Original Contribution

Association of Recent Bacterial Vaginosis With Acquisition of *Mycoplasma genitalium*

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We assessed the association between recent bacterial vaginosis (BV) and incident *Mycoplasma genitalium*, a sexually transmitted bacterium associated with adverse female reproductive health outcomes. Female sex workers in Mombasa, Kenya, completed a monthly sexual behavior interview and clinical examination. During February 2005–February 2006, vaginal fluid specimens collected from women every other month were tested for *M. genitalium* by nucleic acid amplification testing. Vaginal microbiota were assessed monthly and categorized by Nugent score (0–3 = normal microbiota, 4–6 = intermediate microbiota disruption, and 7–10 = BV). A discrete failure time analysis for multiple events using logistic regression was employed to estimate the odds of incident *M. genitalium* infection at follow-up visits among women with BV (vs. normal microbiota) at the preceding visit. Among the 280 women, 54.3% were positive for human immunodeficiency virus. At baseline, 16.1% had prevalent *M. genitalium* infection and 40.4% had prevalent BV. There were 59 incident *M. genitalium* infections among 50 women, for an incidence rate of 34.6 cases per 100 person-years. Following adjustment for age, human immunodeficiency virus status, and time, prior BV was associated with a 3.5-fold increase in odds of incident *M. genitalium* (adjusted odds ratio = 3.49, 95% confidence interval: 1.86, 6.56). This strong association suggests that BV may enhance susceptibility to *M. genitalium* infection.

bacterial vaginosis; *Mycoplasma genitalium*; Nugent score; vaginal microbiota

Abbreviations: aOR, adjusted odds ratio; BV, bacterial vaginosis; CI, confidence interval; FSW, female sex worker; HIV, human immunodeficiency virus; HSV-2, herpes simplex virus type 2; IQR, interquartile range; STI, sexually transmitted infection.

Mycoplasma genitalium, a sexually transmitted bacterium, has been associated with increased risk of adverse female reproductive health outcomes, including mucopurulent cervicitis, pelvic inflammatory disease, infertility, and preterm birth (1), as well as increased risk of human immunodeficiency virus (HIV) infection (2–4). However, little is known about factors that increase susceptibility to *M. genitalium* infection. Bacterial vaginosis (BV), which is present in approximately 11%–37% of women (5–9), has been associated with increased risk of acquiring other sexually transmitted infections (STIs) (10–16) and may also be associated with increased risk for *M. genitalium*. However, data are limited and inconsistent, with some studies demonstrating increased risk of *M. genitalium* infection among women with

BV (2, 17, 18), one demonstrating decreased risk (19), and others demonstrating no relationship (20–22).

One potential explanation for conflicting evidence about the association between BV and *M. genitalium* is inadequate assessment of the temporal relationship. Most prior analyses employed cross-sectional study designs (2, 17, 19–21). Furthermore, in the 2 published longitudinal studies (18, 22), time between BV assessment and *M. genitalium* testing ranged from several months to over a year. This may not be the correct time frame in which to assess an etiological link, as levels of vaginal microbiota fluctuate over time (23, 24).

A second explanation for these conflicting results may be differing methods of assessing BV, which is typically diagnosed using either clinical (Amsel's) or microbiological

(Nugent) criteria. Under the criteria of Amsel et al. (25), BV is defined as 3 of the following 4 signs: 1) thin, homogenous, white vaginal discharge; 2) clue cells on wet mount; 3) amine odor when exposed to a drop of 10% potassium hydroxide; and 4) pH > 4.5. Amsel's criteria, however, fail to capture women who have disrupted vaginal microbiota but do not have clinically detectable signs. In contrast, a Nugent score is derived by scoring Gram-stained vaginal smears based on the quantity of *Lactobacillus* morphotypes, Gram-variable rods, and curved rods, allowing for assessment of vaginal microbiota in the absence of clinical signs. The Nugent score (26) represents a continuum of increasing disruption of vaginal microbiota, with scores of 0–3 being classified as normal microbiota, scores of 4–6 as intermediate, and scores of 7–10 as BV. Most studies have compared women with BV (score ≥7) to women without BV (score 0–6), which combines the normal and intermediate categories. However, increasing evidence suggests that an intermediate level of microbiota disruption is associated with adverse outcomes (27, 28). Therefore, this categorization may miss the contribution of milder disruptions of the microbiota to adverse outcomes, such as STI acquisition, attenuating estimates of an underlying association.

