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Mycoplasma genitalium infection in Kenyan and US women

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Abstract

Background—Little is known about the natural history of *Mycoplasma genitalium* (MG) infection. We retrospectively tested archived samples and assessed MG prevalence, incidence, persistence, recurrence and antimicrobial resistance markers among women participating in the Preventing Vaginal Infections trial, a randomized trial of monthly presumptive treatment to reduce vaginal infections.

Methods—High-risk, nonpregnant, HIV-negative women aged 18–45 from Kenya and the US were randomized to receive metronidazole 750mg + miconazole 200mg intravaginal suppositories

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POTENTIAL CONFLICTS OF INTERESTS

JEB has received donated reagents and test kits from Hologic. RSM currently receives research funding, paid to the University of Washington, from Hologic. J. Schwebke has received consultancy payments from Akesis, Hologic, Symbiomix, and Starpharma, and has grants/pending grants from Akesis, BD Diagnostic, Hologic, Cepheid, Quidel, Symbiomix, Starpharma, and Viamet. LEM has received donated reagents and test kits from Hologic and honoraria for scientific advisory board membership from Hologic and Qiagen, Inc. JSJ has received speaker fees and travel support and research funding via Serum Statens Instiutue (SSI) from Hologic and SSI has performed contract work for SpeeDx, Diagenode, Nytor, Cempra, Angelini, and Nabriva. All other authors declare that they do not have a commercial or other association that might pose a conflict of interest.

AUTHOR CONTRIBUTIONS

JEB, LEM, and RSM conceptualized the article and analysis plan. JEB conducted the analysis in collaboration with LEM, JSJ and RSM. JEB drafted the initial report and LEM, JSJ, and RSM contributed to the content and revisions. OA, JK, J Schwebke, J Shafi, CR, and EK contributed to data collection. All authors contributed to article content and approved the final manuscript.

or placebo for 5 consecutive nights each month for 12 months. Cervicovaginal fluid specimens were tested for MG using Hologic nucleic acid amplification testing at enrollment and every other month thereafter. Specimens that were MG+ underwent additional testing for macrolide resistance mediating mutations (MRMM) by DNA sequencing.

Results—Of 221 women with available specimens, 25 (11.3%) had MG at enrollment. Among 196 women without MG at enrollment, there were 52 incident MG infections (incidence=33.4 per 100 person-years). Smoking was independently associated with incident MG infection (adjusted HR=3.02; 95% CI 1.32, 6.93), and age <25 years trended towards an association (adjusted HR=1.70; 95% CI 0.95, 3.06). Median time to clearance of incident MG infections was 1.5 months (interquartile range 1.4, 3.0). Of the 120 MG+ specimens, 16 specimens from 15 different women were MRMM+ (13.3%), with no difference by country.

Conclusions—*M. genitalium* infection is common among sexually active women in Kenya and the Southern US. Given associations between MG and adverse reproductive health outcomes, this high burden of *M. genitalium* in reproductive-aged women could contribute to substantial morbidity.

Keywords

Mycoplasma genitalium; natural history; women; macrolide resistance mediating mutations; antibiotic resistance; Africa

INTRODUCTION

Mycoplasma genitalium is a sexually transmitted, fastidious bacterium that colonizes the female genital tract [1–6]. There is increasing evidence that *M. genitalium* infection is associated with adverse reproductive health outcomes in women, including cervicitis, urethritis, pelvic inflammatory disease (PID), infertility, ectopic pregnancy, adverse birth outcomes and HIV-1 acquisition [1, 7–9]. However, data are limited on the natural history of *M. genitalium* infection, particularly with respect to duration of infection (persistence) and recurrence. Natural history studies are critical to improving our understanding of the contribution of *M. genitalium* to adverse health outcomes in women and informing guidelines for screening and treatment.

In addition over the past several years, *M. genitalium* treatment has been complicated by to the poor efficacy of doxycycline eand increasing resistance to azithromycin in high-income countries. Azithromycin resistance in *M. genitalium* is mediated by macrolide-resistance mediating mutations (MRMM) in the 23S rRNA gene [10]. The majority of studies assessing *M. genitalium* macrolide resistance in women have been conducted in Europe and Australia [11–17], where circulating resistance greater than 50% has been reported in some settings. To date, only one published report has estimated macrolide-resistant *M. genitalium* in the US. Among women with *M. genitalium* from 7 sites across the US, MRMM prevalence was 51% [18]. In East Africa, the prevalence of macrolide-resistant *M. genitalium* is unknown. Using data and stored specimens from women who previously participated in a randomized controlled trial (RCT) of monthly periodic presumptive treatment to reduce vaginal infections, we conducted a retrospective cohort study to assess

the natural history of *M. genitalium* infection and estimate the frequency of macrolide-resistant *M. genitalium*.

