Recent bacterial vaginosis is associated with the acquisition of *Mycoplasma genitalium*

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Abstract

Recent bacterial vaginosis is associated with the acquisition of *Mycoplasma genitalium*

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Background: *Mycoplasma genitalium* has been associated with adverse female reproductive tract outcomes such as cervicitis, pelvic inflammatory disease, infertility, pre-term birth, and HIV infection, yet little is known about factors predisposing women to acquiring *M. genitalium*. Bacterial vaginosis (BV) is the most prevalent female reproductive tract condition and has been associated with increased risk of acquiring several sexually transmitted pathogens, and may also be associated with *M. genitalium*.

Methods: Utilizing data from a prospective cohort of HIV positive and negative female sex workers in Mombasa, Kenya, we examined the relationship between recent BV and incident *M. genitalium* infection detected by a transcription mediated amplification assay (Hologic, Inc. San Diego, CA). At monthly clinic visits, women completed a sexual behavior interview and clinical examination, including collection of genital samples. Vaginal swab specimens from visits every other month were tested for *M. genitalium.* BV was defined on the basis of Nugent scoring (normal microbiota (scores 0-3), intermediate microbiota (scores 4-6), and BV (scores 7-10). A discrete time failure analysis for multiple events using logistic regression was used to estimate the odds of incident *M. genitalium* infection at follow-up visits in women with and without BV at the visit prior.

Results: Two hundred eighty women contributed 2,454 visits for a total of 148.5 person-years at risk for acquiring *M. genitalium*. At baseline, 16.1% of women had prevalent *M. genitalium* infections, 40.4% had prevalent BV, and 18.2% had an intermediate microbiota. During follow-up, 50 women experienced at least one incident infection, for a total of 59 incident infections. The overall incidence rate of *M. genitalium* infection was 39.7 per 100 person-years and 43.3% (45/104) of the prevalent or incident *M. genitalium* infections were persistent, with an average duration of infection of 93 days. BV was detected at 38.3% (940/2,448) of visits and of these, women reported concurrent vaginal itching and/or discharge at only 8.4% (79/940) of visits. With adjustment for age and HIV status, prior BV was associated with a 3.5-fold increase in the odds of incident *M. genitalium* infection (aOR=3.49; 95%CI: 1.86, 6.55) and prior intermediate microbiota was associated with a modest, but not statistically significant, increase in odds (aOR=1.70; 95%CI: 0.69, 4.18). In the test for linear trend, the odds of incident *M. genitalium* infection increased by 16% for each increase in the Nugent score, after adjustment for age and HIV infection (aOR: 1.16, 95%CI: 1.07, 1.26).

Conclusions: These analyses suggest a strong association between BV and acquisition of *M. genitalium*. If recent BV increases susceptibility to *M. genitalium*, effective treatment of BV might have dual benefit, reducing both the female reproductive tract morbidity associated with BV, as well as reducing susceptibility to *M. genitalium* and the consequences of its sequelae.

Introduction

Mycoplasma genitalium is a sexually transmitted bacterium that infects the genital tract and is found in 0.7% to 3.3% of women in the general population (1,2), prevalences that are typically lower than *Chlamydia trachomatis*, but somewhat higher than *Neisseria gonorrhoeae* (3,4). Prevalence in high-risk groups, such as sex workers and sexually transmitted disease (STD) clinic attendees is higher, ranging from 7-22% (5–8). Incidence in these high-risk groups has been less frequently assessed, but ranges from 22.7–33.5 per 100 women-years in African sex workers (9,10). Furthermore, the duration of infection can be relatively long. In sex workers in Kenya, 47% of the *M. genitalium* infections persisted for ≥3 months (9). In Ugandan sex workers, the median time to clearance of *M. genitalium* infection was 2.1 months (IQR: 1.5-4.8 months) (6).

M. genitalium has been associated with mucopurulent cervicitis (8,11–13), pelvic inflammatory disease (PID) (14–16), infertility (17,18), and preterm birth (19) and a recent meta-analysis demonstrated a 1.7-2.5 fold increase in risk of these adverse reproductive outcomes among *M. genitalium* infected women (20). In addition to this association with adverse female reproductive tract outcomes, *M. genitalium* has also been associated with increased risk of both prevalent and incident HIV infection (21–23). Although these associations suggest *M. genitalium* infections should be identified and treated, diagnostic tests for *M. genitalium* infection are not readily available, and *M. genitalium* responds poorly to standard syndromic therapies for female reproductive tract syndromes (24). Given the health and economic costs associated with PID, infertility, pre-term birth, and HIV infection, prevalence similar to that of *C. trachomatis* and *N. gonorrhea* (3,4), and poor response to standard therapies, *M. genitalium* raises significant public health concern. Risk factors for acquiring *M. genitalium* infection may be suitable prevention targets, yet little is known about factors predisposing women to acquiring *M. genitalium*. Some data suggest that bacterial vaginosis (BV) may be one of those factors (1,25).

