-19].

The impact of ART on the microbiota is unclear. Some studies suggest that long-term ART partially restores the microbiota to a level between untreated HIV and HIV-uninfected [4, 5, 7]. Others suggest ART has little impact [6, 8] or even disrupts the microbiota [7]. Taken

together, the stage of HIV disease and the timing and duration of ART may impact the microbiota differently.

The objective of this study was to evaluate the rectal microbiota among three groups: HIV-uninfected, untreated HIV, and ART-treated HIV.

## Materials and Methods

### Study design and Population

Our cross-sectional rectal microbiota study was nested in the Abuja, Nigeria, site of the parent cohort, TRUST/RV368. Data on demographics, sexual behaviors, HIV and sexually transmitted infections (STIs) among Nigerian men who have sex with men (MSM) were previously collected [20, 21]. The prevalence of high-risk human papillomavirus (HR-HPV) was characterized in a subset of 165 participants [22]. After HPV testing, seven samples had insufficient volume and 28 failed the 16S rRNA amplification criteria of 1000 reads, leaving a final sample size of 130 for the rectal microbiota analysis.

The study was approved by the Federal Capital Territory Health Research Ethics Committee in Nigeria and the University of Maryland Baltimore Institutional Review Board.

### Laboratory Procedures

DNA was extracted and processed as previously described [10]. Sequencing targeted an amplicon size of ~469bp within the V3 and V4 hypervariable regions using primers 319F and 806R. The paired-end reads (300bp each) were sequenced on the Illumina MiSeq platform with an overlap of ~90bp, resulting in entire coverage of the amplicons. Paired-end reads were assembled using FLASH [23] with error correction. Dual barcoding information was used to index individual samples [24], and the sequences were de-multiplexed by binning sequences with the same barcodes in QIIME (version 1.8.0) [25]. QIIME quality trimming was performed. Sequences with less than 3% dissimilarity were clustered together using USEARCH (v5.2.32) [26] and *de novo* chimera detection was conducted in UCHIME v5.1 [27]. Genus-level taxonomic ranks were assigned for a representative sequence of each operational taxonomic unit using Ribosomal Database Project (RDP) Naïve Bayes Classifier v.2.2 [28], using 0.8 confidence values as the cutoff to greengenes 16S rRNA gene sequences (Aug, 2013 vers.) [29].

### Statistical Analyses

Baseline characteristics were compared between groups using Pearson's chi square test, Kruskal-Wallis test or Wilcoxon rank sum test.

The Shannon index, a measure of alpha diversity, was calculated as the negative of the sum of the relative abundance multiplied by the natural log of the relative abundance for each *genus* and further stratified by phyla. Shannon indices were compared using the Kruskal-Wallis test. Associations with CD4+ T cell counts and viral loads were quantified using Spearman's Rank Correlation ( $R_s$ ).

Shifts in composition were estimated from the relative abundance of the 15 most abundant *genera* within the top 5 phyla. The remaining *genera* were pooled within their phyla category prior to calculating a relative abundance. This resulted in relative abundances for 21 taxa categories within the 5 phyla that were compared between the three HIV infection/treatment groups. Age, rectal gonorrhea, and HR-HPV were tested separately for associations with relative abundance using  $R_s$  and Wilcoxon rank sum test in order to determine if these could be confounding any associations with HIV/ART status.

## Results

### Participant characteristics

Of the 130 participants, 55 were HIV-uninfected, 41 had untreated HIV, and 34 had ART-treated HIV. HIV-uninfected were younger than untreated and ART-treated HIV-infected men [median (interquartile range, IQR) 23 (20-28), 25 (21-27) and 27 (24-32), respectively,  $p<0.01$ ]. The HIV treated group had the lowest prevalence of rectal gonorrhea than untreated and HIV-uninfected men [11.8%, 39%, 27.8%, respectively,  $p=0.03$ ] and more HR-HPV [median (IQR) 3 (1-4), 2 (1-4), 0 (0-1), respectively,  $p<0.01$ ]. The three groups did not differ significantly on rectal chlamydia, treatment for symptomatic STIs, number of male partners with whom they engaged in receptive sex, condomless receptive sex, and concurrent partnerships. The ART-treated HIV group had higher CD4 counts ( $p=0.05$ ) and lower  $\log_{10}$  transformed viral loads ( $p<0.01$ ) than the HIV-untreated group. Years since HIV diagnosis was longer for the ART-treated vs. untreated group [median (IQR) 2 (1-4) and 1(0-2), respectively,  $p=0.02$ ].