MATERIALS AND METHODS

Study population and procedures

The Preventing Vaginal Infections (PVI) trial was a double-blinded, randomized controlled trial that assessed the effect of monthly metronidazole 750mg + miconazole 200mg intravaginal suppositories versus matching placebo to reduce rates of bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC) [#NCT01230814]. Detailed methods and results have been previously described [19]. Briefly, 234 high-risk women from three sites in Kenya and one in the United States were enrolled between May 2011 and August 2012. Eligible women were 18–45 years of age, HIV-1 uninfected, not pregnant or breastfeeding, sexually active, and had one or more vaginal infections at screening (BV, VVC, or *Trichomonas vaginalis*). All participants provided written informed consent, including a separate consent for the storage and future testing of biological specimens. The trial was approved by the human subjects research committees at Kenyatta National Hospital (Nairobi, Kenya), the University of Washington (Seattle, WA), and the University of Alabama at Birmingham (Birmingham, AL).

At enrollment, structured face-to-face interviews were conducted to collect data on demographic, clinical and behavioral characteristics. At monthly follow-up visits, a urine pregnancy test was performed and data were collected on sexual behaviors, intravaginal practices, contraceptive use, product use and genital tract symptoms (abnormal itching or discharge). Non-pregnant participants received a month's supply of study product and free male condoms. In addition, during follow-up visits at months 2, 4, 6, 8, 10, and 12, participants underwent a physical examination including pelvic speculum examination with collection of genital swabs for diagnosis of genital tract infections. If a participant missed an examination visit, a physical examination was performed at her next follow-up visit.

Laboratory methods

Cervicovaginal fluid was collected using the Hologic APTIMA Combo 2 system (Hologic Inc.; San Diego, CA) at enrollment and months 2, 4, 6, 8, 10 and 12. Specimens collected at enrollment were tested for the presence of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. The remainder of the enrollment specimen and all follow-up specimens were stored at −80°C for future testing. At the completion of the study, stored specimens were tested for *M. genitalium* using a research-use only transcription mediated amplification (TMA) assay with reagents provided by Hologic as part of their ongoing research program. Specimens with a value >40,000 relative light units were considered positive [20]. Samples that were *M. genitalium* positive underwent DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). MRMM in region V of the *M. genitalium* 23S ribosomal RNA gene were detected by PyroMark (Qiagen) DNA sequencing of PCR amplicons [10, 21].

Vaginal Gram stained slides were evaluated for BV using the Nugent's score [22]. Saline and KOH wet preparations were examined for the presence of motile trichomonads, clue cells, and yeast. Endocervical Gram stained slides were scanned at low power to identify polymorphonuclear leukocytes (PMNs) in three non-adjacent oil immersion fields. Cervicitis defined as ≥30 PMNs on Gram stain. Yeast culture was performed on Sabouraud's agar with a germ tube test to identify presumptive *Candida albicans*.

Statistical analysis

The objectives of this analysis were to (1) estimate the prevalence, infection duration, and correlates of *M. genitalium* identified at enrollment; (2) assess the incidence, infection duration and correlates of *M. genitalium* during follow-up; (3) assess the frequency of *M. genitalium* recurrence; and (4) determine MRMM frequency among women who tested positive for *M. genitalium* at any point during the trial. *Mycoplasma genitalium* clearance was defined as testing negative after a previous positive result, and time of clearance was estimated as the mid-point between the last positive and first negative result. Recurrent *M. genitalium* infection required a previous positive result, with one or more interim negative results, followed by a new positive result. Time of recurrence was considered to be the visit at which recurrent *M. genitalium* was first detected. Strain typing was not performed, so it was not possible to determine whether recurrences represented infection with a new strain versus recurrence or reinfection with an identical strain.