BV is the most prevalent female reproductive tract condition (26) and is characterized by disruption of the normal vaginal microbiota through the loss of lactobacilli and an overgrowth of anaerobic and Gram

negative bacteria, genital mycoplasmas (e.g., *Mycoplasma hominis)*, and other microorganisms (27). BV is assessed clinically using Amsel's criteria, and diagnosed when any three of the following four signs are present: thin, homogenous, white, and malodorous vaginal discharge; clue cells on wet mount, amine odor, and pH>4.5. BV can also be defined by the Nugent score, which scores Gram stained vaginal smears based on the quantity of three morphotypes (lactobacilli, gram variable rods, curved rods). Because Amsel's criteria is reliant on clinical symptoms and BV is often asymptomatic, Nugent criteria is a more sensitive diagnostic technique (27). Due to the differences in diagnostic methods, as well as the diverse populations studied, prevalence estimates for BV vary widely, with studies reporting population-based prevalence estimates ranging from 17.8% (28) to 29% (26).

BV has been associated with an increased risk of acquiring Herpes Simplex Virus-2 (29,30), *Trichomonas vaginalis, N. gonorrhoeae*, and *C. trachomati*s (31–34), as well as HIV infection (35). This is thought to be due to the disruption of the normal vaginal microbiota that occurs in women with BV. A normal vaginal microbiota is predominated by *Lactobacillus* species and there is increasing evidence suggesting that the loss of lactobacilli increases a woman's susceptibility to sexually transmitted infections (STI) (36). BV may therefore also be associated with an increased risk of *M. genitalium* infection, but few studies have been conducted and data are inconsistent.

Both cross-sectional and prospective studies have evaluated the relationship between *M. genitalium* and BV. In a cross-sectional assessment of women attending an STD clinic in the United States, women with BV were less likely to have *M. genitalium* infection than women without BV (8), while other studies among female students in London and Zimbabwean women who recently acquired HIV found that women infected with *M. genitalium* were significantly more likely to have concurrent BV compared to women without *M. genitalium* (1,25). Two longitudinal studies provide conflicting data on the association between BV diagnosed at an earlier time point and incident *M. genitalium* infection. BV detected at baseline was associated with increased risk of *M. genitalium* among female students in London (1), while there was no association between BV since enrollment and incident *M. genitalium* among female sex workers in Kenya (9).

Given these inconsistencies and the adverse reproductive health outcomes associated with *M. genitalium* infection, further prospective study of the relationship between BV and *M. genitalium* is warranted. If recent BV increases susceptibility to *M. genitalium*, effective treatment of BV might reduce susceptibility to *M. genitalium*. Utilizing data from a prospective cohort of female sex workers in Mombasa, Kenya, we examined the relationship between recent BV and incident *M. genitalium* infection.

Methods

Study setting

Study participants were women participating in the Mombasa Cohort, a prospective open cohort study of female sex workers that was initiated in 1993, with the goal of identifying risk factors for HIV-1 and STI acquisition (37). Women in the cohort attend monthly clinic visits, including standardized clinical examinations and interviews. The study and these analyses were approved by the Kenyatta National Hospital Ethics and Research Committee and the University of Washington Human Subjects Review Division. All participants provided written informed consent for participation.

Clinic procedures, specimen collection and laboratory methods

At each clinic visit, women completed a standardized sexual behavior interview and clinical examination, including a pelvic examination and collection of genital and blood samples. Participants were asked about smoking and alcohol use at enrollment into the cohort, but not at subsequent visits. A vaginal swab specimen was collected for Gram staining and Nugent scoring to characterize BV status (38), but only symptomatic BV was treated with metronidazole or miconazole. Vaginal swab specimens were also plated on Rogosa agar to culture lactobacilli and positive cultures were tested for H_2O_2 production using a tetramethylbenzadine assay (37). Yeast were detected by wet mount microscopy of vaginal secretions. Cervical swab specimens were inoculated onto modified Thayer-Martin media for *N. gonorrhoeae* culture. Cervical inflammation was evaluated by Gram staining of cervical secretions with ≥30 peripheral mononuclear cells averaged in three high-power fields on microscopy indicative of cervicitis. *T. vaginalis* diagnosis was based on detection of motile trichomonads by wet mount microscopy and through culture

of vaginal swab specimens inoculated into Diamond's media, with a positive on one or both tests characterized as a *T. vag*inalis infection. HIV-1 infection was detected in serum using the ELISA-Detect HIV 1/2 (BioChem Immunosystems, Montreal, Canada) and confirmed using ELISA-Recombigen (Cambridge Biotech, Worchester, MA, USA). Women who were seropositive for HIV-1 also underwent venipuncture every three months for CD4 cell count assessment (FACScount, Becton Dickinson, San Jose, CA, USA).

