Penile Microbiota and Female Partner Bacterial Vaginosis in Rakai, Uganda

Cindy M. Liu,a,b,c Bruce A. Hungate,d Aaron A. R. Tobian,a Jacques Ravel,e Jessica L. Prodg, David Serwadda,g Godfrey Kigozi,g Ronald M. Galiwango,g Fred Nalugoda,g Paul Keimg,h Maria J. Wawerg,h Lance B. Price,c,i Ronald H. Grayh

Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, Maryland, USAa; Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, Arizona, USAe; Division of Pathogen Genomics, Translational Genomics Research Institute, Flagstaff, Arizona, USAc; Center for Ecosystem Science and Society and Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona, USAf; Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, Maryland, USAh; Department of Medicine, University of Toronto, Toronto, Ontario, Canadai; Rakai Health Sciences Program, Entebbe, Uganda; Department of Epidemiology, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, Maryland, USAc; Department of Environmental and Occupational Health, Milken Institute School of Public Health, Washington, DC, USAi

L.B.P. and R.H.G. contributed equally to this article.

ABSTRACT Bacterial vaginosis (BV) is a common vaginal bacterial imbalance associated with risk for HIV and poor gynecologic and obstetric outcomes. Male circumcision reduces BV-associated bacteria on the penis and decreases BV in female partners, but the link between penile microbiota and female partner BV is not well understood. We tested the hypothesis that having a female partner with BV increases BV-associated bacteria in uncircumcised men. We characterized penile microbiota composition and density (i.e., the quantity of bacteria per swab) by broad-coverage 16S rRNA gene-based sequencing and quantitative PCR (qPCR) in 165 uncircumcised men from Rakai, Uganda. Associations between penile community state types (CSTs) and female partner’s Nugent score were assessed. We found seven distinct penile CSTs of increasing density (CST1 to 7). CST1 to 3 and CST4 to 7 were the two major CST groups. CST4 to 7 had higher prevalence and abundance of BV-associated bacteria, such as Mobiluncus and Dialister, than CST1 to 3. Men with CST4 to 7 were significantly more likely to have a female partner with a high Nugent score (P = 0.03). Men with two or more extramarital partners were significantly more likely to have CST4 to 7 than men with only marital partners (CST4 to 7 prevalence ratio, 1.84; 95% confidence interval [CI], 1.16 to 2.92). Female partner Nugent BV is significantly associated with penile microbiota. Our data support the exchange of BV-associated bacteria through intercourse, which may explain BV recurrence and persistence.

IMPORTANCE Bacterial vaginosis (BV) is sexually associated but not considered a sexually transmitted disease. Our findings suggest that the uncircumcised penis is an important niche for BV-associated genital anaerobes. In addition, we found a link between extramarital sexual relationships and BV-associated bacteria in men, which parallels earlier findings of the association between sexual activity and BV in women. This suggests the sexual transmissibility of BV-associated bacteria. Reducing bacterial exchange by barrier methods and managing carriage of BV-associated bacteria in men may decrease BV persistence and recurrence in women.

Typified by vaginal discharge, discomfort, and malodor, bacterial vaginosis (BV) results in millions of health care visits annually in the United States. The prevalence of BV varies globally (1), from 29% in the United States (2) to nearly 50% in rural Uganda (3). Key characteristics of BV include an elevated pH and vaginal microbial communities with reduced proportions of Lactobacillus spp. and increased proportions of anaerobes, including species of Mobiluncus, Atopobium, Gardnerella, Prevotella, and other taxa of the order Clostridiales (4). The same anaerobic taxa have recently been reported in the penile microbiota, particularly among uncircumcised men (5–7). However, BV is not recognized as a sexually transmitted condition (8), partly because the communicability of BV has not been established and the condition might have multiple etiologies.