Given the high prevalence of BV and adverse outcomes associated with *M. genitalium*, it is critical to determine whether BV enhances a woman's susceptibility to *M. genitalium*. To address this question, we investigated the association between recent BV and incident *M. genitalium* infection in a longitudinal study with frequent assessment of the vaginal microbiota using the Nugent score.

METHODS

Study population

Participants were women in the Mombasa Cohort, a prospective open cohort study of female sex workers (FSWs) in Mombasa, Kenya, with ongoing enrollment. The cohort was created in 1993 to identify risk factors for HIV and STI acquisition (29). There is no set follow-up period. Women were enrolled in the cohort if they were at least 16 years of age and reported exchanging sex for cash or in-kind payments.

The Kenyatta National Hospital/University of Nairobi Ethics and Research Committee and the University of Washington Institutional Review Board approved the study protocol. All participants provided written informed consent for participation.

Clinic procedures, specimen collection, and laboratory methods

At enrollment in the cohort and at monthly follow-up visits, women underwent a standardized face-to-face interview eliciting information on demographic and clinical characteristics and sexual behaviors. A clinical examination was performed at each visit, including collection of vaginal and cervical specimens for STI diagnosis. Women with STI symptoms were treated syndromically according to Kenyan national guidelines, which follow the World Health Organization's guidelines for syndromic management (30). Additional treatment for STIs was

based on laboratory results. Asymptomatic BV was not treated, as there is currently no indication for treatment in asymptomatic nonpregnant women. HIV-positive women were offered antiretroviral therapy according to Kenyan national guidelines, which recommended treatment for persons with a CD4-positive T-lymphocyte (CD4 cell) count less than 200 cells/mm^3 during the study period.

Vaginal swabs were collected for Gram staining and Nugent scoring (26). Cervical swab specimens were inoculated onto modified Thayer-Martin media for *Neisseria gonorrhoeae* culture. Cervicitis was defined as an average of ≥30 polymorphonuclear leukocytes in 3 high-power microscopic fields on a Gram-stained slide of cervical secretions. Trichomoniasis was diagnosed by detection of motile trichomonads on wet mount microscopy or positive culture of vaginal swab specimens inoculated into Diamond's media. In HIV-negative women, herpes simplex virus type 2 (HSV-2) was detected in serum using the HerpeSelect 2 ELISA immunoglobulin G test (Focus Diagnostics, Cypress, California) (31), with an optical density index value greater than 1.1 indicating positivity. Given the high prevalence of HIV/HSV-2 coinfection in this cohort (32), HIV-positive women did not undergo routine HSV-2 testing. Infection with HIV type 1 (HIV-1) was detected in serum using an enzyme-linked immunosorbent assay (Detect HIV-1/2; BioChem Immunosystems, Montreal, Quebec, Canada) and confirmed using a second enzyme-linked immunosorbent assay (Recombigen; Cambridge Biotech, Worchester, Massachusetts). In HIV-positive women, quantitation of CD4 cell count was performed every 3 months (FACScount; Becton Dickinson, San Jose, California).

Between February 2005 and February 2006, additional vaginal swabs were collected and stored at −80°C. Financial constraints prohibited testing of all specimens; therefore, we randomly sampled women for *M. genitalium* testing from among those with 2 or more additional vaginal specimens (approximately 1:1 HIV-positive to HIV-negative). Specimens from visits made every other month were shipped to Seattle, Washington, for testing with the Aptima TMA assay (Hologic, Inc., San Diego, California) using analyte-specific reagents for *M. genitalium*. Because testing was conducted several years after sample collection, women were not specifically treated for *M. genitalium*.

