

Anal Microbial Patterns and Oncogenic Human Papillomavirus in a Pilot Study of Nigerian Men Who Have Sex with Men at Risk for or Living with HIV

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Abstract

To cluster anal microbiota and define microbial patterns associated with biological, clinical, and behavioral correlates among Nigerian men who have sex with men (MSM) living with or at risk for HIV. In this cross-sectional pilot study, the 15 most abundant 16S taxa in the anal microbiota of 113 MSM underwent unsupervised K-means clustering and z-score comparisons to define similarities and dissimilarities among 4 microbiota taxonomic profiles. Distributions of oncogenic HPV (high-risk human papillomavirus [HR-HPV]), concurrent HIV, antiretroviral therapy (ART), and other clinical and behavioral data were evaluated using Fisher's exact and Kruskal–Wallis tests to determine biological signatures of cluster membership. *Prevotella* was consistently represented in each cluster, but the average composition ranged from 14% to 44%. Cluster 2 was enriched with a member of the *Fusobacteria* phylum, *Sneathia* (29%). More participants of cluster 2 were HIV infected and taking ART (83%, 5/6), were virally suppressed (80%, 4/5), had HPV-16 (66.7%, 4/6), and reported no vaginal sex partners (83%, 5/6). HPV-35, a highly prevalent oncogenic HPV in Nigeria, was observed in all clusters except cluster 2 (0%, 0/6). Other covariates were similar across clusters (all $p > .05$). K-means unsupervised clustering, a canonical pattern recognition method, generalized the microbial community composition and structure while accounting for among sample variability. Further studies are needed to evaluate whether an anal microbial community enriched with members of the *Fusobacteria* phylum is associated with HIV-infected MSM who are virally suppressed and have a concurrent HPV-16.

Keywords: K-means clustering, MSM, *Sneathia*, *Fusobacterium*, HPV-16

Introduction

COMMENSAL BACTERIA AND their metabolites are important symbiotic partners in the host's immune response to local pathogenic viruses.¹ Shifts in the microbial composition have been described for men who have sex with men (MSM) living with HIV as compared with HIV-uninfected MSM.^{2–4}

During HIV infection as compared with healthy controls, there is a reduction in commensal genera, such as *Faecalibacterium*, *Roseburia*, *Blautia*, and *Ruminococcus* and an enrichment in other taxa, such as *Prevotella*, *Fusobacteria*, *Anaerococcus*, *Porphyromonas*, and *Campylobacter* in the lower gastrointestinal tract and rectum.^{2–7} Additional studies suggest that these compositional changes are influenced

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by anal sex, antiretroviral therapy (ART), and dietary patterns.^{2, 8–11} Whether these compositional changes are an indication of a less “healthy” microbiome and/or a loss of local immunity has not been well established.

One way to determine the downstream effect of an altered microbial composition is to evaluate its impact on the natural history of a local viral infection. Human papillomavirus (HPV) is a pervasive infection of the anogenital tract that undergoes states of clearance, regression, and/or recurrence depending on local immunity.^{12,13} It remains unclear whether an altered microbiota favors persistence or clearance of oncogenic HPV (high-risk human papillomavirus [HR-HPV]), and/or contributes to the higher burden of anal cancer, most attributed to HPV-16,¹⁴ among HIV-infected MSM on ART.

There has been limited study of the relationship between HIV-altered anal mucosa and the pathogenesis of HR-HPV. One small study among 42 HIV-infected MSM evaluated the association of bacterial taxa from biopsied samples with normal low-grade squamous intraepithelial lesions and high-grade squamous intraepithelial lesions (HSIL), finding that minority species including *Peptostreptococcus*, *Campylobacter*, and *Gardnerella* were associated with anal precancer. However, there was no consistency in the taxa across stages of precancer.¹⁵ Like most rectal microbial studies, Serrano-Villar *et al.* compared the enrichment or depletion of individual taxa.^{2,7–9,15,16}

Individual taxa do not necessarily represent the ecologic niche that embodies cooperative and competitive interactions among multiple bacteria.^{17–19} Data from vaginal microbiome studies suggest that community state types, derived from unsupervised hierarchical clustering, characterize the microbial patterns and provide insight on local immunity.^{20,21} For HPV pathogenesis, a characteristic cluster or a type of microbial community would be expected to occur at all stages of anal dysplasia if part of the causal pathway.