The analysis population was restricted to participants who provided consent for future testing of stored specimens. Descriptive statistics were used to summarize participant characteristics and chi-squared and T-tests were performed as appropriate. Logistic regression models were used to assess correlates of prevalent *M. genitalium* infection at enrollment. Age and study site were included in multivariable models based on *a priori* assumptions. In a prior analysis, we observed a trend towards a lower incidence of *M. genitalium* in the intervention arm compared to placebo [23]; therefore, initial analyses assessing correlates of incident infection were adjusted for study arm and stratified by site. Cox proportional hazards models were used to assess baseline and time-varying correlates of incident *M. genitalium* infection. Factors associated with *M. genitalium* infection with a p-value <0.10 in bivariate analyses were considered for inclusion in multivariable models. Factors that continued to have a p-value <0.10 were retained in final multivariable models. Descriptive statistics were used to summarize the proportion of successfully tested specimens with MRMM. All statistical tests were assessed using a 2-sided α of 0.05. Analyses were conducted using Stata version 14.1 (StataCorp, Inc., College Station, TX).

RESULTS

Of 234 women enrolled, 221 (94%) returned for follow-up and provided consent for future testing. Participant characteristics are presented in Table 1. Median age was 29 years (interquartile range [IQR] 24–34), 24% of participants were from the US, and 54% reported ever having sex in exchange for money, goods or services. Overall, 77(35%) participants tested positive for *M. genitalium* either at enrollment or during follow-up. A higher proportion of participants from Kenya experienced *M. genitalium* infection at during study

participation compared to those from the US (64/168[38%] versus 13/53[25%], respectively; p=0.07).

Prevalence, clearance and persistence

Mycoplasma genitalium prevalence at enrollment was 11.3% and did not differ between Kenyan (11.3%) and US participants (11.3%). Of note, the prevalence of *M. genitalium* was higher than other curable STIs (*C. trachomatis* = 7%; *N. gonorrhoeae* = 1%; *T. vaginalis* = 7%), and no *M. genitalium* co-infections with *C. trachomatis* or *N. gonorrhoeae* were observed (Table 1). Associations between participant characteristics and prevalent *M. genitalium* are presented in Table 1. In unadjusted analysis, having a new sexual partner (odds ratio [OR]=2.50; 95% CI 1.02, 6.11) and having VVC (OR=2.44; 95% CI 1.02, 5.83) were associated with prevalent *M. genitalium*. In multivariable analyses that adjusted for age and study site, the effect estimate was similar for VVC (adjusted OR=2.45; 95% CI 1.00, 5.99) and attenuated slightly for having a new sexual partner (adjusted OR=2.38; 95% CI 0.87, 6.49). Condom use and the prevalence of cervicitis did not differ between women with prevalent *M. genitalium* infection versus those that were *M. genitalium* negative. Among 25 women with prevalent *M. genitalium* at enrollment, 22 participants cleared their *M. genitalium* infection prior to the end of the study, while 3 participants had *M. genitalium* detected at every study visit. Median time to clearance of prevalent infection was 3.9 months (interquartile range [IQR] 1.0, 8.4) [Figure 1]. Use of doxycycline was uncommon and did not appear to correlate with *M. genitalium* clearance (Figure 1). No azithromycin was dispensed to women with *M. genitalium* infection at any point in the trial.

Incidence, clearance, persistence and recurrence

Among 196 women without *M. genitalium* at enrollment, there were 52 incident *M. genitalium* infections during 155.7 person years of follow-up (overall incidence *M. genitalium* [excluding recurrences]=33.4 per 100 person-years). *Mycoplasma genitalium* incidence was higher among Kenyan participants compared to US participants (38.4 per 100 person-years [95% CI 28.7, 51.4] versus 18.2 per 100 person-years [95% CI 8.7, 38.1]; p=0.06). In univariate analysis, being a current smoker was associated with incident infection, while younger age and concurrent BV trended towards an association (Table 2). In a multivariable model that included these three factors in addition to study arm and stratified by site, smoking was independently associated with increased risk of incident *M. genitalium* (adjusted HR=3.02; 95% CI 1.32, 6.93), while the effect estimates for concurrent BV (adjusted HR=1.59; 95% CI 0.87, 2.88) and age <25 years (adjusted HR = 1.70; 95% CI 0.95, 3.06) continued to trend towards an association.

The duration and patterns of incident *M. genitalium* are presented in Figure 2. Median time to *M. genitalium* clearance was 1.5 months (IQR 1.4, 3.0) and did not differ by study arm (p=0.65). Of 52 women who experienced an incident infection during follow-up, 43 (83%) experienced one incident infection (i.e. no recurrence) and the majority had *M. genitalium* detected at only one study visit (26/43[60%]). Again, reported use of doxycycline was uncommon and did not appear to correlate with clearance of incident or recurrent infection (Figure 2). Of 61 participants who had either prevalent (n=22) or incident (n=39) *M.*

genitalium and cleared their infection, 20(34%) had a recurrence of *M. genitalium* during study follow-up, with a median time to recurrence of 4.1 months (IQR 3.7, 7.3).