During February 2005-February 2006, additional vaginal swab specimens were collected for *M. genitalium* testing and frozen at -80ºC. Stored vaginal swab specimens from visits every two months were selected and shipped to Seattle, WA for *M. genitalium* testing using the APTIMA *M. genitalium* transcription mediated amplification (TMA) assay (Hologic, Inc., San Diego, CA, USA). Because *M. genitalium* testing was conducted on stored samples, women were not specifically treated for *M. genitalium*, although women were prescribed antibiotics for other suspected or diagnosed bacterial STIs when indicated.

Analysis

Prevalent *M. genitalium* infection was defined as a positive *M. genitalium* test at the baseline visit. Incident *M. genitalium* infection was defined as a positive *M. genitalium* test at any follow-up visit that was preceded by a negative test at the prior visit with *M. genitalium* testing. *M. genitalium* was considered persistent if a woman had a positive test at more than one sequential visit. The duration of *M. genitalium* infection was calculated using the median time between positive and negative tests. BV status in most analyses was defined using Nugent score and categorized as normal (scores 0-3; reference group), intermediate (scores 4-6), and BV (scores 7-10). BV was considered persistent if detected at more than one visit in a row, and considered recurrent if there was at least one visit without BV between visits with a normal or intermediate microbiota (Nugent score <7). We did not assume treatment failure for BV occurred because symptomatic BV was uncommon in the cohort, asymptomatic BV was not routinely treated unless women were pregnant, and treatment for BV was rare (prescriptions for metronidazole or miconazole provided at 3.3% (31/940) of BV positive visits).

Primary Analysis:

A randomly sampled subset of women in the cohort (~1:1 HIV+ to HIV-) who had two or more visits with *M. genitalium* testing and did not acquire HIV-1 infection during the study period were included in these analyses. Baseline demographic and sexual behavior characteristics of women with and without prevalent BV (Nugent score ≥7 vs Nugent score <7) and prevalent *M. genitalium* were compared using Fisher's exact test for categorical variables and t-tests for continuous variables to assess statistical significance.

The primary outcome was incident *M. genitalium* infection and the primary exposure was BV status at the visit prior to *M. genitalium* testing. A discrete time failure analysis for multiple events using logistic regression was used to estimate the odds of incident *M. genitalium* infection at follow-up visits. Observations were clustered by woman to account for within-person correlation. Women without *M. genitalium* at baseline entered the risk set at their first follow-up visit, which corresponded to the first time point with exposure data (BV at the previous visit). Women with prevalent *M. genitalium* infection at baseline were excluded until their first negative *M. genitalium* test, at which point they joined the risk set. Follow-up time for women with incident *M. genitalium* infection began after the first negative *M. genitalium* test, at which point they returned to the risk set. Women were removed from the risk set after their last *M. genitalium* test.

Discrete time logistic regression models were adjusted *a priori* for age (continuous) and HIV-1 status, characteristics known to be associated with BV and *M. genitalium*. Time-varying covariates at each follow-up visit, such as hormonal contraceptive use, presence of other STIs (*T. vaginalis, N. gonorrhoeae,* HSV-2), number of sex partners in the last week, and receipt of antibiotic therapy at the prior visit, were assessed as potential confounders. Characteristics associated with incident *M. genitalium* infection at $p \leq 0.20$ in bivariate assessment were evaluated in multivariable models and only those covariates that changed the odds ratio by ≥10% (crude versus adjusted) were retained in the final model. A linear trend test was performed to evaluate the presence of a linear relationship between increasing Nugent score (continuous) and the odds of incident *M. genitalium*. Data were analyzed using Stata 13.0 (StataCorp, College Station, TX) and statistical significance was defined as $p<0.05$ for all analyses.