The objective of this study was to define anal microbial patterns for MSM and their correlations with HR-HPV, HIV, and other biological and behavioral characteristics. These correlates were explored as indicators of the anal microbial communities much like Nugent score and pH are used as indicators of the vaginal microbiota.

Materials and Methods

Study design and population

Sampling and analytic approaches for this cross-sectional study of the anal mucosa microbiota among Nigerian MSM have been previously described.^{4,22} In brief, the prevalence of HR-HPV and the composition of the mucosal microbiota were characterized from a subset of archived baseline anal swab samples from the Abuja site of the parent cohort, TRUST/RV368 ($n = 130$). Selection criteria included completion of HIV testing at baseline, an available rectal swab sample, and among the first 165 enrolled participants. Data on demographics, sexual behaviors, prevalent and incident HIV, and sexually transmitted infections (STIs) were available from the parent cohort.^{23,24}

All participants provided written informed consent. 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

In the parent cohort, whole blood was tested for HIV using rapid test kits (Abbott Determine HIV-1/2, Chembio HIV-1/2 Stat Pak, and Trinity biotech Uni-Gold HIV test for discordant results) as outlined by the parallel testing algorithm for high-risk individuals in Nigeria.²⁵ HIV-uninfected individuals were tested for HIV at every quarterly visit. If a participant was HIV positive, HIV RNA was quantified using the COBAS TaqMan HIV-1 Test (Roche Molecular Diagnostics, Pleasanton, CA) and CD4 count was estimated using the Partec CyFlow Counter. Rectal *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) were detected using the Aptima Combo 2 CT/NG Assay (Hologic, San Diego, CA) from anal swabs that were inserted by a doctor ~2” into the anorectum and rotated for sample collection.

For our cross-sectional study, 37 high- and low-risk HPV genotypes were detected using the Linear Array HPV Genotyping Test (Roche Molecular Diagnostics, Indianapolis, IN). High-risk HPV included 13 type-specific infections: 16, 18, 31, 35, 39, 45, 51, 52, 56, 58, 59, and 68.²² HPV-52 was considered positive if there was no cross-reaction with HPV-33, HPV-35, or HPV-58. HPV-52 status in men with HPV-33, HPV-35, or HPV-58 is, therefore, unknown. The remaining 24 genotypes detected were considered low risk. Quality control samples were included during DNA extraction, polymerase chain reaction (PCR) amplification, and HPV genotype detection steps. Any sample missing human β -globin, an internal control target, was excluded from the analysis because of insufficient cellular material to detect HPV.

Anal microbiota data were generated by sequencing the V3 and V4 hypervariable regions of the 16S ribosomal RNA (rRNA) on the Illumina MiSeq platform. Negative and positive controls were included in both extraction and PCR steps. In addition, negative controls without a template were included for each barcoded primer pair. To maintain consistency, 50 ng of DNA and equimolar amounts of PCR amplicons were used for each sample. QIIME, USEARCH, and UCHIME were used for further sequence processing steps.^{26–28} Quality control of the sequence reads included review of the sequence tag (barcode) and 16S rRNA gene primer, a minimal length of 200 bp, and a 60% or more match to previously determined 16S rRNA. Sequences were clustered into operational taxonomic units (OTUs) with $\geq 97\%$ similarity and classified to the taxonomic level of *Genera* using the RDP classifier in QIIME and the Greengenes database (gg13.8).²⁹

Microbial community analyses

First, OTUs with at least 10 or more reads in $>5\%$ ($n = 7$) of samples were retained for clustering analysis, reducing the total OTUs from 213 to 67. Second, samples with at least 500 reads were included in the analysis, reducing the sample size from 130 to 113. Third, the relative abundance of the 15 most abundant genera⁴ was clustered into 4 characteristic signatures using K-means clustering. These genera included *Prevotella*, *Faecalibacterium*, *Peptoniphilus*, *Roseburia*, *WAL_1855D*, *Fingoldia*, *Sneathia*, *Bacteroides*, *Anaerococcus*, *Porphyromonas*, *Ruminococcaceae*-unclassified, *Fusobacterium*, *Campylobacter*, *Blautia*, and *Ruminococcus*.