The diagnosis of BV relies on Gram stain using Nugent’s criteria (i.e., a Nugent score of 7 to 10) (9) or on clinically based Amsel criteria (i.e., at least three of the four criteria, including vaginal discharge, elevated pH, clue cells, and fishy odor). While the Nugent score is less sensitive and specific for diagnosing symptomatic BV than the Amsel criteria (10), the Nugent score is commonly used in research settings because it is objective, replicable, and feasible in large-scale epidemiological studies, including those conducted in resource-limited countries (3, 11–16). Thus, Nugent-based BV diagnosis (Nugent-BV) forms the basis of most data linking BV to sexually transmitted infection (STI) and HIV susceptibility and transmission (12, 17–20) and to gynecologic and obstetric complications (21, 22). Findings based on
Nugent-BV can be interpreted in the context of its epidemiological associations (9, 12, 17, 18, 21, 22).

There is growing evidence for the exchange of BV-associated bacteria through sexual intercourse. Previous studies have shown that the penis can harbor BV-associated bacteria and that these bacteria are reduced by male circumcision (5–7). Furthermore, male circumcision significantly reduces Nugent-BV in female partners (11). Persistence or recurrence of Nugent-BV after antibiotic treatment is common and may be driven by the reintroduction of BV-associated bacteria through sexual intercourse (23, 24). However, microbiological evidence establishing the exchange of BV-associated bacteria in heterosexual partners is lacking.

The primary goal of this study was to assess the relationship between penile microbiota of uncircumcised men and Nugent-BV in female partners. We characterized the penile microbiota in 165 uncircumcised men from Rakai, Uganda, and the association between the penile microbiota and female partner Nugent-BV status.

RESULTS

Study participants. All 165 participants were HIV-negative, uncircumcised men from Rakai, Uganda. Approximately 40% of participants had a female partner with Nugent-BV, 45% had a partner with a normal Nugent score, and 15% had a partner with an intermediate Nugent score (Table 1). Most participants were in monogamous heterosexual relationships (148/165 [89.7%]), and nonmarital sexual relationships were uncommon (24/165 [14.5%]). Condom use was also uncommon, and most participants who reported using condoms used them inconsistently (58/60 [96.7%]) (Table 1).

The seven penile CSTs. We characterized the penile microbiota using hierarchical clustering and found seven distinct community state types (CSTs) (25), which could be divided into two major groups: CST1 to 3 and CST4 to 7 (Fig. 1B; see Fig. S1 in the supplemental material). Bacterial densities differed significantly among the seven CSTs (by analysis of variance [ANOVA], \( P < 0.001 \) (Fig. 1A), indicating that the composition of penile microbiota varied along the bacterial density gradient. The lowest-density CST (CST1) had \( 1.0 \times 10^6 \) 16S rRNA gene copies per swab on average (standard deviation [SD], 8.2 \( \times 10^5 \)), and the highest-density CST (CST7) had \( 1.0 \times 10^9 \) 16S rRNA gene copies per swab on average (SD, 9.1 \( \times 10^6 \)) (Fig. 1C).

High prevalence of BV-associated bacteria in men with CST4 to 7. We identified the bacteria associated with female partner Nugent-BV (i.e., Nugent-BV indicators) versus a normal Nugent score (i.e., normal Nugent score indicators) among our participants using indicator analysis (see Table S1 in the supplemental material). As indicators of a normal female partner Nugent score, Corynebacterium and Staphylococcus were significantly more prevalent in CST1 to 3 than in CST4 to 7; however, even though Lactobacillus was 10% more prevalent in CST1 to 3 than in CST4 to 7, the difference was not statistically significant (Table 2; see Table S1). This discrepancy may be explained in part by the profile of Lactobacillus iners. For example, both L. iners and Lactobacillus vaginalis were associated with having a female partner with a normal Nugent score. However, L. iners was equally prevalent in CST1 to 3 and in CST4 to 7 (L. iners prevalence in CST1 to 3 of 22.8% and prevalence in CST4 to 7 of 9.4%; \( P = 0.046 \)). Taken together, our findings suggest that Lactobacillus associates consistently with a normal female partner Nugent score but variably with CSTs in a species-dependent manner (see Table S2 in the supplemental material).