Macrolide resistance-mediating mutations

Among 77 women who tested positive for *M. genitalium* during the trial, specimens from 26 women were unevaluable for MRMM testing. Fifty-one women had a total of 120 evaluable *M. genitalium* samples that were tested for MRMM. Sixteen specimens (13.3%) from 15 different women tested positive for MRMM; mutations A2059G and A2058G were detected. The proportion of specimens with MRMM did not differ by country (Kenya: 12 of 82 [14.6%]; US: 4 of 38 [10.5%]; p=0.54). Patterns of *M. genitalium* infection among women with MRMM are displayed in Figure 3. Ten of 15 women with MRMM were *M. genitalium* negative at the prior visit, suggesting that macrolide resistant *M. genitalium* was acquired from a sex partner. In most cases of MRMM, infection was cleared by the next assessment. Mean duration of infection differed by MRMM status. Women with wild type *M. genitalium* infection had a longer median duration of infection (48 days; IQR 43, 106) compared to those with *M. genitalium* with MRMM (42 days; IQR 40, 44) [p=0.01].

DISCUSSION

In this prospective cohort of Kenyan and US women, we observed a high prevalence of *M. genitalium* infection (11.3%), which was detected more frequently than other curable STIs. We observed a similar prevalence of *M. genitalium* between Kenyan and US women, with an overall prevalence that was consistent with observations from studies of high-risk women in Uganda (14%) [24], Kenya (12.9%-16%) [25–27], South Africa (8.7%) [28] and the US (7.7%-19.2%) [18, 29, 30]. We also observed a high incidence of *M. genitalium* infection (33.4 per 100 person-years), which again, was substantially higher than the incidence of other curable STIs in this cohort reported previously (*C. trachomatis* incidence=11.7 per 100 person-years; *N. gonorrhoeae* incidence=7.2 per 100 person-years) [23]. Data on *M. genitalium* incidence are sparse, but our estimates are similar to those reported by other Kenyan studies (22.7–34.6 per 100 person year) [26, 27]. This high prevalence and incidence of *M. genitalium* highlights the significant burden of this pathogen among reproductive-aged women.

Differences were observed in factors associated with prevalent *M. genitalium* infection versus incident infection. Detection of VVC and having a new sex partner were associated with prevalent *M. genitalium*, but were not associated with incident infection. Conversely, being a current smoker was associated with increased risk of incident infection, while younger age and concurrent BV trended towards an association. For smoking, age and concurrent BV, no association was observed for prevalent *M. genitalium*. These differences may be due to the fact that risk factor assessment was performed most likely after *M. genitalium* infection occurred (i.e. prevalent infections could have been recent or persisted for months), whereas risk factor assessment for incident infections occurred closer to the time of actual infection. The association of smoking, age and concurrent BV with incident, but not prevalent infection is likely due to limited study power and the smaller number of prevalent infections at baseline. Of note, the effect estimates for the association of smoking,

age and concurrent BV with prevalent MG were in the same direction as those in the incidence analysis and of generally similar magnitude. Our findings regarding BV and incident *M. genitalium* differed from a study by Lokken *et al.* that reported an association with BV at the preceding visit and incident infection. Both cohorts were from Kenya and had sampling every two months [27]. However, the inclusion of women receiving a vaginal health intervention as part of present study decreased the overall prevalence of BV and could have impacted the relationship between BV and *M. genitalium* susceptibility. Lastly, in contrast to other reports [5, 29, 31, 32], no association was observed between cervicitis by Gram stain and prevalent *M. genitalium*.

Among women with prevalent *M. genitalium*, median time to clearance was approximately 4 months. This represents the lower bound for infection duration among those with prevalent infection, since we were unable to account for person-time prior to enrollment. Median infection duration among women with incident *M. genitalium* was 1.5 months. This infection duration was similar to the median clearance time reported in a study of Ugandan women (2.1 months) [24], but slightly shorter than those observed in another cohort of Kenyan women (2.9 months) [27]. Overall, there was little reported use of azithromycin and doxycycline in our cohort, and no women who tested positive for *M. genitalium* during follow-up ever received azithromycin. In all cases where doxycycline was dispensed during the trial, it was either received at *M. genitalium*-negative time points or did not result in eradication of the organism in infected women, suggesting that the vast majority of infections were cleared spontaneously. Recurrent *M. genitalium* infection occurred in approximately one-third of women, which is also consistent with the recurrence rate among women in the Ugandan study (39%) [24].