TABLE 1. CENTROID MEANS OF 15 MOST ABUNDANT GENERA AFTER K-MEANS CLUSTERING (N=113)

Phylum	Genera	Cluster 1 N=58	Cluster 2 N=6	Cluster 3 N=38	Cluster 4 N=11
Bacteroidetes	<i>Prevotella</i>	20.0	24.0	44.3	13.8
Firmicutes	<i>Faecalibacterium</i>	7.1	0.9	6.4	3.4
Firmicutes	<i>Peptoniphilus</i>	4.2	3.0	2.3	8.5
Firmicutes	<i>Roseburia</i>	4.4	0.9	5.1	2.9
Firmicutes	<i>WAL_1855D</i>	5.5	2.7	1.3	1.4
Firmicutes	<i>Finegoldia</i>	3.0	4.0	3.5	21.6
Fusobacteria	<i>Sneathia</i>	1.2	28.9	0.8	0.2
Bacteroidetes	<i>Bacteroides</i>	4.0	1.4	1.4	2.8
Firmicutes	<i>Anaerococcus</i>	3.0	2.0	1.5	10.7
Bacteroidetes	<i>Porphyromonas</i>	3.0	1.8	0.9	0.9
Firmicutes	<i>Ruminococcaceae-unclassified</i>	2.5	0.6	2.0	1.2
Fusobacteria	<i>Fusobacterium</i>	2.7	4.3	1.1	1.5
Proteobacteria	<i>Campylobacter</i>	2.6	4.5	1.1	0.7
Firmicutes	<i>Blautia</i>	2.7	0.5	2.5	2.0
Firmicutes	<i>Ruminococcus</i>	3.0	0.4	1.5	3.1

K-means clustering randomly assigns K number of centroid means and then calculates the Euclidian distance between the individual data point and the closest centroid. Clusters begin to form, comprising the centroid and the closest data points. After initial clustering, a new centroid mean is calculated and the Euclidian distances for the individuals are recalculated to see whether they remain closest to the centroid mean or belong to another centroid. Individual data points may shift into a new cluster. This process of recalculating the centroid and the distances is repeated until all data points are clustered with the smallest distances to the K number of centroid means. K-means clustering analysis was performed using SPSS software (version 23; SPSS, Inc., Chicago, IL).

To evaluate the compositional differences across clusters, the centroid means of each genera were converted into z-scores. Z-scores were estimated as the difference between the centroid mean of the genera in the cluster as compared with the average of the centroids outside of that cluster, standardized by the joint standard error $(\text{mean}_i - \text{mean}_j) / \sqrt{(\text{sem}_i^2 + \text{sem}_j^2)}$. A correlation matrix heat map was generated using RStudio (v. 1.0.153) with the ggplot2 package (v.2.1.0). Centroid means that were similar to one another were shaded in white. Centroid means in the clusters that were above the population average were shaded in red and those below the population average were shaded in blue. Z-scores more than two standard errors from the mean of zero were considered statistically significant ($p < .05$).

The primary exposure variable was microbial cluster. Concurrency was defined as having multiple sexual partnerships at the same time, further categorized as none, men only, or both men and women. Viral suppression was defined as those with <1,000 copies/mL of HIV RNA. Fisher's exact and Kruskal-Wallis tests were used to explore distributions of baseline demographics, clinical characteristics, sexual risk behavior, oncogenic HPV, prevalent and incident STIs, and antibiotic use by microbial cluster. Associations with $p < .05$ were considered statistically significant and those with $p < .10$ were suggestive of a trend. A bivariate analytic approach was used, as opposed to multivariable analysis, because of a limited sample size. Analyses were performed using Stata Statistical Software: Release 13 (StataCorp LP, College Station, TX).

Results

Cluster composition

Patterns of bacterial frequency from a total of 113 participants were distributed into 4 clusters based on the 15 most abundant genera in the population (Table 1). The composition of the cluster included the centroid mean of each taxon for all participants categorized within that cluster (Table 1). *Prevotella* was consistently represented in each cluster, ranging from 14% to 44%. Cluster 1 ($n=58$) had 20% *Prevotella*, 7% *Faecalibacterium*, and 6% *WAL_1855D*. Cluster 2 ($n=6$) had 24% *Prevotella* and 29% *Sneathia*. Cluster 3 ($n=38$) had 44% *Prevotella*, 6% *Faecalibacterium*, and 5% *Roseburia*. Cluster 4 ($n=11$) had 14% *Prevotella*, 9% *Peptoniphilus*, 22% *Finegoldia*, and 11% *Anaerococcus*. All remaining taxa within the clusters had centroid means <5%.