Measures

Prevalent *M. genitalium* was defined as a positive *M. genitalium* test at the baseline study visit. Incident *M. genitalium* was defined as a positive test at any follow-up visit that had been preceded by a negative *M. genitalium* test at the most recent prior visit. Persistent *M. genitalium* was defined as a positive test at more than 1 sequential visit with *M. genitalium* testing. Time to clearance of incident infections was defined as the number of days from the beginning of infection (midpoint between negative and positive tests) to the end of infection (midpoint between the last positive and first negative tests). Disruption of the vaginal microbiota was characterized by Nugent score (0–3 = normal microbiota (reference group), 4–6 = intermediate microbiota disruption, and 7–10 = BV). BV was considered persistent if it was detected at more than 1 sequential visit and recurrent if there

was at least 1 visit with normal or intermediate microbiota between visits with BV.

Statistical methods

Baseline demographic, clinical, and sexual behavior characteristics of women with and without prevalent BV (Nugent scores of ≥7 and <7, respectively) and prevalent *M. genitalium* were compared using Fisher's exact test for categorical variables and Wilcoxon's rank-sum test for continuous variables to assess statistical significance. *M. genitalium* incidence and 95% confidence intervals were estimated using Poisson regression with robust standard errors (clustering by woman), allowing for multiple incident infections. Time at risk was defined using the beginning and end of *M. genitalium* infections as defined above. In sensitivity analyses, we also calculated *M. genitalium* incidence after excluding women with prevalent infections and censoring women after their first incident infection.

To evaluate the relationship between BV (either new or recurrent) at the visit prior to *M. genitalium* testing and incident *M. genitalium*, we employed a discrete failure time analysis for multiple events using logistic regression, clustering by woman ([33]). Women without *M. genitalium* at baseline entered the risk set at the first visit with exposure data (BV assessment at prior visit); women with prevalent *M. genitalium* at baseline entered the risk set at their first negative *M. genitalium* test. Women with incident infections reentered the risk set after their first negative *M. genitalium* test. All women were censored after their last *M. genitalium* test.

The models adjusted a priori for age (years; continuous), HIV-1 status, and time (visit number). Hormonal contraceptive use, presence of other STIs (*Trichomonas vaginalis, N. gonorrhoeae*), number of sex partners in the last week, frequency of protected and unprotected sex in the last week (no sex, all sex acts protected, some sex acts protected, no acts protected), and receipt of a doxycycline or metronidazole prescription at the prior visit were assessed as potential confounding factors. Variables associated with *M. genitalium* ($P < 0.2$) in bivariate models were added to the multivariable model one at a time and subsequently removed if the odds ratio for the association between BV and incident *M. genitalium* changed by less than 10% (after adjustment for age, HIV status, and time). No additional characteristics changed the estimates appreciably, and none were included in the final model. A term for interaction between BV and HIV-1 status was tested but was not statistically significant (Wald test: $P = 0.87$) and therefore not retained. We also performed a linear trend test to evaluate the relationship between increasing Nugent score (continuous) and the odds of incident *M. genitalium* infection. Data were analyzed using Stata 13.0 (StataCorp LP, College Station, Texas). Statistical significance was defined as $P < 0.05$ (2-sided) for all analyses.

To further investigate the timing of BV episodes, we conducted a secondary analysis using the same discrete failure time analysis approach and categorized women on the basis of their BV status at both the visit with *M. genitalium* testing and the visit prior to *M. genitalium* testing. Women without BV at either visit served as the referent group and were compared with 3 groups of women: 1) no BV at the prior visit but BV at the visit with *M. genitalium* testing; 2) BV at the prior visit but no BV at the visit with *M. genitalium* testing; and 3) BV at both the visit prior to and the visit with *M. genitalium* testing. For this analysis, women with intermediate microbiota were excluded.