### Alpha Diversity

Overall, there was no significant difference in the alpha diversity between the HIV-uninfected, untreated and ART-treated HIV-infected men [median (IQR) 2.68 (2.43-2.89), 2.58 (2.27-2.94), 2.68 (2.34-2.87), respectively,  $p=0.73$ ]. When the Shannon index was stratified by phyla, the treated HIV group had a decrease in species richness and evenness in the *Bacteroidetes* phylum ( $p=0.002$ ) and a non-significant increase in the *Fusobacteria* phylum ( $p=0.12$ ). For men who were HIV-infected, the alpha diversity of the *Bacteroidetes* phylum did not differ by CD4 counts ( $R_s=-0.10$ ,  $p=0.39$ ) but was positively correlated with viral load ( $R_s=0.32$ ,  $p<0.01$ ).

### Shifts in microbial composition

The HIV-uninfected and untreated HIV groups did not differ in their relative abundances of the 21 different taxa categorizations (Table 1, Figure 1). Compared to the HIV-uninfected group, the ART-treated had significant shifts in the composition of their rectal microbiota (Table 1). Within the *Firmicutes* phylum, there was an increase in the *Peptoniphilus*, *Finexgoldia* and *Anaerococcus* genera. Within the *Bacteroidetes* phylum, there was a decrease in *Prevotella*. Within the *Proteobacteria* phylum, there was an increase in *Campylobacter*. Within the *Fusobacteria* phylum, there was a non-significant increase in *Fusobacterium* ( $p=0.11$ ). These shifts were not significantly associated with age or number of HR-HPV. Participants with rectal gonorrhea had increased abundance of *Prevotella* compared to those without gonorrhea [median (IQR) 29.3 (16.2-43.1) vs. 22.7 (14.2-33.4),  $p=0.05$ ].

## Discussion

Nigerian MSM with untreated HIV did not differ in alpha diversity or composition of their rectal microbiota as compared to HIV-uninfected MSM. Treated HIV was associated with a decreased diversity of the *Bacteroidetes* phylum and compositional shifts towards more pathogenic bacteria. Lower prevalence of rectal gonorrhoea may have contributed to a lower abundance of *Prevotella* in the ART-treated group. The alpha diversity and relative abundance of *Fusobacteria* was similar across the groups.

Interestingly, participants on ART had a shift in abundance of common bacteria, which may have been confounded by antibiotic therapy. WHO guidelines recommend long-term co-trimoxazole prophylaxis for adults receiving ART since it has been shown to reduce early mortality by 58% [30-33]. The extent of the confounding is unclear since none of the treated HIV-infected men (n=34) reported using co-trimoxazole and 44% (15/34) reported either an allergy or a CD4+ T cell count of >350 cells/mm<sup>3</sup> that contraindicated them from receiving co-trimoxazole [34]. This suggests only a fraction of the men were on antibiotic prophylaxis.

Other antibiotics have been associated with reduced gut and fecal microbial diversity [35-37] but the impact of co-trimoxazole has not been well investigated. Yu et al. found co-trimoxazole was only significantly associated with an altered rectal microbiota in one of six alpha and beta diversity measures [10]. Another study among HIV-exposed uninfected infants found co-trimoxazole was associated with an increase in blood levels of lipopolysaccharide, a marker of microbial translocation and poor gut integrity, but not a direct measure of rectal microbiota changes [38].

Alternatively, HIV treatment itself may negatively impact the rectal microbiota. A decrease in species diversity after ART initiation was observed in two longitudinal studies, one in humans [7] and the other in macaques [39]. Other studies suggest that ART impacts the phylum, *Bacteroidetes* [4, 6], and in particular the genus *Prevotella*, as opposed to *Bacteroides* [5, 7]. *Bacteroidetes* may be an important commensal because it is preserved in HIV-positive elite controllers [7] and is depleted among those with inflammatory bowel disease [40]. The dominance of *Prevotella* may be attributed to sexual behavior [8] or a diet comprised mostly of simple carbohydrates [41].

There was also an increase in more Gram-positive anaerobic bacteria such as *Peptoniphilus*, *Finegoldia*, and *Anaerococcus*, all of which have been previously found to be associated with vaginal or genitourinary tract infections [42] and were reduced in the penile microbiota after circumcising HIV-uninfected men [43]. Additionally there was an increase in *Campylobacter* in the gut microbiota of ART-treated HIV-infected individuals [6]. Further studies of the influence of anaerobic species on local immunity and long term susceptibility to STIs are needed.