When comparing the z-scores for each taxon within the clusters, cluster 1 had a significantly higher abundance of multiple genera than the other clusters (*Faecalibacterium*, *WAL_1855D*, *Bacteroides*, *Porphyromonas*, *Ruminococcaceae-unclassified*, *Campylobacter*, and *Ruminococcus*) and a lower abundance of *Prevotella* (Fig. 1). Cluster 2 had a high abundance of a single genus, *Sneathia*. Cluster 3 had one of the strongest differences in abundance, indicated by a z-score >10, of a single genus, *Prevotella*. Cluster 4 had a high abundance of a few genera (*Peptoniphilus*, *Finegoldia*, and *Anaerococcus*) and lower abundance of *Prevotella*.

Participant characteristics by microbiota cluster

The majority of participants were in their 20s (65%), not married (84%), self-identified as bisexual (70%), and had their anal sexual debut within the past 9 years (57%) (Supplementary Table S1). Age, marital status, sexual orientation, years since first anal sex, number of receptive partnerships, condomless sex, and having concurrent partnerships were not significantly associated with clustering of anal microbiota (all $p > .10$) (Table 2 and Supplementary Table S2). Those with a cluster 2 microbiota trended toward self-reporting not having any vaginal sex partners (83% compared with 32%–50% in the other clusters) (Fig. 2).

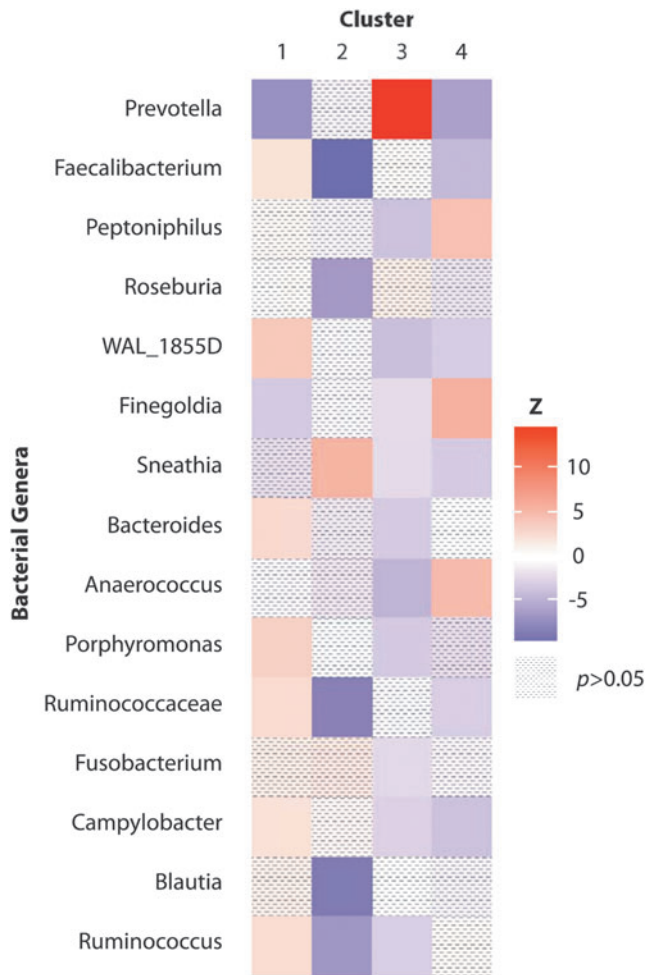


FIG. 1. Z-score distributions of 15 centroid means by cluster. *Note:* Z-scores represent the deviation in the centroid mean for each cluster-specific genera relative to the population centroid mean, standardized by the joint standard error. Genera with values between -2 and $+2$ were no different from the population average ($p > .05$). Centroid means in the clusters that were above the population average are shaded in red and those below the population average are shaded in blue.

Clinical characteristics by microbiota cluster

Approximately half of the participants were HIV uninfected (47%), a third had untreated HIV (31%), and one-fifth were taking ART for their HIV infections (22%) (Supplementary Table S1). HIV and ART status differed by clusters with cluster 2 having the highest proportion of MSM taking ART (83%) with viral suppression (80%). Cluster 4 had the second highest proportion of MSM taking ART (46%) with viral suppression (83%). The remaining clusters had higher proportions of MSM with untreated HIV (35%–37%) and less viral suppression (24%–28%) (Table 2 and Fig. 2).