In contrast, indicators of female partner Nugent-BV included Dialister, Mobiluncus, Prevotella, and Porphyromonas, which were significantly more prevalent in CST4 to 7 than in CST1 to 3 (\( P < 0.05 \)) (Table 2; see Table S1 in the supplemental material). Notably, even though Gardnerella is classically associated with BV, its prevalence was not significantly higher in CST4 to 7 (Table 2).

High proportional abundance of BV-associated bacteria in men with CST4 to 7. The proportional abundances of BV-associated bacteria were also significantly higher in CST4 to 7 than CST1 to 3, including Prevotella, Porphyromonas, and unclassified Clostridiales (\( P < 0.05 \)) (Table 2); however, Lactobacillus or Gardnerella again did not differ significantly across CST group (Table 2). Further analysis of CST1 to 3 and CST4 to 7 indicator bacteria recapitulated the microbiological link between BV-associated bacteria and CST4 to 7 (see Table S3 in the supplemental material).

Men with CST4 to 7 were more likely to have a partner with Nugent-BV. Men with CST4 to 7 were significantly more likely to have a partner with Nugent-BV than men with CST1 to 3 (CST4 to 7 versus CST1 to 3 prevalence rate ratio [PRR], 1.55; 95% confi-

| Parameter | No. (%) of participants (n = 165) |
|-----------|----------------------------------|
| Age, yr   |                                  |
| 15–19     | 2 (1.2)                          |
| 20–24     | 30 (18.2)                        |
| 25–29     | 50 (30.3)                        |
| 30–49     | 83 (50.3)                        |
| Partner bacterial vaginosis status (Nugent score) |                          |
| Normal (0–3) | 75 (45.4)                                  |
| Intermediate (4–6) | 30 (18.2)                                  |
| Bacterial vaginosis (7–10) | 60 (36.4)                                  |
| Marital status |                                  |
| Currently married, monogamous | 148 (89.7)                                  |
| Currently married, polygamous | 17 (10.3)                                  |
| No. of sexual partners in past yr |                                  |
| 1         | 93 (56.4)                        |
| 2         | 53 (32.1)                        |
| ≥3        | 19 (11.5)                        |
| Nonmarital sexual relationships |                                  |
| No        | 141 (85.5)                       |
| Yes       | 24 (14.3)                        |
| Condom use in past yr |                                  |
| None      | 105 (63.6)                       |
| Inconsistent use | 58 (35.2)                       |
| Consistent use | 2 (1.2)                        |
| Current condom use |                                  |
| No        | 148 (89.7)                       |
| Yes       | 17 (10.3)                        |
| Self-reported symptoms of sexually transmitted infection in past yr |                                  |
| Genital ulcer disease | 10 (6.0)                        |
| Urethral discharge | 4 (2.4)                        |
| Dysuria   | 7 (4.2)                          |
Likewise, men with CST4 to 7 were less likely to have a partner with a normal Nugent score than men with CST1 to 3 (CST4 to 7 PRR, 0.68; 95% CI, 0.48 to 0.97) (Table 3). In contrast, there was no significant association between intermediate Nugent-BV and CST4 to 7 (CST4 to 7 PRR, 1.21; 95% CI, 0.65 to 2.25) (Table 3). Thus, men with CST4 to 7 had higher abundance of BV-associated bacteria and were more likely to have a female partner with Nugent-BV.

