

Vaginal and Penile Microbiome Associations With Herpes Simplex Virus Type 2 in Women and Their Male Sex Partners

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Background: We determined how the vaginal and penile microbiomes contribute to herpes simplex virus type 2 (HSV-2) serostatus within sexual partnerships.

Methods: Microbiomes were characterized in cervicovaginal lavage and penile meatal swab specimens through high-throughput 16s ribosomal RNA gene amplicon sequencing. HSV-2 antibody was detected in serum specimens. We modeled vaginal and penile taxa and covariates contributing to HSV-2 status in women and men using bivariate probit analysis.

Results: Among 231 couples, HSV-2 was detected in both partners in 78 couples (33.8%), in the woman only in 52 (22.5%), in the man only in 27 (11.7%), and in neither in 74 (32.0%). Among the women (median age, 22 years) 10.9% had human immunodeficiency virus (HIV), and 21.4% had Bacterial vaginosis. Among men (median age, 26 years), 11.8% had HIV, and 55.0% circumcised. In an analysis with adjustment for sociodemographics and Bacterial vaginosis, enrichment of vaginal *Gardnerella vaginalis* and *Lactobacillus iners* was associated with increased likelihood of HSV-2 in both partners. Penile taxa (including *Ureaplasma* and *Aerococcus*) were associated with HSV-2 in women.

Conclusions: We demonstrate that penile taxa are associated with HSV-2 in female partners, and vaginal taxa are associated with HSV-2 in male partners. Our findings suggest that couples-level joint consideration of genital microbiome and sexually transmitted infection or related outcomes could lead to new avenues for prevention.

Keywords. HSV-2; genital herpes; vaginal microbiome; penile microbiome; Kenya; HIV; bivariate probit; amplicon sequencing.

Human immunodeficiency virus (HIV) remains a global pandemic, with an estimated 1.8 million new infections in 2017; more than half of new infections occurred in sub-Saharan Africa, where women accounted for 59% of the new infections in adults [1]. This is in part due to a high burden of cofactors for HIV infection, specifically herpes simplex virus type 2 (HSV-2) and Bacterial vaginosis (BV). As reviewed by Torrone et al [2], the prevalence of HSV-2 ranges from 34% to 79% among clinic- and community-based 15–24-year-olds in Eastern Africa, and from 46% to 66% in high-risk populations. The prevalence of BV is estimated to be 33% among clinic- and community-based women aged 25–49 years in Southern and Eastern Africa [2]. In Kenya, our studies and those of others find similarly high prevalence and incidence of HSV-2 in men [3], as well as BV and HSV-2 in women [4, 5]. HSV-2 is associated with a 2–3-fold

increased risk of HIV acquisition among women and men [6, 7], and BV is associated with a 1.6-fold increased risk of HIV acquisition [8]. BV and HSV-2 are linked epidemiologically: in a meta-analysis of prospective studies, the risk of BV was 1.55 times higher for women with HSV-2 [9].

BV is considered a “sexually enhanced” condition based on substantial epidemiologic evidence [10]. A meta-analysis of 28 studies located worldwide estimated a 20% protective effect of condom use on BV, and a 60% increased risk of BV for women with new or multiple male sex partners [11]. Microbiologic findings support the sexual exchange of penile and vaginal microbiota. Through high-resolution microbial genotyping, Eren et al [12] showed that within 53 monogamous, heterosexual couples, *Gardnerella vaginalis* was detected in 44 (83%) of the male partners, and the oligotype correlation was >0.9 in 24 couples and >0.5 in 12 couples. Comprehensive measures of the penile microbiome have identified a high prevalence and abundance of common BV-associated bacteria in uncircumcised men [13, 14], and this penile microbiome composition is associated with increased prevalence of BV in female sex partners [15].

Given that HSV-2 is sexually transmitted and correlated within couples [16–18], that there is sexual exchangeability between the vaginal and penile microbiome, and that BV and HSV-2 are associated, we sought to determine how the vaginal microbiome and penile microbiome contribute to

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women's and men's HSV-2 serostatus, within sexual partnerships (Supplementary Figure 1). While we have mounting understanding of how the vaginal and penile microbiome composition may together contribute to BV, it is necessary to understand whether vaginal and penile microbiome composition extends to HSV-2 for treatment and intervention development.

METHODS

This study was approved by the Institutional Review Board of the University of Illinois at Chicago and the Ethical Review Committee of Maseno University (Kisumu, Kenya).

Study Design and Participants

This study used data and biologic specimens from Afya Jozi, Afya Jamii (Kiswahili for "Healthy Pair, Healthy Community"), a prospective cohort study of heterosexual couples in Kisumu, Kenya. Recruitment, eligibility criteria, and description of study cohort have been published [4]. Eligible members of cisgender male-female couples independently confirmed that they had been in a sexual relationship for ≥ 6 months. We included men aged 18–35 years and their female partners aged ≥ 16 years. In private examination rooms, men and women separately underwent a standardized medical history, physical examination and a personal interview to obtain information on sociodemographics and sexual behavior. Trained clinicians and counselors interviewed participants in their language of choice (English, Dholuo, or Kiswahili).

Detection of HSV-2, BV, and HIV

Serum specimens were tested for HSV-2 antibody (Kalon HSV-2 immunoglobulin G enzyme-linked immunosorbent assay; Kalon Biological Limited Kingdom) using the manufacturer's recommended cutoff. We selected Kalon HSV-2 test owing to its superior accuracy in our target population [19]. A vaginal swab specimen was collected by the clinician and taken to the on-site laboratory for assessment of BV according to Nugent criteria; a morphologic score of 7–10 was defined as BV [20]. Testing for HIV infection was conducted using a serial rapid test protocol that followed the Kenyan national guidelines [21]. Subjects who reported themselves as HIV positive at baseline were not required to have confirmatory testing.