The proportion of *M. genitalium* infections with MRMM was relatively low (13.3% of *M. genitalium* positive specimens had MRMM detected) compared to reports from other regions, where MRMM prevalence has approached 60% [15–18]. The frequency of MRMM in our study population was similar to that observed in a cohort of women in South Africa (MRMM prevalence=9.8%) [28]. Azithromycin-resistant infections emerge after azithromycin therapy in approximately 10% of *M. genitalium* positive individuals [33]. The lower frequency of MRMM observed in these African studies may be due to lower rates of background azithromycin use, reducing selective pressure. Despite the low overall frequency of MRMM detection, the majority of *M. genitalium* infections with MRMM were detected as incident infections, demonstrating that macrolide resistant *M. genitalium* is circulating in these regions. Interestingly, the duration of infection with macrolide resistant *M. genitalium* was shorter than wild type *M. genitalium*, which suggests that macrolide resistant *M. genitalium* may be less fit or more easily cleared than wild type strains. Given the rapid emergence of macrolide resistance [34], it is important to characterize the prevalence of MRMM in populations at high risk for *M. genitalium* infection to better inform treatment guidelines and potentially minimize treatment failure.

Our study should be interpreted in the context of several limitations. Specimens for *M. genitalium* testing were collected every other month, which may have inflated estimates of infection duration and may have also resulted in failure to detect infections of short duration. More frequent, monthly sampling would improve the precision of infection duration

estimates and incidence. A proportion of *M. genitalium* positive specimens were unevaluable for MRMM testing, limiting our ability to assess MRMM among all women with *M. genitalium*. Women participating in the trial were considered to be at higher risk for STIs as approximately half reported past or present transactional sex. As a result, the observed prevalence and incidence in our study population may be higher than that in the general population. Lastly, data on *M. genitalium* strain were not available. Therefore, we were unable to determine if women who experienced a second *M. genitalium* infection experienced an infection with a new strain or if the *M. genitalium* infection actually persisted, with intervening negative visits being false negatives (i.e. low organism concentration) rather than true negatives.

In summary, our data show that *M. genitalium* infection is very common among sexually active women in Nairobi and Mombasa, Kenya and Birmingham, Alabama. Given its association with adverse reproductive health outcomes in women, a high prevalence and incidence of *M. genitalium* could contribute to substantial morbidity. Additional work is needed to definitively demonstrate that *M. genitalium* causes adverse reproductive health outcomes. [35, 36]. Studies of *M. genitalium* treatment and prevention will be critical to improving our understanding of this pathogen's contribution to adverse reproductive outcomes and developing appropriate public health control strategies.

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SHORT SUMMARY

Using retrospective testing of archived specimens, a natural history study of *Mycoplasma genitalium* among Kenyan and US women reported a high prevalence (11.3%) and incidence of *M. genitalium* (33.4 per 100 person-years) of *M. genitalium*.

#	Country	Study arm	Enrollment	Month 2	Month 4	Month 6	Month 8	Month 10	Month 12
1	US	Placebo	+	-	-	-	-	+	-
2	Kenya	Placebo	+	+	+	-	+	+	+
3	Kenya	Placebo	+	+	+	+	-	+	-
4	Kenya	Placebo	+	-	-	-	-	+	-
5	Kenya	Placebo	+	+	+	+	-	+	-
6	Kenya	Placebo	+	-	-	-	-	-	-
7	Kenya	Placebo	+	+	+	+	+	-	-
8	Kenya	Placebo	+	+	+	+	(+)	+	-
9	Kenya	Placebo	+	-	+	+	+	+	+
10	US	PPT	+	+	+	+	+	+	-
11	US	PPT	+	+	+	-	+	//	-
12	US	PPT	+	+	-	-	-	-	-
13	US	PPT	+	-	-	-	-	-	-
14	US	PPT	+	+	+	+	+	//	-
15	Kenya	PPT	+	+	(-)	+	-	-	-
16	Kenya	PPT	+	+	-	-	-	+	-
17	Kenya	PPT	+	-	-	-	-	-	-
18	Kenya	PPT	+	+	+	+	-	+	-
19	Kenya	PPT	+	+	-	-	-	(-)	-
20	Kenya	PPT	+	+	+	+	+	//	-
21	Kenya	PPT	+	+	+	+	+	+	+
22	Kenya	PPT	+	-	-	-	-	-	-
23	Kenya	PPT	+	+	(+)	+	+	-	+
24	Kenya	PPT	+	+	+	+	+	+	+
25	Kenya	PPT	+	+	+	+	+	+	+

+	= *M. genitalium* detected
-	= *M. genitalium* not detected
//	= Missed visit
(+) or (-)	= Doxycycline dispensed at the visit or in the preceding 30 days. No azithromycin was dispensed to women who had *M. genitalium* at any point during the trial

Figure 1.