RESULTS

Baseline

These analyses included 280 women with 2 or more *M. genitalium* tests during follow-up. The median age of participants was 35.3 years (interquartile range (IQR), 31.3–39.2), and 54.3% (152/280) were HIV-positive (Table 1). At baseline for this study, 40.4% (113/280) of women had BV, 18.2% (51/280) had intermediate microbiota disruption, and 41.4% (116/280) had normal vaginal flora. In addition, 16.1% had *M. genitalium* infection. Women with BV (Nugent score ≥7) were more likely to be HIV-positive than women without BV (Nugent score 0–6) (63.7% vs. 47.9%; $P = 0.01$). Similarly, women with *M. genitalium* were more likely to be HIV-positive (68.9% vs. 51.5%; $P = 0.04$) and younger (median age, 33.5 years vs. 35.5 years; $P = 0.003$) than those without *M. genitalium*. The distributions of all other baseline factors were similar between women with and without *M. genitalium* and with and without BV.

Follow-up

Participants contributed 2,454 study visits during 196.9 person-years of observation between February 2005 and February 2006. The median number of visits per woman was 10 (IQR, 5–12), and the median time between visits was 29 days (IQR, 28–34). After excluding time in which women had *M. genitalium* infections, there were 170.5 person-years at risk for acquiring *M. genitalium*.

BV was detected at least once in 73.9% (207/280) of women at 38.4% (940/2,448) of visits with BV data. Only 8.4% (79/940) of visits with BV were accompanied by symptoms (vaginal discharge and/or itching), and metronidazole was prescribed at only 2.6% (24/940) of visits with BV. Among women with BV, 55.1% (114/207) had at least 1 recurrence of BV, with a maximum of 4 recurrences.

During a median follow-up time of 318.5 days, 50 women experienced an incident *M. genitalium* infection, and 9 women experienced 2 episodes, for a total of 59 incident infections and an incidence rate of 34.6 per 100 person-years (95% confidence interval (CI): 26.0, 42.0). In sensitivity analyses excluding women with prevalent *M. genitalium* infections and censoring women after their first incident infection, the incidence rate was 25.0 per 100 person-years (95% CI: 18.2, 34.4). Of the 104 *M. genitalium* infections (prevalent or incident), 45 (43.3%) were persistent, and the maximum duration of infection was 332.5 days. The median time to clearance of incident *M. genitalium* infections was 85.5 days (IQR, 59.5–183). Although time to clearance was longer for HIV-positive women, this difference was not statistically significant (Figure 1; log-rank $P = 0.09$). Doxycycline was prescribed at 2.5% (54/2,174) of follow-up visits for syndromic treatment of STI, but not specifically for *M. genitalium*.

Table 1. Baseline Demographic, Clinical, and Sexual Behavior Characteristics of 280 Female Sex Workers Enrolled in a Study of Bacterial Vaginosis and Incident *Mycoplasma genitalium* Infection Between February 2005 and February 2006, Mombasa, Kenya

Baseline Characteristic	No. of Women	%	Median (IQR)
Demographic characteristics			
Age, years			35.3 (31.3–39.2)
Smoking, cigarettes/week[a]			
0	247	88.2	
1–9	27	9.6	
≥10	6	2.1	
Alcohol consumption, drinks/week			
0	61	21.8	
1–7	147	52.5	
≥8	72	25.7	
Clinical characteristics[b]			
M. genitalium	45	16.1	
Vaginal microbiota			
Bacterial vaginosis (Nugent score ≥7)	113	40.4	
Intermediate disruption (Nugent score 4–6)	51	18.2	
Normal flora (Nugent score 0–3)	116	41.4	
HIV-1 seropositivity	152	54.3	
CD4 cell count, cells/mm^{3c}			308 (195–482)
Initiation of antiretroviral therapy (ever)[d]	20	13.2	
HSV-2 seropositivity	148	92.5	
Trichomonas vaginalis	10	3.6	
Neisseria gonorrhoeae	2	0.7	
Cervicitis[e]	10	3.6	
Vaginal washing in past week			
None	8	2.9	
Water	60	21.6	
Soap/other[f]	210	75.5	
Method of contraception			
None	185	66.3	
Oral contraceptive pills	19	6.8	
Medroxyprogesterone acetate injectable	53	19.0	
Intrauterine device	2	0.7	
Implant	6	2.2	
Other	14	5.0	
Hormonal contraception	80	28.7	
Vaginal itching/burning	24	8.6	
Vaginal discharge	8	2.9	
Sexual behavior			
No. of sex partners in past week[g]			1 (0–1)
Frequency of sex in past week			1 (0–2)