Specimen Collection for Penile and Vaginal Microbiome Characterization

Using premoistened minitip flocked swabs (Copan Diagnostics), clinicians twirled the swab at the meatal opening for 3–5 rotations. In uncircumcised men, the foreskin was retracted before sampling. Swabs were immediately placed in the collection kit tube and stored at -80°C until shipment. The vaginal microbiome was characterized based on cervicovaginal lavage samples, which were immediately aliquoted to 2.5-mL cryovials and stored at -80°C until shipment.

Microbial Community Characterization

Genomic DNA was used as template for polymerase chain reaction amplification of the V3–V4 variable region of bacterial 16S ribosomal RNA gene, according to a 2-step polymerase chain reaction protocol using primers 341F and 806R, as described elsewhere [22]. After pooling of bar-coded samples, amplicons were sequenced on an Illumina MiSeq instrument, implementing V3 chemistry (600 cycles). Forward and reverse reads were merged using the software package PEAR [23]. Quality and primer-trimmed sequence data were then processed using a standard bioinformatics pipeline for chimera removal, and annotation was conducted by the University of Maryland Institute for Genomic Science [24]. Subsequently, a biologic observation matrix was generated at the lowest taxonomic level identifiable. Data were filtered separately by anatomic site to retain taxa that contributed $\geq 0.05\%$ of the total sequence reads, resulting in retention of 53 vaginal taxa and 73 penile taxa. Vaginal community state types (CSTs) were identified in a reference data set using nearest centroid classification, as described elsewhere [25].

Statistical Analysis

Descriptive Analyses and Visualization

Of 252 couples with paired measures, 231 (91.7%) had both HSV-2 and penile or vaginal microbiome measure with ≥ 5000 sequence read depth, and these 231 couples are used in the current analysis. Descriptive and inferential analyses were conducted in an R statistical computing environment, except as otherwise reported, and packages are reported. We visualized the vaginal and penile taxa using Spearman correlation heat maps (*ggplot2*, *hclust*) to assess correlations between relative abundances of vaginal and penile taxa and with HSV-2. Stacked bar plots were created using Stata/SE 15 software. Alpha diversity indices were calculated separately for vaginal and penile microbiomes after each data set was rarefied to 6000 sequence depth (*vegan*).

We tested for global differences in vaginal and penile bacterial community composition by HSV-2 status at the couples level using analysis of similarity of the Bray–Curtis resemblance matrix; we visualized the relationship of global bacterial communities by joint HSV-2 status using nonmetric multidimensional scaling of bootstrapped averages of centroids with 500 replicates per group (Primer-E, version 7).

Inferential Analyses

We jointly modeled the binary HSV-2 outcomes in men and women with taxa and covariates using bivariate probit analysis, because HSV-2 is highly correlated within couples and bivariate probit analysis appropriately accounts for the correlation parameter of the marginal distributions of HSV-2 status. In the first step of the analysis, we sought to identify specific penile and vaginal taxa that differed by HSV-2 status. Following geometric

Bayesian multiplicative prior imputation of zeros [26], filtered sequence data were transformed using centered log ratios [27] (*zCompositions*). After zero imputation and transformation, the data were scaled from 0 to 1.

Next, 4 random forest (*randomForest*) models were fit to select bacterial taxa separately for female and male HSV-2 status: (1) penile taxa influencing HSV-2 status in men, (2) vaginal taxa influencing HSV-2 status in women, (3) penile taxa influencing HSV-2 status in women, and (4) vaginal taxa influencing for HSV-2 status in men. In the second step of the analysis, the top 25 female and top 25 male taxa (50 features total) identified in the 4 random forest models were entered in a bivariate probit regression model to test for association with male and female HSV-2 status (*Zelig*).

The final multivariable model was selected based on minimized Akaike information criterion. In addition to the selected bacteria, in a separate bivariate probit model, we tested covariates of interest. These included, from both partners, age, educational status, having multiple sex partners, condom use, and HIV status; female BV status; and male circumcision status. The final multivariable bivariate probit regression modeled penile and vaginal taxa associated with HSV-2 in women and men, while adjusting for significant covariates.

RESULTS

Overall, 130 women (56%) and 108 men (45%) were HSV-2 positive. The 231 couples included 78 (33.8%) that were jointly infected, 52 (22.5%) in which only the woman was infected, 27 (11.7%) in which only the man was infected, and 74 (32.0%) in which neither was infected. Women had a median age of 22 years; 10.9% had HIV, and 21.4% had BV. Men had a median age of 26 years, 11.8% had HIV, and 55.0% were circumcised. The prevalence of HSV-2 increased with age, lower educational attainment, and HIV positivity for both women and men, and HSV-2 was more prevalent in women with BV (Tables 1 and 2) for findings in women and men, respectively).

In crude bivariate probit analyses (Supplementary Table 1), older male age, lower male and female educational attainment, and male or female HIV positivity were associated with greater odds of HSV-2 in both women and men; older female age and higher BV prevalence were associated with increased odds of HSV-2 in women. In multivariable adjusted bivariate probit analysis, only 3 covariates were significantly associated with HSV-2 status in women or men: older male age, lower educational attainment among women, and BV in the female partner (Supplementary Table 2).