M. genitalium infection and resolution among participants with *M. genitalium* detected at enrollment

#	Country	Study arm	Enrollment	Month 2	Month 4	Month 6	Month 8	Month 10	Month 12
26	US	Placebo	-	-	-	+	+	-	-
27	US	Placebo	-	+	+	+	+	+	+
28	US	Placebo	-	-	-	-	+	+	-
29	Kenya	Placebo	-	-	+	-	-	-	-
30	Kenya	Placebo	-	-	+	-	-	-	-
31	Kenya	Placebo	-	-	+	-	-	-	-
32	Kenya	Placebo	-	-	-	(-)	+	-	+
33	Kenya	Placebo	-	-	+	-	-	-	+
34	Kenya	Placebo	-	-	+	-	-	-	+
35	Kenya	Placebo	-	-	-	+	-	-	-
36	Kenya	Placebo	-	-	+	+	-	-	-
37	Kenya	Placebo	-	-	-	//	-	-	+
38	Kenya	Placebo	-	+	-	-	-	+	-
39	Kenya	Placebo	-	+	-	-	+	-	-
40	Kenya	Placebo	-	-	-	+	-	-	-
41	Kenya	Placebo	-	-	-	-	+	-	-
42	Kenya	Placebo	-	-	-	-	+	+	(-)
43	Kenya	Placebo	-	-	-	+	-	-	-
44	Kenya	Placebo	-	-	-	-	-	-	+
45	Kenya	Placebo	-	-	-	+	-	-	-
46	Kenya	Placebo	-	-	+	+	-	-	-
47	Kenya	Placebo	-	-	-	-	+	-	-
48	Kenya	Placebo	-	-	-	-	+	+	+
49	Kenya	Placebo	-	+	+	+	+	//	-
50	Kenya	Placebo	-	-	-	-	+	+	-
51	Kenya	Placebo	-	-	//	//	+	+	-
52	Kenya	Placebo	-	-	+	-	-	-	-
53	Kenya	Placebo	-	-	-	-	-	-	+
54	Kenya	Placebo	-	-	+	-	-	-	-
55	Kenya	Placebo	-	+	+	//	//	//	+
56	Kenya	Placebo	-	-	-	-	-	+	-
57	Kenya	Placebo	-	-	-	-	-	+	-
58	US	PPT	-	-	+	+	+	+	+
59	US	PPT	-	-	+	-	-	+	-
60	US	PPT	-	-	-	-	-	+	-
61	US	PPT	-	+	+	-	+	+	+
62	US	PPT	-	-	-	-	-	-	+
63	US	PPT	-	-	+	-	-	-	-
64	US	PPT	-	-	-	+	//	//	-
65	US	PPT	-	-	-	+	//	//	+
66	US	PPT	-	-	-	-	+	-	-
67	Kenya	PPT	-	+	-	-	+	+	+
68	Kenya	PPT	-	-	+	//	//	//	//
69	Kenya	PPT	-	-	-	+	-	+	-
70	Kenya	PPT	-	+	-	-	-	-	-
71	Kenya	PPT	-	-	+	-	-	-	-
72	Kenya	PPT	-	-	-	-	+	-	-
73	Kenya	PPT	-	-	-	+	-	-	-
74	Kenya	PPT	-	-	-	(-)	+	+	-
75	Kenya	PPT	-	-	-	-	+	+	+
76	Kenya	PPT	(-)	//	//	//	//	//	+
77	Kenya	PPT	-	-	-	-	-	+	-

\+ = *M. genitalium* detected
\- = *M. genitalium* not detected
// = Missed visit
(+) or (-) = Doxycycline dispensed at the visit or in the preceding 30 days. No azithromycin was dispensed to women who had *M. genitalium* at any point during the trial