The rectal microbiota did not differ between untreated HIV and HIV-uninfected participants, but it is possible that HIV infection was too recent within this cohort to observe long-term shifts. Simian studies have found that SIV does not immediately impact gut microbial diversity [44, 45]. In a human study, diversity was not different for those with untreated HIV and >500 CD4+ T cell counts as compared to HIV-uninfected [8]. Furthermore, diversity

and number of taxa were not altered among men who had been living with HIV for seven to eleven years [10]. Alterations may only become apparent during later stages of HIV disease.

A few limitations should be acknowledged. First, this cross-sectional study cannot assess directionality, but is one of the first studies to describe the rectal microbiota in sub-Saharan Africa. Second, self-reported data on co-trimoxazole prophylaxis were incomplete and the component of treatment regimens contributing to observed shifts could not be determined. Third, co-infection with rectal gonorrhea may have confounded the abundance differences in one genus, *Prevotella*, but a larger sample size would be needed to account for this effect. Lastly, diet, antibiotic use and smoking status were not collected and may have confounded our main association.

In summary, there were subtle shifts in the rectal microbiota among HIV-positive individuals receiving treatment, including a decrease in diversity of the *Bacteroidetes* phylum driven primarily by a loss in *Prevotella*. There was a shift towards more pathogenic bacteria. Prospective studies are needed to evaluate the long-term consequences of alterations of the microbiota with HIV infection and its treatment.

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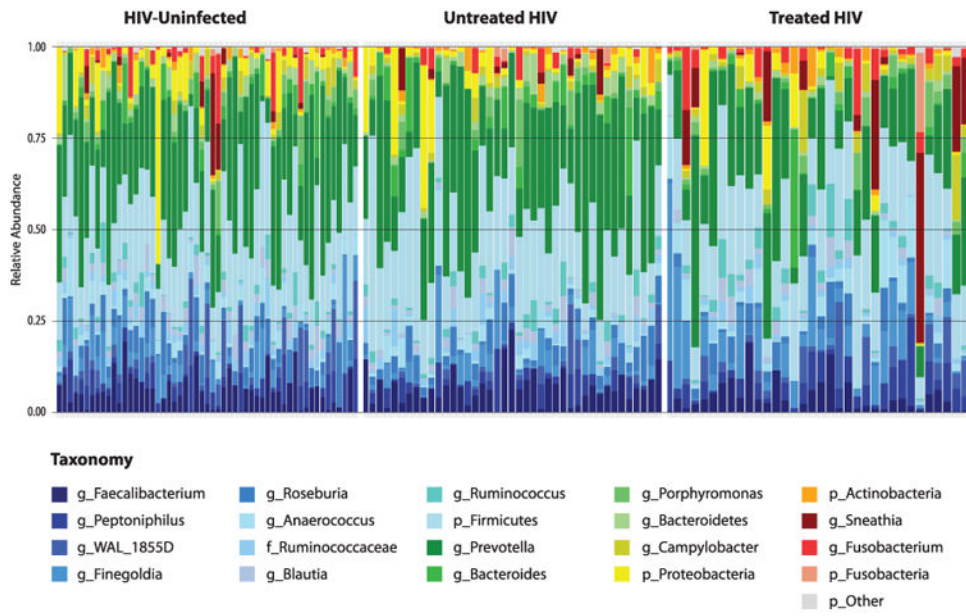
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**Figure 1.**  
 Relative Abundance of Rectal Taxa, by HIV and ART Status.  
 Color shades of phyla (*Firmicutes*: blue; *Bacteroidetes*: green; *Proteobacteria*: yellow; *Actinobacteria*: orange; *Fusobacteria*: red; other: gray)