Table continues

Table 1. Continued

Baseline Characteristic	No. of Women	%	Median (IQR)
Protected and unprotected sex in past week[h]			
No sex	128	45.7	
All sex acts protected	104	37.1	
Some sex acts protected	9	3.2	
No sex acts protected	39	13.9	

Abbreviations: HIV, human immunodeficiency virus; HIV-1, human immunodeficiency virus type 1; HSV-2, herpes simplex virus type 2; IQR, interquartile range.

[a] Participants were asked about smoking and alcohol use at enrollment in the Mombasa female sex worker cohort. Median time from enrollment to baseline for this study was 2.9 years (IQR, 0.82–7.76).

[b] Baseline data were available for 142 participants for CD4 cell count, for 160 participants for HSV-2, for 279 participants for *N. gonorrhoeae* infection, method of contraception, and hormonal contraception, and for 278 for cervicitis and vaginal washing in the past week.

[c] Quantitation of CD4 cell count was performed every 3 months. For HIV-positive participants who did not have a measured CD4 cell count at the baseline of this study (dates of which varied by woman), we carried forward their most recent Mombasa cohort CD4 cell count to baseline.

[d] HIV-positive participants who had ever initiated antiretroviral therapy (receipt of antiretroviral therapy at the Mombasa clinic or self-report of receipt of antiretroviral therapy at another location) by baseline of this study.

[e] ≥30 cervical peripheral mononuclear cells.

[f] "Other" includes detergent and antiseptic solution.

[g] "Number of partners" includes both regular partners and paying partners.

[h] "Protected sex" was defined as condom use during the sex act.

Recent BV and incident *M. genitalium*

In bivariate analysis, compared with women with normal vaginal microbiota (Nugent score 0–3) at the visit prior to *M. genitalium* testing, BV (Nugent score ≥7) at the preceding visit was associated with a 3.5-fold increase (odds ratio = 3.48, 95% CI: 1.87, 6.48) in the odds of incident *M. genitalium* infection (Table 2). Intermediate microbiota disruption (Nugent score 4–6) was associated with a 1.7-fold increase in the odds of incident *M. genitalium* infection (odds ratio = 1.70, 95% CI: 0.69, 4.19), but this was not statistically significant. After adjustment for age, HIV status, and time (visit number), BV at the prior visit remained associated with an increase in the odds of subsequent incident *M. genitalium* infection (adjusted odds ratio (aOR) = 3.49, 95% CI: 1.86, 6.56). Considering the continuous Nugent score, the odds of incident *M. genitalium* increased by 16% for each incremental point increase, after adjustment (aOR = 1.16, 95% CI: 1.07, 1.26; *P*-trend < 0.001).