Figure 2.
Incident *M. genitalium* infection by study arm

#	Country	Study arm	Enrollment	Month 2	Month 4	Month 6	Month 8	Month 10	Month 12
11	US	PPT	wild type	wild type	wild type	-	A2058G	//	-
61	US	PPT	MG-	wild type	A2059G/ wild type[1]	-	wild type	A2059G	wild type
14	US	PPT	wild type	wild type	wild type	wild type	A2059G	//	-
30	Kenya	Placebo	-	-	A2059G	-	-	-	-
31	Kenya	Placebo	-	-	A2059G	-	-	-	-
33	Kenya	Placebo	-	-	NT	-	-	-	A2059G
64	Kenya	PPT	-	-	-	A2059G	//	//	-
34	Kenya	Placebo	-	-	A2059G	-	-	-	NT
35	Kenya	Placebo	-	-	-	A2059G	-	-	-
36	Kenya	Placebo	-	-	NT	A2059G	-	-	-
66	Kenya	PPT	-	-	-	-	A2059G	-	-
46	Kenya	Placebo	-	-	A2059G	NT	MG-	-	-
5	Kenya	Placebo	NT	wild type	wild type	wild type	MG-	A2059G	-
76	Kenya	PPT	-	//	//	//	//	//	A2059G
57	Kenya	Placebo	-	-	-	-	-	A2059G	-

MG- = Specimen was negative for *M. genitalium*

Wild type = Specimen was positive for *M. genitalium* and no MRMM detected

A2059G = Specimen was positive for *M. genitalium* and MRMM A2059G detected

A2058G = Specimen was positive for *M. genitalium* and MRMM A2058G detected

// = Missed visit, no specimens collected

NT = Specimen was positive for *M. genitalium* but not typeable

[1]Both wild type M. genitalium and A2059G were detected in this sample.

Figure 3.

Patterns of *M. genitalium* infection and MRMM among women with MRMM at any point during participation in the PVI trial

Table 1

Correlates of prevalent *M. genitalium* infection at enrollment

	All participants N=221		*M. genitalium* detected n=25		*M. genitalium* not detected n=196		Univariate OR (95% CI)	
Demographics and behaviors								
Age < 25 years	62	(28)	9	(36)	53	(27)	1.52	(0.63, 3.64)
Partnership status								
Married or living with a partner	63	(29)	5	(20)	58	(30)	1.00	—
Separated, divorced or widowed	87	(40)	10	(40)	77	(39)	1.51	(0.49, 4.65)
Never married	71	(32)	10	(40)	61	(31)	1.90	(0.61, 5.90)
Number of live births	2	(1–3)	1	(1–2)	2	(1–3)	0.93	(0.69, 1.27)
Family planning method								
No modern method	39	(18)	5	(20)	38	(20)	1.00	—
Condoms only	61	(28)	9	(36)	52	(27)	1.32	(0.40, 4.24)
Oral contraceptives	23	(10)	2	(8)	21	(11)	0.72	(0.13, 4.06)
Injectable contraceptives	49	(22)	3	(12)	46	(23)	0.50	(0.11, 2.11)
IUD	14	(6)	2	(8)	12	(6)	1.27	(0.22, 7.39)
Implant	14	(6)	2	(8)	12	(6)	1.27	(0.22, 7.39)
Tubal ligation	15	(7)	2	(8)	15	(8)	1.01	(0.18, 5.80)
Currently smoke cigarettes	30	(14)	5	(20)	25	(13)	1.71	(0.59, 4.97)
Vaginal washing in the past month	111	(50)	13	(52)	98	(50)	1.08	(0.47, 2.49)
Sex in exchange for goods/money/services	119	(54)	17	(68)	102	(52)	1.96	(0.81, 4.75)
Sexual behaviors in the past week								
Vaginal sex								
None	41	(19)	3	(12)	38	(19)	1.00	—
1–2 acts	89	(40)	10	(40)	79	(40)	1.60	(0.41, 6.17)
3–4 acts	36	(16)	3	(12)	33	(17)	1.15	(0.22, 6.10)
5 or more	55	(25)	9	(36)	46	(23)	2.48	(0.63, 9.81)
Unprotected sex								
No sex	41	(19)	3	(12)	38	(19)	1.00	—
100% condom use	95	(43)	14	(56)	81	(41)	2.19	(0.59, 8.07)