**Table 1**  
**Relative abundance of most common rectal genera within 5 phyla, stratified by HIV and ART status**

|                        | HIV-Uninfected          | Untreated HIV       | HIV- vs. Untreated | Treated HIV             | HIV- vs. Treated | Abundance shift |
|------------------------|-------------------------|---------------------|--------------------|-------------------------|------------------|-----------------|
|                        | Median % (IQR) n=55     | Median % (IQR) n=41 | P*                 | Median % (IQR) n=34     | P*               |                 |
| <i>Firmicutes</i>      |                         |                     |                    |                         |                  |                 |
| g_faecalibacterium     | 5.2 (2.3-8.1)           | 6.6 (4.1-9.0)       | 0.15               | 3.4 (1.1-6.1)           | 0.07             |                 |
| <b>g_peptoniphilus</b> | <b>2.1 (0.6-6.7)</b>    | 2.1 (0.5-5.4)       | 0.54               | <b>4.8 (1.3-7.0)</b>    | <b>0.04</b>      | ↑               |
| g_wal_1855D            | 0.7 (0.0-5.0)           | 0.0 (0.0-1.4)       | 0.05               | 1.3 (0.0-4.8)           | 0.90             |                 |
| <b>g_finegoldia</b>    | <b>2.6 (0.7-5.9)</b>    | 1.7 (0.9-5.8)       | 0.91               | <b>5.1 (1.9-14.1)</b>   | <b>0.01</b>      | ↑               |
| g_roseburia            | 3.5 (1.0-6.9)           | 3.0 (1.7-6.6)       | 0.86               | 1.7 (0.8-3.7)           | 0.09             |                 |
| <b>g_aerococcus</b>    | <b>1.7 (0.6-4.5)</b>    | 1.5 (0.4-2.9)       | 0.47               | <b>3.0 (1.3-9.5)</b>    | <b>0.03</b>      | ↑               |
| f_ruminococcaceae      | 1.8 (1.0-2.9)           | 1.5 (0.8-3.3)       | 0.90               | 1.2 (0.7-2.1)           | 0.07             |                 |
| g_blaugia              | 2.2 (1.0-3.7)           | 1.6 (0.9-3.0)       | 0.24               | 1.1 (0.5-3.1)           | 0.08             |                 |
| g_ruminococcus         | 1.2 (0.5-2.7)           | 1.4 (0.4-3.4)       | 0.62               | 2.1 (0.6-3.8)           | 0.38             |                 |
| g_other                | 20.6 (16.5-26.6)        | 21.9 (15.7-25.6)    | 0.92               | 19.6 (12.7-25.7)        | 0.26             |                 |
| <i>Bacteroidetes</i>   |                         |                     |                    |                         |                  |                 |
| <b>g_prevotella</b>    | <b>26.4 (15.6-38.2)</b> | 27.8 (16.2-39.7)    | 0.53               | <b>18.7 (10.8-27.2)</b> | <b>0.02</b>      | ↓               |
| g_bacteroides          | 1.2 (0.4-3.0)           | 2.1 (0.3-4.9)       | 0.44               | 0.5 (0.3-2.4)           | 0.24             |                 |
| g_porphyromonas        | 0.5 (0.0-2.2)           | 0.3 (0.1-2.1)       | 0.82               | 0.8 (0.2-3.4)           | 0.27             |                 |
| <b>g_other</b>         | <b>1.3 (0.5-2.9)</b>    | 2.0 (0.5-2.9)       | 0.78               | <b>0.7 (0.2-1.4)</b>    | <b>&lt;0.01</b>  | ↓               |
| <i>Proteobacteria</i>  |                         |                     |                    |                         |                  |                 |
| <b>g_campylobacter</b> | <b>0.5 (0.0-3.2)</b>    | 0.6 (0.0-1.4)       | 0.53               | <b>1.7 (0.4-4.1)</b>    | <b>0.03</b>      | ↑               |
| <b>g_other</b>         | <b>2.0 (0.7-4.7)</b>    | 1.7 (0.8-4.5)       | 0.88               | <b>0.5 (0.3-1.9)</b>    | <b>0.01</b>      | ↓               |
| <i>Actinobacteria</i>  |                         |                     |                    |                         |                  |                 |
| g_all                  | 0.4 (0.1-0.9)           | 0.4 (0.2-1.2)       | 0.71               | 0.6 (0.2-1.3)           | 0.21             |                 |
| <i>Fusobacteria</i>    |                         |                     |                    |                         |                  |                 |
| g_sneathia             | 0.0 (0.00-0.2)          | 0.0 (0.0-0.0)       | 0.32               | 0.0 (0.0-4.2)           | 0.32             |                 |
| g_fusobacterium        | 0.2 (0.00-2.2)          | 0.1 (0.0-1.6)       | 0.44               | 1.2 (0.0-3.6)           | 0.11             | ↑               |
| g_other                | 0.0 (0.00-0.0)          | 0.0 (0.0-0.0)       | 0.29               | 0.0 (0.0-0.0)           | 0.49             |                 |
| Other phyla            |                         |                     |                    |                         |                  |                 |
| g_all                  | <b>0.2 (0.0-0.6)</b>    | 0.1 (0.0-0.3)       |                    | <b>0.1 (0.0-0.3)</b>    | <b>0.02</b>      | ↓               |

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; g<sub>-</sub>, Genus, f<sub>-</sub>, Family

\* Wilcoxon rank sum test

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