Results

Between February 2005 and February 2006, a total of 280 women had two or more *M. genitalium* tests and were included in these analyses. Median time from enrollment in the Mombasa Cohort to the baseline visit for this study was 3.0 years (IQR: 0.82-7.76). Included women had a mean age of 35.6 years (SD: 6.51) and 54.6% (153/280) were HIV positive. At baseline, 16.1% (n=45) of women had prevalent *M. genitalium* infections, 40.4% (n=113) had prevalent BV, and 18.2% (n=51) had an intermediate microbiota.

Demographic, clinical, and sexual behavior characteristics at baseline are presented in Table 1 by BV and *M. genitalium* status. Women with *M. genitalium* were younger than those without *M. genitalium* (mean: 32.9 vs 36.1 years, p<0.01) and were more likely to be HIV positive (68.9% vs 51.5%, p=0.04). Women with BV (Nugent score ≥7) were also more likely to be HIV positive (63.7% vs 47.9%, p=0.01) than women without BV (Nugent score <7). They were somewhat less likely to have candida (7.1% vs 14.4%, p=0.06) and to be current users of hormonal contraception (23.0% vs 32.3%, p=0.12), although these findings were not statistically significant. Women with BV were somewhat more likely to have concurrent *M. genitalium* infection compared to women without BV (OR=1.68, 95%CI: 0.89, 3.20), but this was not statistically significant. However, when intermediate microbiota was considered separately, both intermediate microbiota and BV were significantly associated with concurrent *M. genitalium* infection (Fisher's exact p=0.01); both women with an intermediate microbiota (Nugent score 4-6) and women with BV (Nugent score ≥7) were more likely to have concurrent *M. genitalium* than women with a normal microbiota (Nugent score 0-3) (BV: 51.1%, Intermediate microbiota: 26.7%, Normal: 22.2%). Smoking, alcohol use, contraceptive use, vaginal symptoms, vaginal washing practices, frequency of condom use, and number of sexual partners were similar between women with and without BV or *M. genitalium*.

The 280 women included in this study contributed 2,454 visits for a total of 196.9 person-years of observation. The median number of visits per woman was 10 (IQR: 5-12) and the median time between visits was 29 days (IQR: 7-119). After excluding visits when women had persistent *M. genitalium* infection, there were 148.5 person-years at risk for acquiring *M. genitalium*.

Forty-five women (16.1%) had prevalent *M. genitalium* infection at baseline. Over the follow-up period, 50 women experienced an incident infection, nine of whom experienced two episodes for a total of 59 incident *M. genitalium* infections. None of the women had greater than two incident *M. genitalium* infections. The overall incidence rate of *M. genitalium* infection was 39.7 per 100 person-years. Of the total 104 *M. genitalium* infections (prevalent or incident), 45 (43.3%) were persistent, with an average duration of infection of 93 days (Range: 14 to 332.5 days). One woman had six consecutive positive *M. genitalium* tests for a maximum duration of infection of 332.5 days. Approximately 12% (33/280) of women had a positive *M. genitalium* test at their last visit.

BV was detected at 38.3% (940/2,448) of visits and in 206 (73.6%) women. Of these, 40.7% (n=114) of women with BV had at least one recurrence. Overall, 32.9% (92/280) of women had a single episode of BV, 25.4% (71/280) had one BV recurrence, 11.8% (33/280) had two recurrences, and 3.6% (10/280) had three or four recurrences. Women with BV reported concurrent vaginal itching and/or discharge at only 8.4% of the 940 visits.

Doxycycline, metronidazole, and/or miconazole were prescribed at 2.4% (52/2,174), 2.7% (59/2,174), and 0.6% (12/2,174) of follow-up visits. Among the small number of women who received antibiotics, the receipt of these medications was not associated with either *M. genitalium* or BV at the subsequent visit ($p>0.05$ for all). Among the 153 HIV positive women, CD4 cell count was available for 5.8% (71/1215) of visits including both baseline and follow-up visits. The mean CD4 count was 368.1 cells/mm^3 (SD: 273.9) and did not differ significantly between women with and without *M. genitalium* infection ($p=0.60$) or BV ($p=0.18$).