Vaginal and Penile Microbiome Composition

Stacked bar charts representing relative abundance of the vaginal and penile taxa with greatest relative abundance sorted by HSV-2 outcomes are shown in Figure 1 (Figure 1A, vaginal taxa; Figure 1B, penile taxa). In women, *Lactobacillus iners* and

G. vaginalis were the taxa with the highest relative abundance, and this is reflected in the distribution of CST (Table 1), with CST-III (*L. iners* dominant) in 97 women (42%) and CST-IV (*G. vaginalis* dominant) in 109 (47%). HSV-2 in women was more prevalent among those with CST-IVB, and less prevalent in those with CST-I (Table 1). Vaginal microbiome alpha diversity measures were greater for women whose male sex partners were HSV-2 positive (Table 1), and penile richness was elevated for men whose female partners were HSV-2 positive (Table 2). In men, the taxa with the highest mean relative abundances were *Corynebacterium* (16.4%), *Anaerococcus* (8.9%), *Streptococcus* (8.1%), *Fingoldia* (7.6%), and *L. iners* (6.8%). Penile microbiome alpha diversity measures did not differ by male HSV-2 status. The mean relative abundance of meatal taxa differed by circumcision status in an expected pattern (Supplementary Table 3).

Figure 2 shows the Spearman rank correlation of the 10 vaginal and 10 penile taxa with the highest relative abundances, and correlations with HSV-2 status and BV. For example, *Staphylococcus* and *Corynebacterium* correlate positively, and both are correlated inversely with the cluster of positively correlated taxa consisting of *Sneathia sanguinegens*, *Sneathia amnii*, *Megasphaera*, Clostridiales BV-associated bacteria 1 (BVAB1), *Atopobium vaginae*, and *G. vaginalis*, which correlated positively with BV. Of note, this group of BV-correlated taxa included penile *S. sanguinegens*. Among penile taxa, *Corynebacterium* and *Streptococcus* positively correlated most strongly, and correlated inversely with penile *Fingoldia*, *Anaerococcus*, *Ezakiella*, and *Peptoniphilus*. Between vaginal and penile taxa, *Streptococcus* and *L. iners* correlated positively. Female and male HSV-2 status correlated positively, were generally positively correlated with BV-correlated taxa (including penile *S. sanguinegens*), and were inversely associated with vaginal *Lactobacillus* spp. (*Lactobacillus* identified at the genus level, but species was not identified) and *Lactobacillus crispatus*, and penile *Staphylococcus* and *Corynebacterium*.

Associations Between Vaginal and Penile Taxa and HSV-2 Status in Women and Men

The global difference in bacterial community composition by HSV-2 outcome was statistically significant (analysis of similarity test, $P = .02$) (Table 3); in pairwise tests only the comparison for both negative versus both positive ($P = .006$) and for woman negative/man positive versus both positive ($P = .04$) were statistically significant. This difference in couples-level genital microbiome composition was apparent in the visualization of the bootstrapped averages of the centroids of the 4 combinations of outcome (Figure 3), with the least overlap between the observations where both members of the couple were HSV-2 positive and those where both were negative.

The top 25 vaginal and penile taxa identified by random forest models as having the greatest influence on joint outcomes are

Table 1. Distribution of Characteristics by Herpes Simplex Virus Type 2 Status in Women

Characteristic	Women, No. (%) ^a		P Value ^b
	HSV-2 Positive (n = 130)	HSV-2 Negative (n = 101)	
Age, median (IQR), y ^c	23 (21–26)	22 (20–25)	.04
Educational attainment			
Primary school or less	80 (61.5)	36 (35.6)	<.001
Some secondary school	39 (30.0)	48 (47.5)	
Postsecondary school	11 (8.5)	17 (16.8)	
Condom used at last sexual encounter	18 (13.9)	20 (19.8)	.23
No. of sex partners in last 6 mo			.39
1	120 (96.8)	95 (99.0)	
≥2	4 (3.2)	1 (1.0)	
Median (IQR) relationship duration, y ^c	3 (2–5)	3 (1.5–5)	.23
Pregnancy or contraceptive use			
Oral contraceptive pills	2 (1.0)	3 (2.3)	.03
Injectable	20 (19.8)	21 (16.2)	
Implant	22 (21.8)	47 (36.2)	
Condoms, calendar, IUD, other, or none ^d	40 (39.6)	51 (39.2)	
Pregnant (urine HCG positive)	17 (16.8)	8 (6.1)	
Clinical characteristics			
BV by Nugent score (7–10)	37 (28.9)	12 (11.9)	.002
HIV positive	19 (14.8)	6 (5.9)	.03
Genital ulcers			
Self-reported in last 6 mo	10 (7.7)	11 (10.9)	.49
Detected at examination	5 (3.9)	0 (0)	.07
Vaginal microbiome alpha diversity measures, median (IQR) ^c			
Shannon diversity index	1.16 (0.30–1.94)	1.15 (0.43–1.78)	.86
Simpson diversity index	0.55 (0.11–0.78)	0.55 (0.17–0.74)	.97
Richness	16 (9–23)	15 (10–24)	.36
Evenness	0.44 (0.14–0.62)	0.42 (0.18–0.57)	.74
Vaginal microbiome CST			
CST-I (<i>Lactobacillus crispatus</i> dominant)	6 (4.6)	14 (13.9)	.02
CST-II (<i>Lactobacillus jensenii</i> dominant)	1 (0.8)	2 (2.0)	
CST-III (<i>Lactobacillus iners</i> dominant)	51 (39.2)	46 (45.5)	
CST-IVA (<i>Gardnerella vaginalis</i> dominant)	7 (5.4)	5 (4.9)	
CST-IVB (<i>G. vaginalis</i> dominant)	60 (46.2)	29 (28.7)	
CST-IVC (<i>G. vaginalis</i> dominant)	5 (3.8)	3 (3.0)	
CST-V (<i>Lactobacillus gasseri</i> dominant)	0 (0.0)	2 (2.0)	
Characteristics of male partner			
Circumcised	71 (54.6)	56 (55.5)	.90
HSV-2 seropositive	78 (60.0)	27 (26.7)	<.001
HIV positive	23 (18.0)	4 (4.0)	.001
Penile microbiome alpha diversity measure, median (IQR) ^c			
Shannon diversity index	1.91 (1.58–2.25)	1.91 (1.39–2.21)	.32
Simpson diversity index	0.77 (0.69–0.84)	0.77 (0.64–0.85)	.55
Richness	29 (24–35)	26 (22–33)	.03
Evenness	0.57 (0.50–0.63)	0.57 (0.45–0.65)	.85