**Link between sexual activity and CST4 to 7.** Compared to men with only marital sexual partner(s), having one nonmarital sexual partner was associated with a trend toward increased CST4 to 7 prevalence (CST4 to 7 PRR, 1.23; 95% CI, 0.79 to 1.92), and having two or more nonmarital sexual partners was associated with significantly increased CST4 to 7 prevalence (CST4 to 7 PRR, 1.84; 95% CI, 1.16 to 2.92) (see Table S4 in the supplemental material). Other sexual behaviors and urogenital symptoms were too infrequent to be investigated (Table 1). This association between CST4 to 7 and multiple nonmarital sexual partners is similar to the association between BV and the female-reported number of male nonmarital sexual partners (26).
TABLE 2 Prevalence and abundance of female partner Nugent score indicators in men with CST1 to 3 versus those with CST4 to 7

| Group and bacterium                      | Prevalence | Proportional abundancea |
|-----------------------------------------|------------|-------------------------|
|                                         | CST1 to 3 (n = 101), n (%) | CST4 to 7 (n = 64), n (%) | Chi-square | CST1 to 3 (n = 101), median % (IQR) | CST4 to 7 (n = 64), median % (IQR) | K-S P valueb |
| Nugent-BV indicator                     |            |                         |            |                                  |                                  |              |
| Dialister                               | 51 (50.5)  | 51 (79.7)               | <0.001     | 1.3 (0.5–2.9)                    | 1.8 (1.0–3.5)                    | 0.41         |
| Gardnerella                             | 40 (39.6)  | 23 (35.9)               | 0.76       | 0.7 (0.4–3.5)                    | 0.5 (0.2–1.2)                    | 0.44         |
| Mobiluncus                              | 22 (21.8)  | 45 (70.3)               | <0.001     | 0.5 (0.2–2.4)                    | 1.0 (0.3–2.5)                    | 0.22         |
| Peptostreptococcus                      | 38 (37.6)  | 31 (48.4)               | 0.23       | 2.1 (0.1–3.9)                    | 1.2 (0.3–2.5)                    | 0.15         |
| Porphyromonas                           | 60 (59.4)  | 61 (95.3)               | <0.001     | 2.7 (1.0–5.2)                    | 6.1 (3.0–10.3)                   | <0.001       |
| Prevotella                              | 81 (80.2)  | 63 (98.4)               | 0.001      | 16.0 (7.3–33.2)                  | 28.8 (17.2–41.9)                 | 0.003        |
| Sarcina fermentans                      | 9 (8.9)    | 40 (62.6)               | <0.001     | 0.06 (NA)c                       | 0.3 (0.2–1.3)                    | 0.5          |
| Sneathia                                | 16 (15.8)  | 12 (18.8)               | 0.79       | 0.2 (0.1–0.7)                    | 0.3 (0.2–2.0)                    | 0.44         |
| Treponema                               | 1 (1.0)    | 20 (31.3)               | <0.001     | 0.02 (NA)                        | 0.1 (0.4–1.6)                    | 0.57         |
| Unclassified Clostridiales              | 82 (81.2)  | 64 (100.0)              | <0.001     | 4.0 (0.8–18.7)                   | 22.8 (12.7–32.0)                 | <0.001       |
| Unclassified Clostridiales family XI    | 50 (49.5)  | 61 (95.3)               | <0.001     | 0.6 (0.3–1.0)                    | 0.9 (0.5–1.6)                    | 0.04         |
| Unclassified phyla                      | 20 (19.8)  | 39 (60.9)               | <0.001     | 0.2 (0.1–0.4)                    | 0.7 (0.2–1.3)                    | 0.01         |
| Normal Nugent score indicators          |            |                         |            |                                  |                                  |              |
| Corynebacterium                         | 90 (89.1)  | 29 (45.3)               | <0.001     | 7.3 (1.4–22.9)                   | 0.5 (0.2–1.2)                    | <0.001       |
| Lactobacillus                           | 40 (39.6)  | 19 (29.7)               | 0.26       | 2.6 (0.3–28.5)                   | 0.9 (0.2–7.4)                    | 0.44         |
| Staphylococcus                          | 55 (54.5)  | 0 (0.0)                 | <0.001     | 1.0 (0.1–3.5)                    | 0 (NA)                          | <0.001       |

a To better delineate the difference in proportional abundance from prevalence, only participants who carried a taxon (i.e., carriers) were included in the proportional abundance comparison.
b P value by Kolmogorov-Smirnov test.
c NA, not applicable.