Primary Analysis:

In bivariate analysis, compared to women with normal vaginal microbiota at the visit prior to *M. genitalium* testing, BV at the preceding visit was associated with a 3.48-fold increase in the odds of incident *M. genitalium* infection (95%CI: 1.87-6.48). Intermediate microbiota was associated with a 1.70 fold increase

in the odds of subsequent incident *M. genitalium* infection (95%CI: 0.69, 4.19), but this was not statistically significant. In multivariable analyses, after adjusting for age and HIV status, BV at the prior visit continued to be associated with an increase in the odds of subsequent incident *M. genitalium* infection (aOR=3.49; 95%CI: 1.86, 6.55) and intermediate microbiota remained associated with a modest, but not statistically significant, increase in the odds of incident *M. genitalium* infection (aOR=1.70; 95%CI: 0.69, 4.18) (Table 2). Additional adjustment for other demographic, behavioral, and clinical characteristics, such as condom use, vaginal washing practices, and concurrent infection with other STIs, did not appreciably change the estimates and none were included in the final model. In the test for linear trend, the odds of incident *M. genitalium* infection increased by 16% for each increase in the Nugent score, after adjustment for age and HIV infection (aOR: 1.16, 95%CI: 1.07, 1.26, p<0.001).

Discussion

In this cohort of HIV-positive and HIV-negative female sex workers in Mombasa, Kenya, the prevalence of *M. genitalium* at baseline was high (16.1%), as was the prevalence of BV (40.4%). The incidence of *M. genitalium* infection during follow-up was also high at nearly 40 per 100 person-years and *M. genitalium* infections persisted for an average of three months. Women with BV had 3.5 times the odds of acquiring *M. genitalium* in the subsequent 30-days compared to women with a normal vaginal microbiota, and the odds of *M. genitalium* infection increased incrementally in parallel with increasing Nugent score.

The incidence rate of *M. genitalium* infection in these Kenyan sex workers from Mombasa was higher than previous observations. In low-risk populations of women in the United Kingdom and Australia, *M. genitalium* incidence rates have ranged from 0.9 to 1.3 per 100 person-years (1,39). In high-risk populations in Kenya and the United States, rates were higher, ranging from 22.7 to 33.5 per 100 person-years (9,10), but still lower than the 40 per 100 person-years that we observed. Our analytic method allowed for multiple infections per woman, whereas other studies only included time to the first incident infection and this may partially explain the higher incidence rate that we observed. Given our bi-monthly testing schedule for *M. genitalium,* it is possible that incident infections were missed during follow-up.

However, we do not expect our incidence rate is markedly underestimated because *M. genitalium* infections were not specifically treated and infections persisted for an average of three months. In cohorts of female sex workers with more antibiotic use than we observed in this cohort, median times to *M. genitalium* clearance were shorter. Among female sex workers in Nairobi, Kenya, where 94.7% of women received antibiotics during follow-up, median time to clearance was 1 month (range: 1-33 months) (9). Median time to clearance was longer (2.1 months (IQR: 1.5-4.8)) in female sex workers in Kampala, Uganda, where 44.1% (49/111) of women who cleared their *M. genitalium* infection were treated with antibiotics for clinical PID or vaginal discharge syndrome during follow-up (6). The longer duration of *M. genitalium* infection observed in our cohort may be attributed to a lower prevalence of antibiotic use, but *M. genitalium* responds poorly to antibiotics, so this does not likely entirely explain the difference (40–42).

These analyses add to the limited research on the relationship between BV and *M. genitalium*. The finding from our cross-sectional analyses that associations between prevalent BV and concurrent *M. genitalium* infection differed depending on how women without BV were defined may shed some light on the inconsistencies in prior assessments of this relationship. The significant association we observed when women with intermediate microbiota were considered separately from those with a normal microbiota is consistent with two other studies that reported women infected with *M. genitalium* had a greater than 2-fold increased odds of having concurrent BV compared to women without *M. genitalium* (1,25). In contrast, the modest and non-significant association we observed when women with intermediate microbiota were included in the group of women without BV was consistent with the two studies that found no association (43,44). Some studies were conducted in developed countries among low-risk women (1,43,44) and were others conducted among high-risk women in Africa and the United States (8,25), and these differences in the study populations may explain the inconsistencies between studies. The inconsistences might also be associated with the BV diagnostic technique and analysis. Most studies diagnosed BV using Amsel's criteria (8,25,43,44) and all of the studies, excluding ours, defined the microbiota in a binary manner (BV vs no BV). Those women characterized as not having BV may have had a normal microbiota or an intermediate microbiota had they been diagnosed by Nugent score, which may have decreased the ability of these studies to identify an association.