Abbreviations: BV, Bacterial vaginosis; CST, community state type; HCG, human chorionic gonadotropin; HIV, human immunodeficiency virus; HSV-2, herpes simplex virus type 2; IQR, interquartile range; IUD, intrauterine device.

^aData represent no. (%) of women unless otherwise specified.

^bP values based on χ^2 test unless otherwise noted; Fisher exact test was used for comparisons with cell sizes <5.

^cWilcoxon rank sum test was used for all comparisons of nonparametric continuously distributed data.

^dIncluding 2 women using an IUD, 11 calendar method, 1 tubal ligation, 1 lactation amenorrhea, 25 condoms only, and 51 using no method for birth control.

shown in [Supplementary Table 4](#). Briefly, the vaginal taxa with the greatest influence (as defined by mean decrease in Gini coefficient) on women's HSV-2 status were *G. vaginalis*, *Lactobacillus* spp., and *L. iners*. Penile taxa most strongly influencing women's HSV-2 status

were *Peptoniphilus*, *Finegoldia* and *Hydrotalea*. Vaginal taxa with the greatest influence on men's HSV-2 status were *G. vaginalis*, *L. iners*, and *Lactobacillus* spp., while the penile taxa most strongly influencing men's HSV-2 status were *Finegoldia*, *Staphylococcus*, and *Peptoniphilus*.

Table 2. Distribution of Characteristics by Herpes Simplex Virus Type 2 Status in Men

Characteristic	Men, No. (%) ^a		P Value ^b
	HSV-2 Positive (n = 105)	HSV-2 Negative (n = 126)	
Age, median (IQR), y ^c	27 (25–31)	26 (23.8–29)	.021
Educational attainment			
Primary school or less	57 (54.3)	36 (28.6)	<.001
Some secondary school	39 (37.1)	63 (50.0)	
Postsecondary school	9 (8.6)	27 (21.4)	
Condom used at last sexual encounter	20 (19.1)	18 (14.3)	.33
No. of sex partners in last 6 mo			
1	82 (79.6)	96 (76.8)	.61
≥2	21 (20.4)	29 (23.2)	
Median (IQR) relationship duration, y ^c	3 (1.9–5)	3 (1.7–5)	.79
Clinical characteristics			
Circumcised	69 (56.2)	68 (54.0)	.74
HIV positive	19 (18.3)	8 (6.4)	.006
Genital ulcers			
Self-reported in last 6 mo	7 (6.7)	0 (0)	.004
Detected at examination	4 (3.8)	0 (0)	.04
Penile microbiome alpha diversity measure, median (IQR) ^c			
Shannon diversity index	1.93 (1.57–2.27)	1.90 (1.42–2.21)	.19
Simpson diversity index	0.77 (0.68–0.85)	0.76 (0.64–0.84)	.22
Richness	29 (22–35)	28 (22–33)	.61
Evenness	0.59 (0.51–0.65)	0.57 (0.46–0.64)	.13
Female partner characteristics			
HSV-2 seropositive	78 (74.3)	52 (41.3)	<.001
HIV positive	17 (16.2)	8 (6.5)	.02
BV (Nugent score, 7–10)	24 (23.1)	25 (20.0)	.57
Vaginal microbiome CST			
CST-I (<i>Lactobacillus crispatus</i> dominant)	6 (5.7)	14 (11.1)	.14
CST-II (<i>Lactobacillus jensenii</i> dominant)	1 (1.0)	2 (1.6)	
CST-III (<i>Lactobacillus iners</i> dominant)	46 (43.8)	51 (40.5)	
CST-IVA (<i>Gardnerella vaginalis</i> dominant)	2 (1.9)	10 (7.9)	
CST-IVB (<i>G. vaginalis</i> dominant)	46 (43.8)	43 (34.1)	
CST-IVC (<i>G. vaginalis</i> dominant)	4 (3.8)	4 (3.2)	
CST-V (<i>Lactobacillus gasseri</i> dominant)	0 (0.0)	2 (1.6)	
Vaginal microbiome alpha diversity measure, median (IQR) ^c			
Shannon diversity index	1.48 (0.69–1.95)	0.81 (0.21–1.68)	<.001
Simpson diversity index	0.66 (0.45–0.78)	0.40 (0.08–0.72)	.001
Richness	19 (10–23.5)	14 (9–23)	.07
Evenness	0.51 (0.31–0.63)	0.35 (0.11–0.55)	<.001

Abbreviations: BV, Bacterial vaginosis; CST, community state type; HIV, human immunodeficiency virus; HSV-2, herpes simplex virus; IQR, interquartile range.