Secondary analysis

Relative to women with normal microbiota at 2 sequential visits, BV at the prior visit but not at the visit with *M. genitalium* testing was associated with substantially increased odds of *M. genitalium* infection (aOR = 4.51, 95% CI: 1.76, 11.6), as was BV at both the prior visit and the visit with *M. genitalium* testing (aOR = 3.93, 95% CI: 1.87, 8.27), after
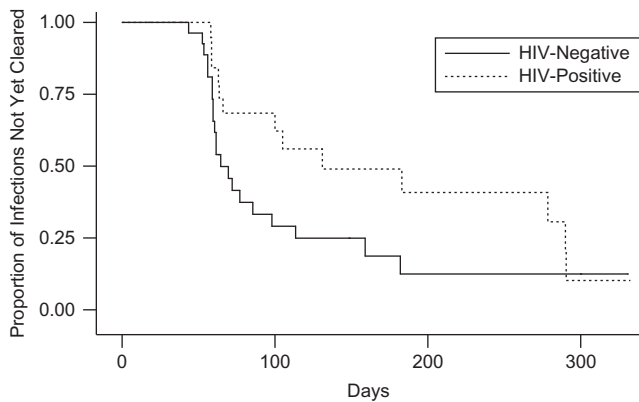
Figure 1. Number of days to clearance of incident *Mycoplasma genitalium* infection by human immunodeficiency virus (HIV) status among female sex workers in Mombasa, Kenya, 2005–2006. The Kaplan-Meier plot includes all 59 incident *M. genitalium* infections (30 HIV-negative and 29 HIV-positive, allowing for multiple incident infections). Infections were censored at the first negative test (clearance) or at the woman's exit from the study, whichever occurred first. At day 100, 7 HIV-negative and 11 HIV-positive women remained infected and in follow-up. At day 200, 2 HIV-negative and 5 HIV-positive women remained infected and in follow-up. At day 300, only 3 women under follow-up (2 HIV-negative, 1 HIV-positive) had not yet cleared their infection. Fifty percent (25/50) of women with any incident infection had a positive *M. genitalium* test at their last study visit. Only 5 of the HIV-positive women had baseline CD4 cell counts less than 200 cells/mm^3.

adjustment (Table 3). In contrast, there was no significant increase in the odds of *M. genitalium* when BV was identified only at the visit with *M. genitalium* testing.

DISCUSSION

In this cohort of high-risk Kenyan women, the prevalences of *M. genitalium* (16.1%) and BV (40.4%) at baseline were high. The incidence of *M. genitalium* infection was also high, at nearly 35 per 100 person-years. Women with BV had 3.5 times the odds of acquiring *M. genitalium* by their next study visit compared with women with normal vaginal microbiota. The odds of incident *M. genitalium* infection increased by 16% with each 1-point increase in the Nugent score, suggesting a dose-response effect of vaginal dysbiosis on the odds of acquiring *M. genitalium*.

The incidence of *M. genitalium* in this study was higher than in previous observations. In low-risk populations of women in the United Kingdom and Australia, *M. genitalium* incidence rates ranged from a cumulative annual incidence of 0.9% to a rate of 1.3 per 100 person-years (18, 34). In higher-risk populations in Kenya and the United States, incidence ranged from 22.7 per 100 person-years to 33.6 per 100 person-years (22, 35). Unlike the case in previous studies, we used a recurrent event analysis, allowing women to have multiple incident infections over the course of follow-up. This may partially explain our higher observed incidence rate.

The median time to clearance of *M. genitalium* infection in these women (85.5 days or 2.8 months) was also higher than

that observed among FSWs in either Nairobi, Kenya (median duration, 1 month) (22) or Kampala, Uganda (median time to clearance, 2.1 months) (36). This difference may be partly due to our bimonthly sampling interval for *M. genitalium* testing. More frequent *M. genitalium* testing may have increased our ability to detect a shorter time to clearance. Antibiotic use may also have influenced time to clearance, and more women in the Nairobi and Kampala studies received antibiotics for other conditions during follow-up than women in our study (22, 36). The higher median time to *M. genitalium* clearance among HIV-positive women that we observed was consistent with results seen among Ugandan FSWs (36), but our finding was not statistically significant, probably reflecting low statistical power.

The strong association between BV and incident *M. genitalium* that we observed when women with BV were compared with women with normal microbiota (Nugent score ≥7 vs. 0–3), as well as the modest association between intermediate microbiota (Nugent score 4–6) and incident *M. genitalium*, suggests that some of the inconsistencies between prior studies may have been due to differences in BV measurement. Prior studies categorized vaginal microbiota disruption in a binary manner (BV vs. no BV), whether diagnosing BV clinically (2, 19, 21) or by scoring Gram stains of vaginal secretions (17, 18, 20, 22). In studies utilizing the Nugent score (17, 18, 22), investigators did not explicitly state how intermediate microbiota disruption was incorporated into their definition of BV, nor did they report the prevalence of intermediate microbiota. However, binary categorizations of vaginal microbiota typically classify women with intermediate microbiota as normal, potentially obscuring a relationship between BV and *M. genitalium*. In addition, studies in which BV was defined by clinical diagnosis (2, 19, 21) may have misclassified women with disrupted vaginal microbiota unaccompanied by clinical signs, again obscuring a relationship with *M. genitalium*.