Utilizing the prospective design of this study, we assessed the temporal association between BV at the prior clinic visit with the acquisition of *M. genitalium* approximately one month later. Two other cohort studies have assessed this relationship; one finding an increased risk for acquiring *M. genitalium* among women with BV and the other finding no such association (1,9). Similar to our study, in a prospective study of 873 female students in the United Kingdom, BV detected at baseline was associated with increased risk of subsequent detection of *M. genitalium* in follow-up samples collected a median of 16 months after baseline (unadjusted RR 6.09, 95% CI: 1.98–18.50) (1). However, the extended time between the detection of BV at baseline and subsequent *M. genitalium* testing weakens the ability to infer that BV is causally related to *M. genitalium* acquisition. In addition, the relative risk is not adjusted for other factors such as condom use or number of sex partners that might put women at increased risk for *M. genitalium* and/or BV. In contrast, among 253 female sex workers in Nairobi, Kenya, there was no association between BV diagnosed during follow-up and incident *M. genitalium* (unadjusted HR: 1.14, 95%CI: 0.70-1.94). While visits were scheduled for every two months, neither the duration of follow-up nor the timing of BV detection in relation to *M. genitalium* testing was summarized. The interval between BV exposure and *M. genitalium* in this study is thus difficult to assess. Lastly, both of these longitudinal studies assessed the association between BV and incident *M. genitalium* utilizing a binary definition of BV which did not consider intermediate microbiota and this might have been a less sensitive measure. Thus, our study benefits from both a more nuanced measurement of the vaginal microbiome and a shorter interval between BV assessment and *M. genitalium* testing

A normal vaginal microbiota is predominated by *Lactobacillus* species and there is increasing evidence suggesting that lactobacilli might act through a number of mechanisms to decrease a woman's susceptibility to STIs (36). For example, the lactic acid produced by some lactobacilli contributes to an acidic vaginal pH, which prohibits the growth of acid-intolerant bacteria (45). Certain lactobacilli species may also prevent the colonization of the vagina with pathogens by sequestering or competing with infectious agents so they cannot attach to the vaginal epithelium (36). Lastly, in women with BV, H_2O_2 producing lactobacilli, which predominate the vaginal microbiota of healthy women, are lost and replaced

by the anaerobic and Gram negative bacteria and genital mycoplasmas (e.g., *M. hominis)* characteristic of BV (27). This suggests H_2O_2 producing lactobacilli might play a direct role in inhibiting infection. However, it is unclear if H_2O_2 is produced *in vivo*, creating some debate about the mechanistic role of H_2O_2 producing lactobacilli (36). The changes to the vaginal flora that are indicative of BV are associated with the acquisition of HIV (35) and many other STIs, including HSV-2, *T. vaginalis, N. gonorrhoeae*, and *C. trachomatis* (29–34). While the mechanisms of the relationship between BV and *M. genitalium* are unknown, the changes to the vaginal microbiota associated with BV offer biological plausibility for an increased risk of acquiring *M. genitalium*. In addition, the observed statistically significant linear trend associated with an increasing risk of acquiring *M. genitalium* with higher Nugent score suggests a possible dose-response relationship with increasing disturbance in the microbiota.

These analyses are characterized by several strengths. The prospective nature of this study allowed us to determine that BV preceded *M. genitalium* infection and the monthly assessments provided more precision in the analyses of the influence of recent BV diagnosis on incident *M. genitalium* infection. In addition, exposure and outcome ascertainment were strong, and vaginal microbiota disturbance was assessed categorically rather than in a binary manner which might have increased our ability to detect an association between BV and incident *M. genitalium*. The APTIMA assay utilized for *M. genitalium* diagnosis is very sensitive and specific (46), and Nugent criteria for BV diagnosis is more sensitive and specific than Amsel's criteria (27). Finally, the discrete time failure analysis using logistic regression permitted the inclusion of more than one *M. genitalium* infection per woman, which more accurately reflects the high-risk women we studied.

There are also a number of limitations to these analyses. Possible confounding by infection with other STIs may not be completely controlled for in these analyses. The prevalence of HSV-2 in HIV positive women in this cohort is high and these women do not undergo routine HSV-2 testing; data on baseline HSV-2 status were missing for almost 80% of the HIV positive women. In addition, women were not screened for *C. trachomatis* and *T. vaginalis* was diagnosed using wet mount and/or culture of vaginal secretions, which are not highly sensitive (47). While *C. trachomatis* and *T. vaginalis* are both associated

with BV (31–33), their relationship with *M. genitalium* is less consistent, and some studies have observed no association (7,8). Nevertheless, there may be residual confounding due to infection with these organisms. There may also be residual confounding associated with incomplete control for sexual behaviors, such as condom use and number of sex partners, due to the under-reporting associated with social desirability and recall bias. Among women diagnosed with multiple incident *M. genitalium* infections during follow-up, it is possible that interim negative results were false negative tests and some of these women may have been persistently infected. If so, this would have overestimated *M. genitalium* incidence. However, given the high sensitivity and specificity of the APTIMA test for *M. genitalium* (46), any effect of this on our results is likely to be small. Lastly, the female sex workers in this study represent a group at high risk for acquiring STIs, including HIV and *M. genitalium*, and these relationships may be different in lower risk populations of women.