^aData represent no. (%) of men unless otherwise specified.

^bP values are based on χ^2 test unless otherwise noted; Fisher exact test was used for comparisons with cells <5.

^cWilcoxon rank sum test used for all comparisons of nonparametric continuously distributed data.

Multivariable Bivariate Probit Analysis Adjustment for Participant-Level Covariates Associated With HSV-2

Results of multivariable bivariate probit regression modeling of taxa are summarized in [Supplementary Table 5](#). Enrichment of 4 vaginal taxa were associated with increased probability of HSV-2 in male partners: *G. vaginalis*, *L. iners*, Lactobacillales, and *Finegoldia*. While enrichment of penile *Eremococcus* was associated with HSV-2 for both women and men, penile enrichment with *Escherichia/Shigella*, *Ureaplasma*, and *S. sanguinegens* were associated with HSV-2 only in women.

Enrichment with penile *Corynebacterium* and *Aerococcus* were inversely associated with HSV-2 in women, and *Staphylococcus*, *Bradyrhizobium*, and *Micrococcus* were inversely associated with HSV-2 in men.

After adjustment for significant covariates (male age, female educational attainment, and BV status), the magnitude and statistical significance of *Eremococcus*, *Escherichia/shigella*, *Anaerococcus*, and *Micrococcus* were little changed ([Table 4](#)). However, there was attenuation of the statistical significance and magnitude of association with HSV-2 for

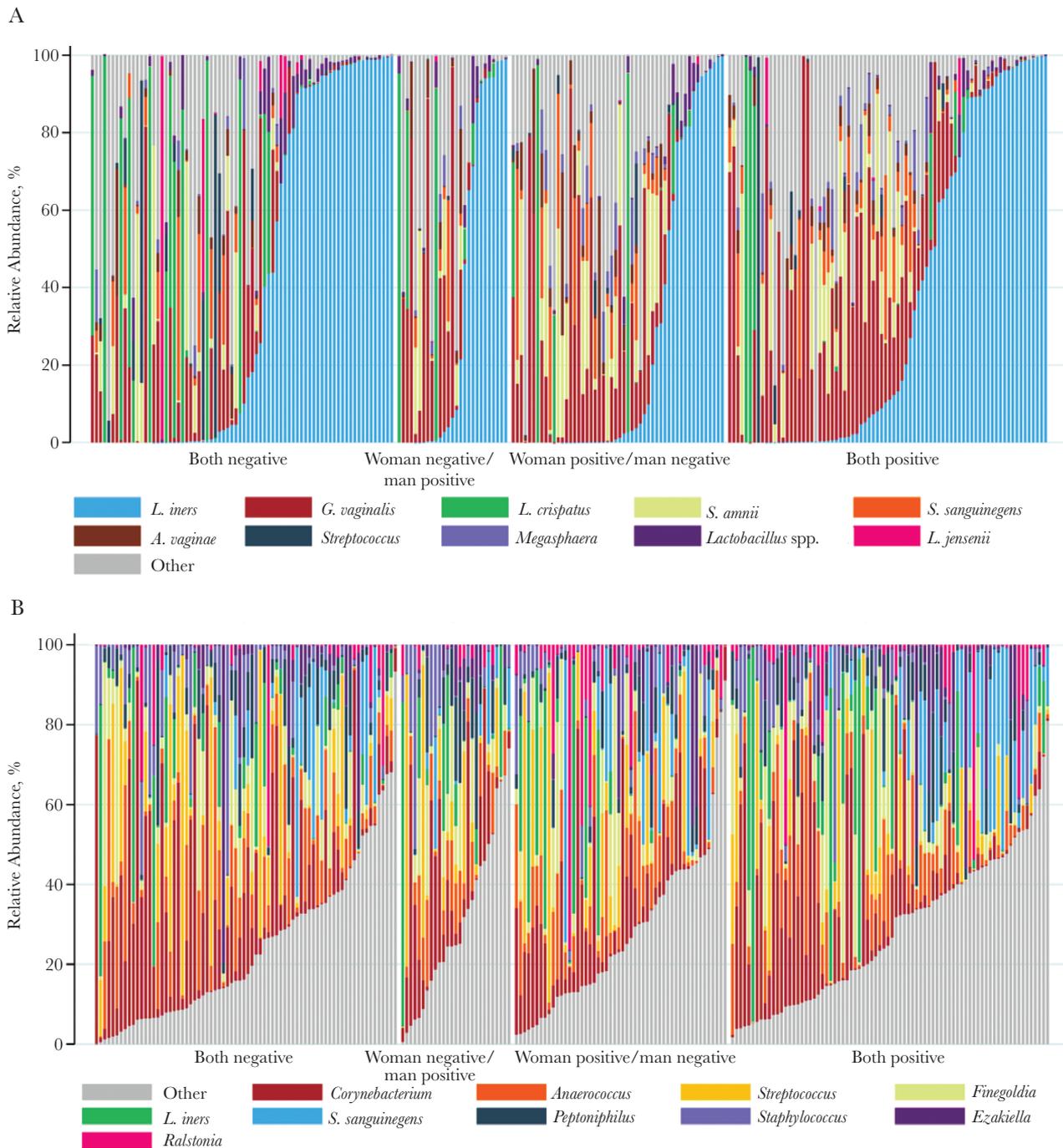


Figure 1. Stacked bar charts of the relative abundance of the 10 vaginal and 10 penile taxa with greatest mean relative abundance for individuals, by herpes simplex virus type 2 (HSV-2) status. Abbreviations: *A. vaginae*, *Atopobium vaginae*; *G. vaginalis*, *Gardnerella vaginalis*; *L. crispatus*, *Lactobacillus crispatus*; *L. iners*, *Lactobacillus iners*; *L. jensenii*, *Lactobacillus jensenii*; *S. amnii*, *Sneathia amnii*; *S. sanguinegens*, *Sneathia sanguinegens*.

penile *Staphylococcus*, *Corynebacterium*, and *S. sanguinegens*. In adjusted analyses, enrichment of vaginal *G. vaginalis* and *L. iners* were associated with increased probability of HSV-2 in women and in men, while the associations between vaginal Lactobacillales and HSV-2 in male partners and between *Senegalimassilia* and HSV-2 in women were attenuated in strength and significance.