Two other prospective studies have assessed the relationship between BV and incident *M. genitalium* (18, 22), and conflicting results were reported. Among FSWs in Nairobi, Kenya, there was no association between BV diagnosed during follow-up and incident *M. genitalium* (unadjusted hazard ratio = 1.14, 95% CI: 0.70, 1.94) (22). Visits occurred every 2 months, but neither the duration of follow-up nor the timing of BV detection in relation to *M. genitalium* testing was summarized, leaving doubt about the temporal relationship. In contrast, BV detected at baseline was associated with increased risk of subsequent detection of *M. genitalium* (unadjusted relative risk = 6.09, 95% CI: 1.98, 18.50) in a study of female students in the United Kingdom (18). Follow-up samples were collected a median of 16 months after baseline (18), yet despite this long interval, there was a strong association between BV and *M. genitalium* acquisition. Similarly, in our study, we observed a strong association between BV and incident *M. genitalium*. This finding, paired with the results from our secondary analysis assessing BV status at the visit prior to and the visit with *M. genitalium* testing, provide additional evidence that recent BV is a more significant driver of *M. genitalium* acquisition than concurrent BV.

The changes in the vaginal microbiota associated with BV offer biological plausibility for an increased risk of acquiring

Table 2. Association Between Prior Bacterial Vaginosis and Incident *Mycoplasma genitalium* Infection Among Female Sex Workers in Mombasa, Kenya, 2005–2006

Nugent Score Category	Unadjusted			Adjusted[a]		
	OR	95% CI	P Value	OR	95% CI	P Value
Normal (0–3)	1.00	Referent		1.00	Referent	
Intermediate (4–6)	1.70	0.69, 4.19	0.25	1.70	0.69, 4.18	0.25
Bacterial vaginosis (≥7)	3.48	1.87, 6.48	<0.001	3.49	1.86, 6.56	<0.001

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Adjusted for age, human immunodeficiency virus status, and time (visit number). Further adjustment for hormonal contraceptive use, presence of other sexually transmitted infections, number of sex partners in the past week, frequency of protected and unprotected sex in the past week, and receipt of an antibiotic prescription at the prior visit did not appreciably change the estimates and were not included in the final adjusted model.

M. genitalium. Normal vaginal microbiota are dominated by *Lactobacillus* species that may decrease a woman's susceptibility to STIs (37). Lactic acid produced by lactobacilli contributes to an acidic vaginal pH, prohibiting the growth of acid-intolerant bacteria (38). In addition, some *Lactobacillus* species may inhibit vaginal colonization by select pathogens (37). Hydrogen peroxide can be produced by some *Lactobacillus* species and is hypothesized to play a role in inhibiting infection, but it is unclear whether hydrogen peroxide is produced in vivo (37). BV has been associated with acquisition of HIV (16) and other STIs, including HSV-2, *T. vaginalis*, *N. gonorrhoeae*, and *Chlamydia trachomatis* (10–15), suggesting that increased susceptibility to *M. genitalium* is plausible. Alternatively, the association between BV and STIs may reflect common sexual risk behaviors (39). However, our secondary analysis demonstrating the strongest synergy between acquisition of *M. genitalium* and recent BV rather than concurrent BV argues in favor of enhanced biological susceptibility.