Female partner Nugent-BV was associated with increased bacterial density among men with CST4 to 7. Among men with CST4 to 7, having a female partner with Nugent-BV was associated with a nearly 2-fold increase in bacterial density (men with partner Nugent-BV mean of 4.5 × 10^6 16S rRNA genes per swab versus men with partner normal Nugent score mean of 2.3 × 10^8 16S rRNA genes per swab; P = 0.06) (Fig. 2). However, the only Nugent-BV indicator that increased significantly in our quasi-Poisson models was Treponema (Δ^Nugent-BV−normal Nugent = +4.9 × 10^2 16S rRNA gene copies per swab; P = 0.01), while Gardnerella showed a borderline increase (Δ = +1.6 × 10^2; P = 0.09). We found no significant increases in other BV-associated bacteria (Fig. 3; see Table S5 and Fig. S2 in the supplemental material).

Female partner Nugent-BV was linked to decreased bacterial density among men with CST1 to 3. Some men also had CST1 to 3, despite having a female partner with Nugent-BV (n = 30/101 [29.7%]). Their penile bacterial densities were 6.5-fold lower on average than those of men whose partner had a normal Nugent score (partner Nugent-BV mean of 8.2 × 10^6 versus partner normal Nugent score mean of 5.3 × 10^7 16S rRNA gene copies per swab; P = 0.04) (Fig. 2). The decrease reflects the lower abundances of Corynebacterium (Δ^Nugent-BV−normal Nugent = −6.0 × 10^3 16S rRNA gene copies per swab; P = 0.05), Staphylococcus (Δ = −2.2 × 10^3; P = 0.007), and Lactobacillus (Δ = −4.5 × 10^2; P = 0.007), based on quasi-Poisson models (see Fig. S3 and Table S5 in the supplemental material).

We also found that female partner Nugent-BV was associated with small but significant increases in 10 of the 12 Nugent-BV indicator species. These 10 Nugent-BV indicators included Prevotella (Δ^Nugent-BV−normal Nugent = +4.6 × 10^3 16S rRNA gene copies per swab; P = 0.002), Porphyromonas (Δ = +5.9 × 10^4; P = 0.005), Dialister (Δ = +1.4 × 10^2; P < 0.001), and unclassified Clostridiales (Δ = +1.6 × 10^2; P = 0.02) (Fig. 3; see Table S5 and Fig. S2 in the supplemental material).

DISCUSSION

Our findings suggest that BV may be sexually transmissible, as indicated by the association between BV-associated bacteria in men and the Nugent score BV of their female partners. Sexual transmissibility of BV is also consistent with the link between multiple partners and BV-associated bacteria in men, which parallels the association between sexual behaviors and BV risk in women (23, 26–28). Sexual transmission of BV may also explain why both topical and oral antimicrobials lack long-term efficacy against BV, since BV-associated bacteria may be reintroduced from the suprapelvic space to the vagina through intercourse.

TABLE 3 Prevalence of female partner Nugent-BV by coronal sulcus community state type

| CST group | Nugent-BV (7–10) | Normal (0–3.0) | Intermediate (4.0–6.0) |
|-----------|------------------|----------------|------------------------|
| CST4 to 7 | (n = 64)         |                |                        |
|           | 30 (56.6)        | 23 (43.4)      | 11 (17.2)              |
|           | 1.55 (1.07–2.24) | 0.68 (0.48–0.97)| 1.21 (0.65–2.25)       |
| CST1 to 3 | (n = 101)        |                |                        |
|           | 30 (36.6)        | 52 (63.4)      | 19 (18.8)              |
In our study of 165 Ugandan men, BV-associated bacteria were prevalent and abundant in the subpreputial space, and the abundance of these bacteria was significantly associated with the Nugent scores of their female partners, except in the instance of intermediate Nugent scores. Together, these findings indicate that the subpreputial space could be an important niche for BV-associated bacteria in men. Earlier studies reported that the subpreputial space and distal urethra can harbor BV-associated bacteria (5, 6), and circumcision can reduce female partner BV (11).