These analyses suggest a strong association between BV and acquisition of *M. genitalium* in a cohort of HIV positive and negative female sex workers in Mombasa, Kenya. If recent BV increases susceptibility to *M. genitalium*, effective treatment of BV might have dual benefit, reducing both the female reproductive tract morbidity associated with BV, as well as reducing susceptibility to *M. genitalium*, and the consequences of its sequelae. Preliminary data from a randomized control trial assessing the efficacy of periodic presumptive treatment (intravaginal metronidazole 750mg + miconazole 200mg versus placebo) for vaginal infections suggests treating BV might reduce susceptibility to *M. genitalium.* Specifically, women who were randomized to the treatment arm were approximately 50% less likely to acquire *M. genitalium* during follow-up compared to women on the placebo (aHR=0.49; 95%CI 0.27, 0.88) (10). This finding, paired with the increase in odds of *M. genitalium* infection with increasing Nugent score observed in this study, suggests that treating BV, and possibly intermediate microbiota, might be a suitable prevention target for reducing susceptibility to *M. genitalium*. Additional studies to assess the association between BV and incident *M. genitalium* infection using definitions of BV that account for intermediate vaginal microbiota and conducted in a variety of populations are warranted.

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Table 1: Demographic, clinical, and sexual behavior characteristics among 280 women by BV and *M. genitalium* status at baseline

Baseline Characteristic	Total N=280 N (%)	BV+* N=113 n (%)	BV- N=167 n(%)	P-value	MG+ N=45 n(%)	MG- N=235 n(%)	P-Value
Demographics							
Age [Mean (SD)]	35.6 (6.51)	35.2 (5.99)	36.0 (6.84)	0.34	32.9 (5.84)	36.1 (6.52)	<0.01
Categorized Age	-	-	-	0.60	-	-	<0.01
<25	10 (3.6)	5 (4.4)	5 (3.0)	-	2 (4.4)	8 (3.4)	-
25-29	35 (12.5)	15 (13.3)	20 (12.0)	-	13 (28.9)	22 (9.4)	-
30-34	82 (29.3)	34 (30.0)	48 (28.7)	-	10 (22.2)	72 (30.6)	-
35-39	85 (30.4)	37 (32.7)	48 (28.7)	-	16 (35.6)	69 (29.4)	-
>40	68 (24.3)	22 (19.5)	46 (27.5)	-	4 (8.9)	64 (27.2)	-
Smoker†	33 (11.8)	10 (8.9)	23 (13.8)	0.26	7 (15.6)	26 (11.1)	0.45
# of cigarettes/week	-	-	-	0.50	-	-	0.56
0	247 (88.2)	103 (91.2)	144 (86.2)	-	38 (84.4)	209 (88.9)	-
1-9	27 (9.6)	8 (7.1)	19 (11.4)	-	6 (13.3)	21 (8.9)	-
10+	6 (2.1)	2 (1.8)	4 (2.4)	-	1 (2.2)	5 (2.1)	-
Alcoholic drinks per week [Mean (SD)]	6.15 (8.13)	6.68 (9.25)	5.78 (7.27)	0.37	5.69 (6.42)	6.23 (8.43)	0.68
Alcoholic drinks per week	-	-	-	0.56	-	-	0.19
0	61 (21.8)	22 (20.4)	38 (22.8)	-	6 (13.3)	55 (23.4)	-
1-7	147 (52.5)	57 (50.4)	90 (53.9)	-	29 (64.4)	118 (50.2)	-
8+	72 (25.7)	33 (29.2)	39 (23.4)	-	10 (22.2)	62 (26.4)	-
Clinical							
M. genitalium	45 (16.1)	23 (20.4)	22 (13.2)	0.14	-	-	-
Vaginal Microbiota		-	-	-	-	-	0.01
BV (Nugent score ≥7)	113 (40.4)	-	-	-	23 (51.1)	90 (38.3)	-
Intermediate (Nugent score 4-6)	51 (18.2)	-	-	-	12 (26.7)	39 (16.6)	-
Normal (Nugent score 0-3)	116 (41.4)	-	-	-	10 (22.2)	106 (45.1)	-
Lactobacillus species	39 (13.8)	11 (9.7)	28 (16.8)	0.11	9 (20.0)	30 (12.8)	0.24
HIV-1 seropositive	153 (54.6)	72 (63.7)	80 (47.9)	0.01	31 (68.9)	121 (51.5)	0.04
HSV-2 seropositive	148 (52.7)	49 (43.4)	99 (59.3)	0.02	18 (40.0)	130 (55.3)	0.12
T. vaginalis	10 (3.6)	6 (5.3)	4 (2.4)	0.21	2 (4.4)	8 (3.4)	0.67
N. gonorrhoeae	2 (0.71)	1 (0.9)	1 (0.6)	0.50	0 (0.0)	2 (0.9)	1.0
Candida	32 (11.4)	8 (7.1)	24 (14.4)	0.06	3 (6.7)	29 (12.3)	0.44
Cervicitis‡	10 (3.6)	3 (2.7)	7 (4.2)	0.20	2 (4.4)	8 (3.4)	0.77
Vaginal washing in last week	-	-	-	0.16	-	-	0.69
None	8 (2.9)	4 (3.5)	4 (2.4)	-	1 (2.2)	7 (3.0)	-