These analyses were characterized by several strengths. The prospective study design allowed us to determine that BV preceded *M. genitalium* infection, and monthly assessment of BV permitted evaluation of a more specific temporal association. In addition, our analysis using the continuous Nugent score (0–10) allowed us to more precisely evaluate the incremental effect of disrupted microbiota on *M. genitalium* incidence. Finally, the discrete failure time analysis that allowed multiple *M. genitalium* infections per woman accounted for spontaneous clearance of *M. genitalium* and the multiple opportunities for acquiring pathogens experienced by these FSWs.

These analyses also had limitations. Women were not screened for *C. trachomatis*, the majority were not tested for HSV-2, and *T. vaginalis* was diagnosed using wet mount and/or culture, methods which are less sensitive than molecular assays (40). While *C. trachomatis* and *T. vaginalis* are both associated with BV (12–14), their relationship with *M. genitalium* is less consistent (19, 41). Nevertheless, there may have been residual confounding due to infection with these organisms. Social desirability bias may have influenced the FSWs' reporting on condom use and number of sex partners, reducing our ability to completely control for

Table 3. Associations Between Prior and Concurrent Bacterial Vaginosis and Incident *Mycoplasma genitalium* Infection Among Female Sex Workers in Mombasa, Kenya, 2005–2006

BV Status[a]		No. of Visits (n = 1,465)	% of Visits	No. of MG Infections (n = 47)[b]	% of MG Infections	Unadjusted			Adjusted[c]		
BV at Prior Visit	Concurrent BV					OR	95% CI	P Value	OR	95% CI	P Value
No	No	684	46.7	10	21.3	1.00	Referent		1.00	Referent	
No	Yes	152	10.4	5	10.6	2.23	0.74, 6.70	0.15	2.31	0.77, 6.91	0.14
Yes	No	152	10.4	9	19.1	4.44	1.73, 11.38	<0.01	4.51	1.76, 11.6	<0.01
Yes	Yes	477	32.6	23	48.9	3.76	1.81, 7.82	<0.001	3.93	1.87, 8.27	<0.001

Abbreviations: BV, bacterial vaginosis; CI, confidence interval; MG, *Mycoplasma genitalium*; OR, odds ratio.

[a] BV (Nugent score ≥7) was compared with normal vaginal microbiota (Nugent score 0–3). Visits at which women had intermediate microbiota disruption (Nugent score 4–6) were excluded from this analysis.

[b] This does not reflect the total number of incident *M. genitalium* infections (n = 59), since visits at which women had intermediate microbiota disruption were excluded.

[c] Adjusted for age, human immunodeficiency virus status, and time (visit number). Further adjustment for hormonal contraceptive use, presence of other sexually transmitted infections, number of sex partners in the past week, frequency of protected and unprotected sex in the past week, and receipt of an antibiotic prescription at the prior visit did not appreciably change the estimates and were not included in the final adjusted model.

the effects of these factors. Time to clearance may have been overestimated if regular sexual partners reinfected women with *M. genitalium*. In contrast, the bimonthly *M. genitalium* testing schedule may have missed short-duration infections; however, *M. genitalium* infections were not treated and antibiotic use was low, and thus our incidence rate was probably not markedly underestimated. Among women diagnosed with multiple incident *M. genitalium* infections, interim negative results may have reflected false-negative tests or been the result of fluctuation around the threshold for detection, which could have overestimated incidence. However, the frequency with which this occurs is unknown. While discrete failure time analysis is essentially equivalent to an interval-censored analysis, the odds ratios may overestimate relative risk and should not be interpreted as such. Lastly, despite a relatively low rate of partner change, risk of HIV/STI was high and HIV/HSV coinfection was common (31, 42); our findings may not be generalizable to lower-risk populations.

These analyses suggest a strong association between recent BV and *M. genitalium* acquisition in a cohort of FSWs in Mombasa, Kenya, and highlight the potential value of categorizing the vaginal microbiota using the full spectrum of the Nugent score. Additional studies will be needed to determine whether BV results in enhanced biological susceptibility to other STIs or whether susceptibility is elevated through common risk factors. If the former is true, effective screening and treatment of BV might reduce women's susceptibility to *M. genitalium*.

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