The human microbiota can be highly dynamic; thus, the term “community state type” (CST) was coined to reflect the transitional nature of human microbiota (25). We showed that the penile microbiota are conserved assemblages of genital bacteria that could be represented by seven community state types. In uncircumcised men, the penile CSTs ranged from low bacterial density comprised primarily of skin-associated bacteria to high bacteria density with abundant BV-associated bacteria and other genital anaerobes.

Given the suspected sexual transmission of BV from this and earlier studies (23, 26–28), the most parsimonious explanation for the association between penile microbiota and female partner Nugent score BV is a bidirectional exchange of genital bacteria. While a simple bacterial exchange (i.e., the transfer of genital bacteria from one partner and consequent establishment in the other) may be a sufficient model to explain the subpreputial bacterial density among men with a BV partner in the high-density group (CST4 to 7) (BV group mean of \(4.5 \times 10^6\) versus normal group mean of \(2.3 \times 10^6\) 16S rRNA gene copies per swab; \(P = 0.06\)). In contrast, there was a 5-fold bacterial density decrease in the low-density CST group (CST1 to 3) (BV group mean of \(8.2 \times 10^6\) versus normal group mean of \(5.3 \times 10^7\) 16S rRNA gene copies per swab; \(P = 0.04\)).

If BV is redefined as a sexually transmitted condition, it has the potential to expand the infectious disease framework from transmission of single pathogens to encompass transmission of bacterial communities. This could affect clinical care of BV and justify new preventative and treatment strategies, such as prebiotic, probiotics, or narrow-spectrum antimicrobials to modify the penile microbiota. It will also be important to determine if our findings could be generalized to non-Ugandan populations. Previous studies suggest that antimicrobial resistance and poor drug penetration can challenge decolonization efforts (41–45). However, study design limitations—including, suboptimal treatment regimen, insufficient randomization methods, limited power, and unknown adherence—may have contributed to previous failed attempts to decrease BV by decolonizing male sexual partners (46).
remained HIV negative together with their partner(s) during the trial, 165 samples were selected at random for this study.

The female partners were enrolled into a parallel study (48) and provided self-collected vaginal swabs, which were evaluated by Nugent’s criteria and scored as normal (Nugent score of 0 to 3), intermediate (Nugent score of 4 to 6), or BV (Nugent score of 7 to 11). Among the 17 polygamous men, 15 only had one female partner enrolled. The female partners for the two other polygamous men had concordant BV assignments. Male herpes simplex virus 2 and syphilis serology were assessed as previously described (5).

**Human subject research.** This study was approved by four institutional review boards: the Science and Ethics Committee of the Uganda Virus Research Institute (Entebbe, Uganda), the National Council for Science and Technology (Kampala, Uganda), the Committee for Human Research at Johns Hopkins University’s Bloomberg School of Public Health (Baltimore, MD), and the Western Institutional Review Board (Olympia, WA).

**Sample processing.** From each sample, 100 μl of the swab eluent was lysed using pressure-cycling technology (Pressure Biosciences, South Easton, MA), purified using the QiaGen AllPrep DNA/RNA minikit (Qiagen, Valencia, CA), and eluted using 100 μl of buffer EB as previously described (5).

**Penile microbiota bacterial density characterization.** Using the purified DNA, we quantified penile microbiota bacterial density, measured as 16S rRNA gene copies per microliter of swab eluent using a broad-range quantitative PCR (qPCR) targeting the 16S rRNA gene (V3 to V6) as previously described (5). The resultant pyrosequences were chimera checked, de-multiplexed, quality checked, and classified taxonomically as previously described (25). We obtained a total of 202,241 reads, with a mean of 1,210 reads per sample (SD, 973; range, 124 to 5,143; median, 930). Taxonomic groups with a single sequence were excluded. *Clostridiales* and *Clostridiales* family XI sequences classified at a <80% bootstrap confidence level were reported as unclassified *Clostridiales* and unclassified *Clostridiales* family XI, respectively. A detailed description of bioinformatics analysis can be found in Text S1 in the supplemental material.