Nearly two-thirds of the participants had at least one HR-HPV (62%) (Supplementary Table S1). Cluster 2 had the highest proportion of MSM with prevalent HPV-16 (67% as compared with 8%–20% in the other clusters) (Fig. 2). HPV-35 did not co-occur with HPV-16 but was prevalent in all the

remaining clusters (0% as compared with 8%–28%) (Fig. 2). Having any type of HPV infection, multiple HPV infections, other high-risk HPVs or HPV genotypes associated with warts did not significantly differ by cluster membership (Supplementary Table S2 and Table 2).

Self-reported years since HIV diagnosis did not differ by cluster membership (Supplementary Table S2). Although only a portion of the HIV-infected participants entering the cohort study knew their status and could report how many years it had been since their first HIV-positive test [59% (19/32) in cluster 1, 100% (5/5) in cluster 2, 35% (6/17) in cluster 3, and 83% (5/6) in cluster 4]. For all those who knew their status from a healthcare provider before study entry ($n = 35$), 88% self-reported <5 years and 11% reported 6–8 years. CD4 T cell counts, prevalent rectal gonorrhea or chlamydia, antibiotics, incident HIV, incident rectal gonorrhea, or chlamydia did not differ by anal microbial cluster (Table 2 and Supplementary Table S2).

Discussion

This study describes the anal microbiota as clustered communities based on their taxonomic profile similarities and explores STIs and specific sexual risk practices as potential correlates among Nigerian MSM living with and without HIV. Microbial communities among MSM were enriched with *Prevotella*, consistent with prior studies,^{2,4,8,9} but interestingly had varying levels of abundance. Much of the prior work reported on differences in individual taxa. Our study evaluated whether these same taxa were shifted in our microbial communities to infer compositional similarities.

Cluster 3 was observed for a number of HIV-uninfected MSM and consistent with prior studies it comprised many commensal species (*Faecalibacterium*, *Roseburia*, and *Blautia*) and fewer minority taxa (*Fusobacterium*, *Anaerococcus*, *Porphyromonas*, and *Campylobacter*).^{5–7} Men who do not engage in receptive sex have a predominance of *Bacteroides*, rather than *Prevotella*, as was observed in cluster 1 where the highest proportion of MSM reported vaginal sex partners.^{2,8,9} Cluster 2 comprised HIV-infected MSM on ART who were virally suppressed and less likely to have vaginal sex partners. Interestingly, this cluster had a reduction in commensal taxa (*Faecalibacterium*, *Roseburia*, *Blautia*, and *Ruminococcus*) and a high abundance of minority taxa (a member of the *Fusobacteria* phyla, *Sneathia*).

Our exploratory analysis with oncogenic HPV suggested that a higher proportion of MSM with prevalent HPV-16 were associated with a cluster enriched with *Sneathia*. *Sneathia* is from the family of *Fusobacteriaceae* and was formerly grouped with *Leptotrichia*.³⁰ *Sneathia* is not a highly abundant bacteria, but is becoming recognized as part of the genitourinary tract in both men and women and has been associated with a variety of clinical conditions such as bacterial vaginosis,^{31,32} urethritis,³³ preterm labor,³⁴ HIV acquisition,³⁵ and cervical cancer.^{36,37} *Leptotrichia amnionii*, *Sneathia amnii*, and *Sneathia sanguinegens* are among the most studied *Sneathia* bacterial group members. *Sneathia* and members of the phylum, *Fusobacteria*, have virulence factors, such as adhesion molecule FadA, that enable them to colonize the mucosa, invade epithelial cells, and alter local