* BV positive was defined as Nugent score ≥7. BV negative was defined as Nugent score <7.

† Participants were asked about smoking and alcohol use at enrollment into the Mombasa Female Sex Worker cohort. Median time from enrollment to baseline for this study was 2.9 years (IQR: 0.82-7.76)

‡ ≥30 cervical peripheral mononuclear cells

Water	60 (21.4)	18 (15.9)	42 (25.0)	-	7 (15.6)	53 (22.6)	-
Soap/Other	210 (75.0)	91 (80.5)	119 (71.3)	-	37 (82.2)	173 (73.6)	-
Method of contraception	-	-	-	0.22	-	-	0.79
None	185 (65.8)	80 (70.8)	105 (62.9)	-	29 (64.4)	156 (66.4)	-
Oral contraceptive pills	19 (6.8)	5 (4.4)	14 (8.4)	-	5 (11.1)	14 (6.0)	-
Depo Provera Injectable	53 (18.9)	16 (14.2)	37 (22.2)	-	9 (20.0)	44 (18.7)	-
Intra-Uterine Device	2 (0.71)	1 (0.9)	1 (0.6)	-	0 (0.0)	2 (0.9)	-
Norplant	6 (2.1)	4 (3.5)	2 (1.2)	-	1 (2.2)	5 (2.1)	-
Other	14 (5.0)	7 (6.2)	7 (4.2)	-	1 (2.2)	13 (5.5)	-
Hormonal contraception	80 (28.6)	26 (23.0)	54 (32.3)	0.12	15 (33.3)	65 (27.7)	0.56
Vaginal itching/burning	24 (8.6)	10 (8.9)	14 (8.4)	1.0	2 (4.4)	22 (9.4)	0.39
Vaginal discharge	8 (2.9)	2 (1.8)	6 (3.6)	0.48	1 (2.2)	7 (3.0)	1.0
Sexual Behavior							
Number of sex partners in prior working week [Mean (SD)]	0.81 (0.06)	0.88 (1.07)	0.76 (1.09)	0.34	0.89 (1.13)	0.80 (1.07)	0.60
Frequency sex last week [Mean (SD)]	1.08 (1.29)	1.16 (1.27)	1.02 (1.30)	0.37	1.22 (1.36)	1.05 (1.28)	0.40
Protected sex last week[§]	-	-	-	0.30	-	-	0.78
No sex	128 (45.7)	44 (38.9)	84 (50.3)	-	18 (40.0)	110 (46.8)	-
All sex acts protected	104 (37.1)	48 (42.5)	56 (33.5)	-	18 (40.0)	86 (36.6)	-
Some sex acts protected	9 (3.2)	4 (3.5)	5 (3.0)	-	1 (2.2)	8 (3.4)	-
No sex acts protected	39 (13.9)	17 (15.0)	22 (13.2)	-	8 (17.8)	31 (13.2)	-

[§] Protected sex defined as condom use at sex act

Table 2: Association between prior BV and incident *M. genitalium*: Crude and adjusted analyses

Nugent Score Category	Crude OR (95% CI)	P value	Adjusted OR (95% CI)*	P value
Normal (0-3)	Ref	-	Ref	-
Intermediate (4-6)	1.70 (0.69, 4.19)	0.248	1.70 (0.69, 4.18)	0.246
BV (≥7)	3.48 (1.87, 6.48)	<0.001	3.49 (1.86, 6.55)	<0.001

***** Adjusting for age and HIV status