**Penile bacterial prevalence, proportional abundance, and absolute abundance calculation.** We characterize the penile microbiota by sequencing the bacterial 16S rRNA gene (V3 to V6) using the same qPCR V3F primer on GS FLX (454 Life Sciences, Branford, CT) as previously described (5). The resultant pyrosequences were chimeras checked, de-multiplexed, quality checked, and classified taxonomically as previously described (5) and specifically for *Lactobacillus* species as previously described (9). Extensive demographic and sexual activity data were collected by interview (47).

**RESULTS**

In the coronal sulcus of men with male partner Nugent-BV, *Porphyromonas* showed a borderline increase (Δ = −0.9×10^3; P = 0.09), and a 16.4-fold increase in *Prevotella* (Δ = +4.9×10^5; P = 0.01) was the only BV-associated bacterium that had significantly higher absolute abundance in men whose female partner had a normal Nugent score. The predicted increases in BV-associated bacteria included an 18.2-fold increase in *Prevotella* (Δ = +4.6×10^9 16S rRNA gene copies per swab; P = 0.01), a 60.3-fold increase in *Porphyromonas* (Δ = +0.9×10^6; P = 0.09), and a 16.4-fold increase in *Diaister* (Δ = +1.4×10^9; P = 0.004), while the abundance of *Lactobacillus* was 12.2-fold lower in men with partner Nugent-BV (Δ = −9.3×10^5; P = 0.01). Among men with CST4 to 7, *Treponema* (Δ = +4.9×10^5; P = 0.01) was the only BV-associated bacterium that had significantly higher absolute abundance in men with female partner Nugent-BV than men whose female partner had a normal Nugent score. *Gardnerella* showed a borderline increase (Δ = −1.6×10^4; P = 0.09).

**FIG 3** Association between female partner Nugent-BV and Nugent-BV indicator in the coronal sulcus based on quasi-Poisson models, stratified by CST. The influence of partner Nugent-BV on coronal sulcus microbiome was especially visible among men with CST1 to 3. In this group, our quasi-Poisson models showed that men with female partner Nugent-BV had significantly higher absolute abundance of BV-associated bacteria than men whose female partner had a normal Nugent score. The predicted increases in BV-associated bacteria included an 18.2-fold increase in *Prevotella* (Δ = +4.6×10^9 16S rRNA gene copies per swab; P = 0.01), a 60.3-fold increase in *Porphyromonas* (Δ = +0.9×10^6; P = 0.09), and a 16.4-fold increase in *Diaister* (Δ = +1.4×10^9; P = 0.004), while the abundance of *Lactobacillus* was 12.2-fold lower in men with partner Nugent-BV (Δ = −9.3×10^5; P = 0.01). Among men with CST4 to 7, *Treponema* (Δ = +4.9×10^5; P = 0.01) was the only BV-associated bacterium that had significantly higher absolute abundance in men with female partner Nugent-BV than men whose female partner had a normal Nugent score. *Gardnerella* showed a borderline increase (Δ = −1.6×10^4; P = 0.09).
nities across human subjects. The 50 most prevalent coronal sulcus bacteria, comprising 99.7% of total sequences, were included in the analysis.

**Microbiota CST assignment by hierarchical clustering.** To identify community state types (CSTs), we used hierarchical clustering by Ward linkage in Euclidean distance using the *cutree* algorithm through an iterative process as previously described (25). Comparisons of the 6-, 7-, and 8-CST solutions revealed the 7-CST solution to be the most parsimonious and effective. We further divided the seven CSTs into two major strata—CST1 to 3 and CST4 to 7—based on the first bifurcation of the clustering dendrogram. Bacterial densities among CSTs were compared using analysis of variance.