	All participants N=221	*M. genitalium* detected n=25	*M. genitalium* not detected n=196	Univariate OR (95% CI)
Intermittent condom use	23 (10)	3 (12)	20 (10)	1.90 (0.35, 10.29)
No condom use	62 (28)	5 (20)	57 (29)	1.11 (0.25, 4.93)
Number of sex partners				
None	41 (19)	3 (12)	38 (19)	1.00 —
1 partner	109 (49)	12 (48)	97 (49)	1.57 (0.42, 5.87)
2 or more partners	71 (32)	10 (40)	61 (31)	2.08 (0.54, 8.03)
New partner	45 (20)	9 (36)	36 (18)	2.50 (1.02, 6.11)
History of anal sex	25 (11)	3 (12)	22 (11)	1.08 (0.30, 3.90)
Clinical characteristics				
Gonorrhea	3 (1)	0 (0)	3 (2)	— —
Chlamydia	16 (7)	0 (0)	16 (8)	— —
Trichomonas vaginalis	16 (7)	3 (12)	13 (7)	1.90 (0.51, 7.19)
Vulvovaginal candidiasis	52 (24)	10 (40)	42 (21)	2.44 (1.02, 5.83)
BV by Nugent score				
0–3	81 (37)	7 (28)	89 (45)	1.00 —
4–6	44 (20)	6 (24)	38 (19)	2.01 (0.63, 6.37)
7–10	96 (44)	12 (48)	69 (35)	2.21 (0.83, 5.91)
Cervicitis[1]	32 (15)	4 (16)	28 (14)	1.14 (0.36, 3.56)

[1] Cervicitis defined as ≥30 PMNs on Gram stain. Two results missing from *M. genitalium* negative women.

Table 2

Correlates of incident *M. genitalium* infection during follow-up

	Number of events	Person-years	Incidence	Bivariate HR (95% CI)[1]	
Baseline factors					
Age					
<25 years	18	40.0	45.1	1.67	(0.93, 2.98)
≥ 25 years	34	115.7	29.4	1.00	–
Partnership status					
Married or living with a partner	14	50.6	27.7	1.47	(0.68, 3.19)
Separated, divorced or widowed	21	61.0	34.4	1.42	(0.61, 3.33)
Never married	17	44.1	38.6	1.00	–
Number of live births					
2 or more live births	34	85.7	39.7	1.17	(0.42, 3.30)
1 live birth	13	46.3	28.1	0.97	(0.32, 2.93)
None	5	23.7	21.1	1.00	–
Currently smoke cigarettes					
Yes	9	16.6	54.1	3.14	(1.42, 6.96)
No	43	139.1	30.9	1.00	–
Sex in exchange for goods/money/services					
Yes	24	83.2	28.8	0.71	(0.27, 1.86)
No	28	72.5	38.6	1.00	–
History of anal sex					
Yes	2	19.1	10.5	0.38	(0.09, 1.72)
No	50	136.6	36.6	1.00	–
HSV-2 status					
Positive	33	95.6	34.5	1.21	(0.68, 2.15)
Negative	17	60.0	31.6	1.00	–
Time-varying factor[2]					
Vaginal sex					
5 or more	11	36.3	30.3	0.86	(0.38, 1.98)
3–4 acts	5	24.8	20.2	0.41	(0.15, 1.18)

	Number of events	Person-years	Incidence	Bivariate HR (95% CI)[1]	
1–2 acts	22	57.6	38.2	0.88	(0.44, 1.77)
None	14	37.1	37.7	1.00	–
Unprotected sex					
100% condom use	21	66.1	31.8	0.82	(0.41, 1.63)
Intermittent condom use	5	14.4	34.6	1.03	(0.37, 2.91)
No condom use	12	37.8	31.8	0.62	(0.27, 1.41)
No sex	14	37.1	37.7	1.00	–
Number of sex partners					
2 or more partners	11	40.6	27.1	0.75	(0.32, 1.75)
1 partner	27	77.8	34.7	0.78	(0.39, 1.54)
None	14	37.2	37.6	1.00	–
New partner					
Yes	9	30.2	29.8	1.04	(0.47, 2.32)
No	43	125.5	34.3	1.00	–
Vaginal washing in the past month					
Yes	13	42.6	30.5	1.18	(0.56, 2.48)
No	39	113.1	34.5	1.00	–
BV by Nugent score[3]					
Yes	19	40.3	47.1	1.71	(0.96, 3.08)
No	33	115.2	28.6	1.00	–
BV by Nugent score at the preceding visit[3]					
Yes	13	41.5	31.3	0.95	(0.50, 1.81)
No	39	114.0	34.2	1.00	–
Vulvovaginal candidiasis					
Yes	4	18.0	22.2	0.68	(0.24, 1.92)
No	48	137.3	35.0	1.00	–

[1] Univariate Cox proportional hazards models stratified by site and adjusted for study arm. Follow-up censored at time of first infection

[2] Factors reported at the same visit as *M. genitalium* testing unless otherwise indicated.

[3] Nugent score 7–10 vs. 0–6.