TABLE 2. BASELINE DEMOGRAPHIC, BEHAVIORAL, AND CLINICAL CHARACTERISTICS BY CLUSTER MEMBERSHIP

	Cluster 1 N=58 n (%)	Cluster 2 N=6 n (%)	Cluster 3 N=38 n (%)	Cluster 4 N=11 n (%)	p*
Age (years)					.85
16–19	9 (15.5)	0 (0.0)	8 (21.1)	1 (9.1)	
20–29	37 (63.8)	5 (83.3)	22 (57.9)	9 (81.8)	
30+	12 (20.7)	1 (16.7)	8 (21.1)	1 (9.1)	
No. of receptive partnerships ^a					.80
None	12 (20.7)	3 (50.0)	8 (22.9)	3 (27.3)	
1–3	20 (34.5)	1 (16.7)	14 (40.0)	4 (36.4)	
4+	26 (44.8)	2 (33.3)	13 (37.1)	4 (36.4)	
Condomless receptive sex					.77
No	21 (45.7)	2 (66.7)	14 (48.3)	3 (33.3)	
Yes	25 (54.4)	1 (33.3)	15 (51.7)	6 (66.7)	
Any vaginal sex partners					.07
No	17 (32.1)	5 (83.3)	12 (33.3)	5 (50.0)	
Yes	36 (67.9)	1 (16.7)	24 (66.7)	5 (50.0)	
HIV and ART status					<.01
HIV negative	26 (44.8)	1 (16.7)	21 (55.3)	5 (45.5)	
Untreated HIV positive	20 (34.5)	0 (0.0)	14 (36.8)	1 (9.1)	
Treated HIV positive	12 (20.7)	5 (83.3)	3 (7.9)	5 (45.5)	
HIV RNA viral load (copies/mL) ^b					<.01
<1,000	9 (28.1)	4 (80.0)	4 (23.5)	5 (83.3)	
≥1,000	23 (71.9)	1 (20.0)	13 (76.5)	1 (16.7)	
High-risk HPV					
16	11 (20.4)	4 (66.7)	3 (8.3)	1 (10.0)	.01
18	11 (20.4)	0 (0.0)	7 (19.4)	1 (10.0)	.79
31	4 (7.4)	1 (16.7)	2 (5.6)	1 (10.0)	.58
33	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	—
35	15 (27.8)	0 (0.0)	3 (8.3)	2 (20.0)	.07
39	11 (20.4)	1 (16.7)	4 (11.1)	1 (10.0)	.69
45	12 (22.2)	1 (16.7)	7 (19.4)	1 (10.0)	.90
51	10 (18.5)	0 (0.0)	5 (13.9)	2 (20.0)	.79
52	2 (3.7)	0 (0.0)	3 (8.3)	2 (20.0)	.19
56	6 (11.1)	0 (0.0)	1 (2.8)	0 (0.0)	.43
58	14 (25.9)	2 (33.3)	6 (16.7)	1 (10.0)	.52
59	7 (13.0)	0 (0.0)	7 (19.4)	0 (0.0)	.44
68	11 (20.4)	0 (0.0)	3 (8.3)	2 (20.0)	.30
Any	36 (66.7)	4 (66.7)	20 (55.6)	6 (60.0)	.75
Prevalent rectal STIs					
NG	14 (24.6)	1 (16.7)	15 (39.5)	2 (18.2)	.35
CT	10 (17.2)	0 (0.0)	6 (15.8)	1 (9.1)	.84
Incident STIs					
HIV ^c	4 (15.4)	0 (0.0)	3 (14.3)	0 (0.0)	1.00
Rectal NG ^d	14 (31.8)	2 (50.0)	8 (29.6)	4 (50.0)	.64
Rectal CT ^d	10 (24.4)	2 (50.0)	5 (19.2)	2 (28.6)	.56
Antibiotics					
Prior treatment ^e	10 (17.2)	1 (16.7)	7 (18.4)	2 (18.2)	1.00
Current cotrimoxazole	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	—

^aIn past year.^bAmong HIV-infected only.^cAmong HIV-uninfected at baseline only.^dNew STI laboratory diagnosis after a negative diagnosis.^eBy healthcare provider in past year for symptomatic STIs.*Fisher's exact test, *bolded* are significant ($p < .05$).ART, antiretroviral therapy; CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; HPV, human papillomavirus; STI, sexually transmitted infection.

immunity.^{30,38–40} Further species-level or even strain-level taxonomic resolution will further assist our understanding of its pathogenic attributes and potentials.