**Identification of indicator taxa for CST, major CST strata, and female partner Nugent score.** We used indicator analysis to identify penile bacteria uniquely associated with CSTs and female partner Nugent score (52). The indicator species analysis is an objective assessment of a particular genus’ representation in an environment or a study group. A genus’ indicator value (IV) for a study group is determined based on its proportional abundance and prevalence in the given study group. The IV can range from 0 to 1, with 0 as no indication to 1 as perfect indication. To test the null hypothesis of no difference between our observation and what might be observed by chance, we built IV null distributions by the Monte Carlo procedure using 1,000 resampled data sets with randomized study group assignments. We determined the P value for each observed IV based on its location within the null distribution and adjusted for false discovery. A significance level of α = 0.10 was used.

**Assessment of relationship between female partner Nugent-BV and penile microbiota across CST strata.** We compared the prevalence of female partner Nugent-BV versus normal Nugent score in CST1 to 3 versus CST4 to 7 based on prevalence rate ratio (PRR) and its 95% confidence interval (CI) by the Breslow test of heterogeneity in EpiR (version 0.9 to 48) (53). We then compared partner Nugent-BV indicator prevalence and proportional abundance in CST1 to 3 versus CST4 to 7. We compared female partner Nugent score indicator and *Lactobacillus* species prevalence by chi-square test. Due to the large differences in prevalence, we only included participants with the taxon in the proportional abundance comparisons, which we performed using the Kolmogorov-Smirnov test. A significance level of α = 0.05 was used.

**Assessment of relationship between female partner Nugent-BV and penile microbiota within CST stratum.** We examined the association between penile microbiota and female partner Nugent-BV (normal and Nugent-BV) within each CST stratum. We compared the bacterial density using a two-tailed t test with unequal variance, after excluding outliers based on the interquartile range rule. We compared Nugent-BV indicator absolute abundance using a quasi-Poisson model to predict each indicator’s absolute abundance by female partner Nugent-BV status for each CST stratum. Polygamy and extramarital relationship were included in the starting model. The predicted absolute abundances were presented as circle plots, where the area of the circle is proportional to mean absolute abundance. Detailed description of the statistical analyses can be found in Text S1 in the supplemental material.

**Nucleotide sequence accession number.** Sequence data have been deposited in GenBank under accession no. SRP058681.

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at http://mbio.asm.orglookup/suppl/doi:10.1128/mBio.00589-15/-/DCSupplemental.

Text S1, DOCX file, 0.04 MB.
Figure S1, TIF file, 2.6 MB.
Figure S2, PDF file, 0.3 MB.
Figure S3, PDF file, 0.2 MB.
Table S1, DOCX file, 0.02 MB.
Table S2, DOCX file, 0.02 MB.
Table S3, DOCX file, 0.02 MB.
Table S4, DOCX file, 0.01 MB.
Table S5, DOCX file, 0.02 MB.

**ACKNOWLEDGMENTS**

This work was provided by R01AI087409-01A1 and U01AI151711 from the National Institutes of Health, the Bill and Melinda Gates Foundation (22006.02), and the Doris Duke Charitable Foundation (no. 2011036). C.M.L. was supported by the Northern Arizona University Technology and Research Initiative Fund (TRIF) and the Crowden Endowment in Microbiology at Northern Arizona University. A.A.R.T. was supported by NIH 1K23AI093152-01A1 and the Doris Duke Charitable Foundation Clinician Scientist Development Award.

The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the funding agencies.

We thank Allison Abraham for assistance with epidemiological methods.

D.S., G.K., R.M.G., F.N., M.J.W., and R.H.G. conducted the field study. F.N. and J.L.P. contributed to metadata collection. C.M.L. contributed to the laboratory analysis. C.M.L., B.A.H., P.K., and L.B.P. contributed to data analysis. C.M.L. drafted the manuscript. B.A.H., A.A.T., J.R., J.L.P., P.K., M.J.W., R.H.G., and L.B.P. contributed to revisions of the manuscript. All authors have reviewed the manuscript.

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