DISCUSSION

Among these community recruited couples in Kenya, there was a high prevalence of HSV-2 and infection concordance within couples was high (66% both positive or both negative). Through joint consideration of HSV-2 and the penile and vaginal microbiomes within heterosexual partnerships, we observed that the penile and vaginal microbiome are correlated

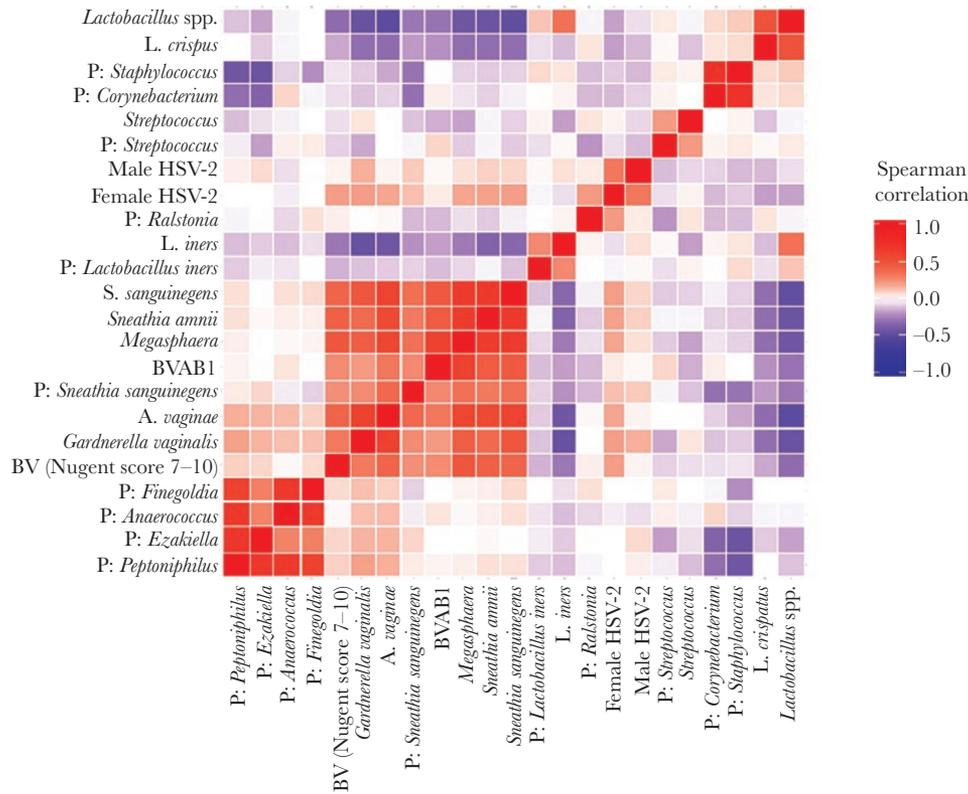


Figure 2. Spearman rank correlation heatmap of the top 10 vaginal and 10 penile taxa with greatest relative abundance, and herpes simplex virus type 2 (HSV-2) and Bacterial vaginosis (BV) status. The correlation heat map represents the direction and magnitude of the Spearman rank correlation between the 10 vaginal and 10 penile taxa with highest relative abundance relative to each other and HSV-2 and BV. Negative correlations are shaded in blue and positive correlations in red, with deeper intensity representing the magnitude of the correlation. Penile taxa are denoted with a "P" before the name. Taxa are represented at the genus level, except where species is noted, for the following: *Atopobium vaginae*, *Gardnerella vaginalis*, *Lactobacillus crispatus*, *Lactobacillus iners*, *Sneathia amnii*, and *Sneathia sanguinegens*.

within couples and the overall bacterial community composition differed by HSV-2 status, with the greatest dissimilarity observed between couples in which both members are HSV-2 negative and those in which both are HSV-2 positive. Vaginal taxa were associated with HSV-2 status in women, and penile taxa with HSV-2 status in men. Furthermore, these HSV-2/genital microbiome relationships show crossover within couples: penile microbiome composition is associated with HSV-2 status in female partners, and vaginal microbiome composition with HSV-2 status in male partners.