Data from studies of the vaginal microbiome suggest that *Sneathia* and other anaerobic bacteria may interact with the

local epithelium in a way that allows oncogenic HPV to persist. In a study of HPV-discordant monozygotic twins, *Fusobacteria* and *Sneathia* species were more abundant in the HPV-positive twins than the HPV-negative twins.⁴¹ An anaerobic vaginal microbial community has been associated

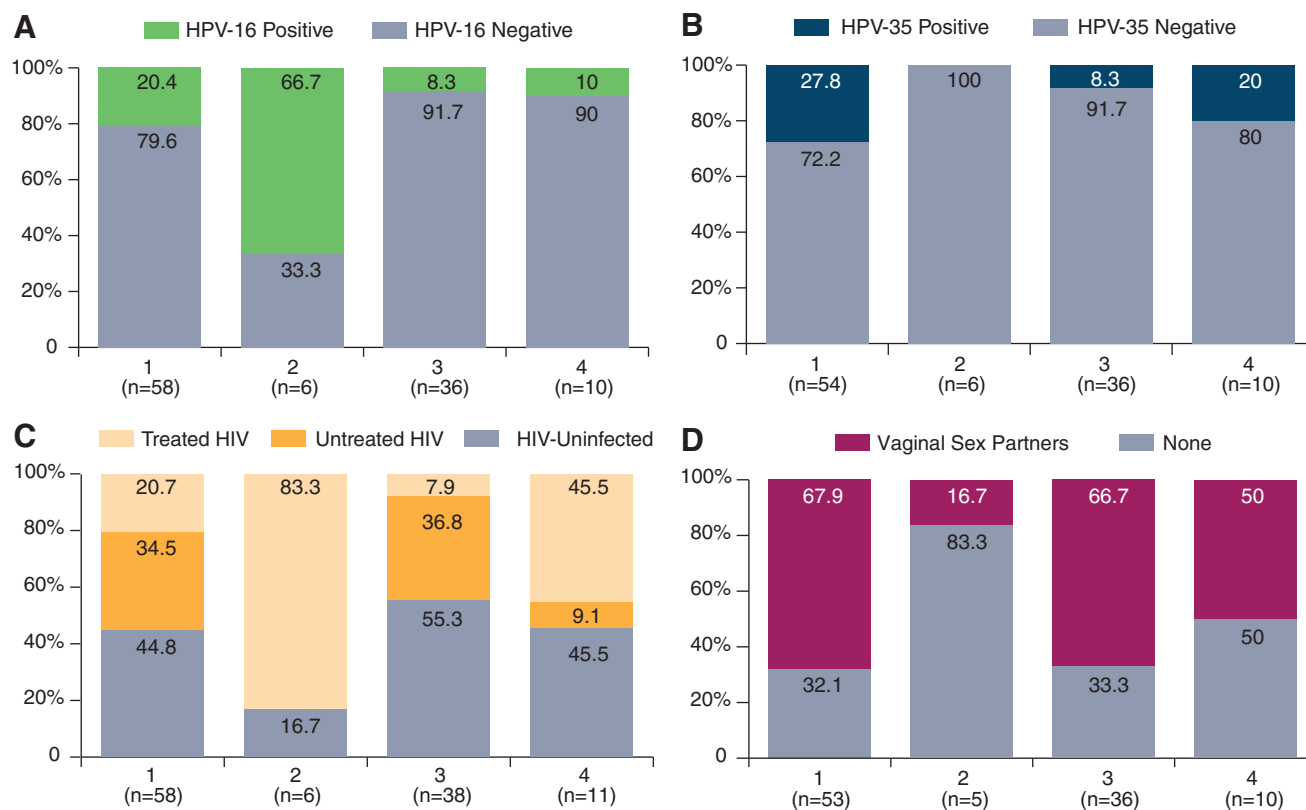


FIG. 2. Prevalence of HPV, ART, and HIV strata, and vaginal sex partners. *Note:* (A) Proportion with prevalent HPV-16. (B) Proportion with prevalent HPV-35. (C) Proportion with treated, untreated, and HIV uninfected. (D) Proportion self-reporting vaginal sex partners. ART, antiretroviral therapy; HPV, human papillomavirus.

with persistent HPV infection,⁴² a critical risk factor for progression to HSIL, leading to cancer. In cross-sectional analyses among women, *Sneathia* has been associated with precancerous lesions,^{37,43} and *Sneathia* and *Fusobacterium* spp. have been associated with cervical cancer.^{36,37} Although all HR-HPVs are similar genetically, HPV-16 confers the highest carcinogenic risk and is the cause of >90% of anal cancers.^{14,44,45} To see HPV-16 emerge with a *Sneathia*-enriched cluster independent of the other HR-HPVs parallels earlier studies that there may be an interesting immunologic interaction. However, this observation arose from a very small sample size and should be interpreted with caution. Furthermore, the vaginal microbial community has a different function and influence of hormones, and thus may not be analogous to the anal microbial community.