As reviewed by Nardis et al [28], the bidirectional relationship between BV and HSV-2 in women is well established epidemiologically. There are limited data on comprehensive measures of the vaginal microbiome and HSV-2 in women, but microbiologic studies demonstrate lack of *Lactobacillus* species and enrichment with *G. vaginalis* are associated with HSV-2 risk [29]. BV increases the risk of HSV-2 acquisition through disruption of mucosa and female genital tract enrichment with HSV-2-reactive CD4⁺ T cells [7]. Fichorova et al [30] found that suppressed cervical immunity in women with BV was predictive of HSV-2 seroconversion. Mechanistic studies could contribute to vaccine or therapeutic development: for example, by

determining whether specific penile and vaginal bacteria may protect mucosal barriers in relation to HSV-2 receptors, mediate host immune response to HSV-2, contribute to epigenetic regulation (or dysregulation) of HSV-2, or interact with critical determinants of pathogenesis, such as virion host shutoff

Table 3. Comparison of Microbial Community Structure by Herpes Simplex Virus Type 2 Status (Analysis of Similarity)

Comparison by HSV-2 Status	R Statistic	P Value
Pairwise tests		
Both HSV-2 negative vs man negative/woman positive	0.026	.08
Both HSV-2 negative vs man positive/woman negative	-0.012	.62
Both HSV-2 negative vs both HSV-2 positive	0.039	.006
Man negative/woman positive vs man positive/woman negative	-0.009	.59
Man negative/woman positive vs both HSV-2 positive	0.033	.04
Man positive/woman negative vs both HSV-2 positive	0.034	.17
Global test	0.026	.02

Abbreviations: HSV-2, herpes simplex virus type 2;

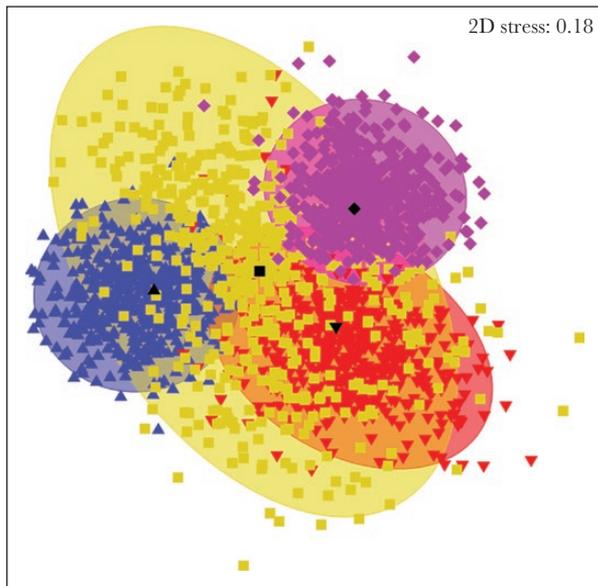


Figure 3. Nonmetric dimensional scaling plot showing the bootstrapped average centroid and 95% coverage region for each of the 4 outcome states for joint herpes simplex virus type 2 (HSV-2) infection. The 4 colors represent the 4 outcome states for HSV-2 infection at the couples level; blue indicates both HSV-2 negative; pink, male partner HSV-2 negative, female partner HSV-2 positive; yellow, male partner HSV-2 positive, female partner HSV-2 negative; and red, both HSV-2 positive. Each colored mark indicates 1 of 500 bootstrappings of the data set, the matching shaded area represents the 95% coverage, and the black symbol at the center of each colored shape represents the average centroid of the 500 bootstraps. Abbreviation: 2D, 2-dimensional.

protein [31, 32]. HSV-2 also can alter the vaginal microbiome. In vitro model systems have identified mechanisms by which HSV-2 may alter microbiome, including differential depletion of cell surface sulfonated heparans (that many bacteria may bind to and that are involved in cell signaling [33]) and altered cell surface hydrophobicity and cell cytoskeletal complex, which could result in altered bacterial adherence [34].

We found that specific penile taxa were associated with HSV-2 status in men and with HSV-2 status in their female sex partners, adjusting for vaginal microbiome composition and the woman's BV status. Given sexual exchangeability of the penile and vaginal microbiome, the correlation of penile microbiome with vaginal microbiome is expected, as is correlation of HSV-2 status within sexual partnerships. Thus, instead of a biologic association similar to that of BV and HSV-2 acquisition, transmission, and pathogenesis, the association between penile microbiome composition with HSV-2 may be merely a cocorrelation rather than a biologic association. However, the penile microbiome does likely have a functional role in infection, as specific penile taxa—especially those commonly associated with BV—are associated with mucosal inflammation and increased risk of HIV acquisition [35]. The increased penile mucosal inflammation associated with greater penile enrichment of these or other BV-associated taxa may also increase

susceptibility to HSV-2 acquisition through disruption of epithelial barrier or increased target cells. Whether the penile microbiome composition affects the risk of HSV-2 acquisition, transmissibility, or course of infection in men (eg, location, frequency, and severity of outbreaks and viral shedding) is biologically plausible but remains unstudied.

In the current study and in a randomized trial of medical male circumcision in Kisumu [36, 37], we did not find that male circumcision status was associated with HSV-2, although the penile microbiome composition differed substantially by circumcision status. Conversely, male circumcision trials in Uganda and South Africa did find that male circumcision was protective of HSV-2 [38, 39]. Subsequent studies have not explored the mechanism by which circumcision may protect against HSV-2 acquisition in men. A relationship between penile and vaginal microbiome may play a role, and it is likely that such associations would vary over time and may interact with or be mediated by sexual practices that should be assessed in a longitudinal study.

Our findings had various clinical and therapeutic implications. BV and enrichment of BV-associated taxa have been shown to increase HSV-2 viral shedding in women [28], thus increasing transmissibility. Screening and treatment of BV among women with HSV-2 may reduce transmission to male sex partners. Live biotherapeutic approaches may provide another intervention option. Consistently, *L. crispatus* has been shown to have beneficial effects on vaginal health. Recent results of randomized controlled study show that Lactin-V [Lactobacillus crispatus CTV-05 (LACTIN-V, Osel Inc)], a live biotherapeutic *L. crispatus* adjuvant applied intravaginally, leads to *L. crispatus* colonization in 77% of women and reduces BV recurrence by 40% [40]. Such a therapeutic option may lead to additional benefits, such as reduced HSV-2 acquisition in women or reduced transmission to male sex partners. Unexpectedly, we observed that enrichment of *L. iners* was associated with increased odds of HSV-2 in women and men, controlling for BV status. However, it is likely that increasing relative abundance of *L. iners* reflects reduced *L. crispatus*, while at the same time *G. vaginalis* is accounted for in the multivariable model.