Suppressive ART appeared to be associated with microbial clusters that had a higher expression of anaerobic microbiota not seen in clusters 1 and 3 where there was more untreated HIV or no HIV. Although our findings are limited by sample size, suppressive ART could potentially impact the composition of the microbial community. Prior studies have suggested that ART impacts the anal microbiota^{4,10,46–48} and is unable to restore the microbial community to a level comparable with HIV uninfected.^{6,7,16,49} Oral zidovudine was observed to have antibacterial effects but this study did not look at alterations in the microbial community.⁵⁰ When tenofovir was applied locally as a rectal gel in a phase I randomized clinical trial, the Combination HIV Antiretroviral Rectal Microbicide (CHARM)-01 study, prevalence of five

rectal bacteria declined.⁵¹ Although only a few species were impacted, the authors were restricted in the bacteria they could detect with culture assays as compared with 16S rRNA sequencing. Recent evidence suggests that bacteria from a community indicative of bacterial vaginosis degraded tenofovir from a microbicide gel applied in the vaginal tract.⁵² However, ART was delivered topically, and oral ART (pre-exposure prophylaxis) has not been shown to affect prevention of HIV for women with bacterial vaginosis.⁵³ Further studies are needed to evaluate the role of oral ART on the microbial community and its impact on local immunity.

There were a few limitations to this study. The sample sizes for cluster 2 and cluster 4 were small and the associations may be spurious. However, the objective of this study was to assess the feasibility of reducing the dimensionality of the microbial data in a way that represented it most similarly to its structure in the data space. This approach uses all of the microbial data as compared with prior studies in men that tested differences in individual bacterial species. Furthermore, this study has access to an information-rich data set that enabled exploration of correlates of the anal microbial clusters. The anal mucosa is not dominated by lactic acid-producing *Lactobacillus*, limiting the applicability of Nugent score or pH to independently confirm the data structure of the clustering algorithms. The cross-sectional design of this study only provides a “snapshot” in time and does not allow analysis of any temporal changes. The preliminary associations are being investigated longitudinally with a larger sample size. In addition, relative abundance measures may

vary depending on sampling techniques and sequencing depth. This bias was minimized by using the same amounts of template DNA and equimolar concentrations of PCR amplicons across samples. As bacteria may have functional redundancy, further studies on the genetic redundancy and/or metabolites would help elucidate whether clustering on the taxa level parallels functionality. Next, we allowed hierarchical clustering for the top 15 most dominant genera instead of all the genera to be consistent with our earlier work.⁴ The top 15 most abundant genera represented a median of 73% (interquartile range: 68%–78%) of all genera observed in the samples. Since the majority of the observed genera were included, potential bias in the formation of clusters was minimized. Lastly, the ART treatment difference by cluster membership may be confounded by duration of HIV infection, although for those who knew their status, their infection duration was within the past 5 years.

In summary, we have demonstrated the potential of clustering of microbial data as an analytic approach that offers a more comprehensive look at multiplexed microbial patterns. The results presented here suggest that a microbial cluster enriched with a member of the *Fusobacteria* Phylum, *Sneathia*, was associated with prevalent HPV-16 among MSM at risk of or living with HIV. Participants with an anal microbiota belonging to that cluster were more likely to be virally suppressed and have no vaginal sex partners. These findings should be validated in larger study populations to establish whether HPV-16 and other HR-HPVs are associated with certain anal microbial communities of ART-treated MSM.

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Authors' Contributions

W.A.B. and M.E.C. designed the TRUST study. R.G.N., S.M.B., and M.E.C. conceived the analysis for the article. Data collection and management were facilitated by R.G.N., W.D., J.R., and B.M. R.G.N. conducted the data analysis with input from S.M.B., J.R., B.M., and H.L. R.G.N. drafted the article and S.M.B., J.R., T.A.C., W.D., B.M., H.L., W.A.B., S.D.B., and M.E.C. provided critical review and editing. All authors have seen and approved the article; the corresponding author had full access to the data and had final responsibility for the decision to submit for publication.

Disclaimer

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Author Disclosure Statement

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Supplementary Material

Supplementary Table S1
Supplementary Table S2

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