If longitudinal study demonstrates that HSV-2 alters the penile microbiome toward a more inflammatory profile, or if penile microbiome composition can lead to increased risk of HSV-2 acquisition, then interventions to alter penile microbiome may prevent HSV-2 acquisitions in men and transmission to female sex partners. Galiwango et al [41] have published a protocol for a trial that seeks to explore the effect of antimicrobial agents on the penile microbiota, immunology, and HIV susceptibility among Ugandan men, which will also evaluate the impact on the same parameters in the vagina of female sex partners. Randomized trials evaluating the effect of antibiotic treatment of male sex partners to reduce BV recurrence may also shed light on the modifiability of the penile

Table 4. Penile and Vaginal Taxa Associated With Herpes Simplex Virus Type 2 Status Among Women and Men in Multivariable Bivariate Probit Analysis, Controlling for Statistically Significant Covariates (N = 229 Couples)

Variable ^a	HSV-2 Females β (95% CI); P value	HSV-2 Males β (95% CI); P value
Penile taxa		
<i>Eremococcus</i>	1.54 (.52–2.55); .003	1.57 (.58–2.57); .002
<i>Escherichia/Shigella</i>	3.96 (1.95–5.98); <.001	–0.21 (–1.94 to 1.52); .813
<i>Staphylococcus</i>	–0.79 (–1.91 to .32); .16	–0.85 (–1.9 to 0.21); .12
<i>Corynebacterium</i>	–0.61 (–1.76 to .53); .30	–0.51 (–1.59 to .57); .36
<i>Bradyrhizobium</i>	1.46 (.24–2.68); .02	–0.95 (–2.14 to .24); .12
<i>Ureaplasma</i>	1.32 (.24–2.41); .02	–0.10 (–1.13 to .93); .85
<i>Sneathia sanguinegens</i>	0.42 (–.59 to 1.42); .42	0.12 (–.85 to 1.08); .81
<i>Aerococcus</i>	–1.43 (–2.74 to –.12); .03	–0.30 (–1.51 to .91); .63
<i>Micrococcus</i>	–0.45 (–1.56 to .65); .42	–1.10 (–2.16 to –.04); .04
Vaginal taxa		
<i>Gardnerella vaginalis</i>	1.38 (.35–2.41); .009	1.56 (.54–2.58); .003
<i>Lactobacillus iners</i>	0.87 (–.00002 to 1.74); .05	1.23 (.38–2.07); .004
Lactobacillales	0.02 (–1.34– to 1.37); .98	1.14 (–.15 to 2.43); .08
<i>Fingoldia</i>	–0.24 (–1.15 to .68); .61	0.99 (.09–1.90); .03
<i>Senegalimassilia</i>	–1.23 (–2.56 to .11); .07	–0.65 (–1.88 to .59); .30
Covariate	OR (95% CI); P value	OR (95% CI); P value
Male partner's age in years	1.05 (1.00–1.11); .06	1.07 (1.02–1.13); .004
Female partner's educational attainment		
Postsecondary school	Reference	Reference
Some secondary school	1.26 (.69–2.32); .45	1.42 (.76–2.66); .27
Primary school or less	2.36 (1.30–4.26); .005	2.03 (1.11–3.74); .02
BV in female partner	1.88 (1.13–3.13); .02	0.92 (.58–1.48); .74

Abbreviations: BV, Bacterial vaginosis; CI, confidence interval; HSV-2, herpes simplex virus type 2; OR, odds ratio.

^aCoefficients are simultaneously adjusted for all variables presented.

microbiome (eg, clinicaltrials.gov NCT02209519 and Australia/New Zealand clinical trials registry ACTRN12619000196145). Trials of new therapeutic approaches are needed in settings such as the site of the current study, where the prevalence and incidence of BV, HSV-2, and HIV are high, so that multiple outcomes in both women and their male partners may be evaluated simultaneously.

Among its strengths, the current study is unique in that it provides HSV-2 measures within heterosexual couples paired with penile and vaginal microbiome findings. Few studies address the interaction of HSV-2 with the vaginal microbiome, and such studies are lacking in relation to the penile microbiome and couple-level association of HSV-2 with the genital microbiome. Other strengths of our study include a large community-recruited sample, minimal missing data, and use of validated methods for HSV-2 detection and microbiome characterization. We demonstrate association of the vaginal and penile microbiome with HSV-2 status in women and men with multiple approaches (ecologic, nonparametric correlation, and inferential modeling), demonstrating robustness of findings.

A limitation of the study is that we did not measure viral shedding or details of HSV-2 symptoms, such as when they initially occurred, their severity, or their frequency. There were insufficient incidents of HSV-2 infections in women and men for longitudinal analysis, and well-powered prospective studies

could provide an understanding of how sex partners' genital microbiome composition may contribute to the acquisition of HSV-2 and the clinical course of disease within partnerships.

In conclusion, our findings suggest that expanded joint consideration of couples-level genital microbiome and sexually transmitted infection or related health outcomes could lead to new avenues for research into prevention.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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editors consider relevant to the content of the manuscript have been disclosed.

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