MAJOR ARTICLE

Prevalence and Correlates of *Mycoplasma genitalium* Infection Among Female Sex Workers in Kampala, Uganda

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Background. The importance of *Mycoplasma genitalium* in human immunodeficiency virus (HIV)–burdened sub–Saharan Africa is relatively unknown. We assessed the prevalence and explored determinants of this emerging sexually transmitted infection (STI) in high-risk women in Uganda.

Methods. Endocervical swabs from 1025 female sex workers in Kampala were tested for *Mycoplasma genitalium* using a commercial Real-TM polymerase chain reaction assay. Factors associated with prevalent *Mycoplasma genitalium*, including sociodemographics, reproductive history, risk behavior, and HIV and other STIs, were examined using multivariable logistic regression.

Results. The prevalence of *Mycoplasma genitalium* was 14% and higher in HIV-positive women than in HIV-negative women (adjusted odds ratio [OR], 1.64; 95% confidence interval [CI], 1.12–2.41). *Mycoplasma genitalium* infection was less prevalent in older women (adjusted OR, 0.61; 95% CI, .41–.90 for women ages 25–34 years vs <25 years; adjusted OR, 0.32; 95% CI, .15–.71 for women ≥35 years vs those <25 years) and in those who had been pregnant but never had a live birth (adjusted OR, 2.25; 95% CI, 1.04–4.88). *Mycoplasma genitalium* was associated with *Neisseria gonorrhoeae* (adjusted OR, 1.84; 95% CI, 1.13–2.98) and with *Candida* infection (adjusted OR, 0.41; 95% CI, .18–.91), and there was some evidence of association with *Trichomonas vaginalis* (adjusted OR, 1.56; 95% CI, 1.00–2.44).

Conclusions. The relatively high prevalence of *Mycoplasma genitalium* and its association with prevalent HIV urgently calls for further research to explore the potential role this emerging STI plays in the acquisition and transmission of HIV infection.

Mycoplasma genitalium (*M. genitalium*) is 1 of 15 *Mycoplasma* species isolated in humans. The predominant colonization site is the urogenital tract of men and women [1]. The organism adheres to epithelial cells, invades them, and uses the intracellular environment as a survival niche where it multiplies and persists [2–4]. *M. genitalium* was first isolated in 1980 from the urethra of 2 men with nongonococcal urethritis [5]. However, because the organism is difficult to culture, clinical and epidemiological studies only became possible following the development of sensitive and specific polymerase chain reaction (PCR) assays in the early 1990s [6, 7]. Studies investigating transmission of *M. genitalium* in couples and DNA sequence typing among sexual partners have concluded that the bacterium is sexually transmitted [8–12].

M. genitalium is now considered a main cause of nonchlamydial nongonococcal urethritis (NCNGU) in men. A meta-analysis of 15 case-control studies reported that the prevalence of *M. genitalium* in men with NCNGU was 22%, compared with 6% in those without urethritis (pooled odds ratio [OR], 5.15; 95% confidence

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interval [CI], 3.6–7.4) [13]. Except for 1, all studies were conducted in high-income countries [14]. *M. genitalium* is also found in the female reproductive tract, and there is increasing evidence that this bacterium may cause cervicitis and urethritis and may be correlated with upper genital tract infections and its sequelae such as tubal factor infertility [15–20].

Sexually transmitted infections (STIs) are important risk factors for acquiring and transmitting HIV, and STI control is recognized worldwide as a priority in the prevention of HIV [21]. A recent meta-analysis of 19 cross-sectional studies showed a strong association between prevalent *M. genitalium* and HIV infection (summary OR, 2.01; 95% CI, 1.44–2.79), with a stronger effect in studies from sub–Saharan Africa (OR, 2.60; 95% CI, 2.17–3.11) [22]. More data on the importance and the epidemiological determinants of *M. genitalium* infection are needed from this HIV-burdened region in order to decide whether to include this emerging infection in the existing STI control strategies.

We investigated the epidemiology of *M. genitalium* infection in a cross-sectional study of women involved in high-risk sexual behavior in Kampala, Uganda. To our knowledge, this is the first study on *M. genitalium* infection in Uganda.

SUBJECTS AND METHODS

Study Population and Clinical Procedures

Our study was carried out in a recently established cohort of female sex workers in Kampala, Uganda. Here we report findings from the baseline survey of this cohort. The study setting and procedures have been described in detail elsewhere [23]. In brief, women engaged in commercial sex work and/or employed in entertainment facilities were recruited from red-light areas in southern Kampala and asked to participate in a cohort study. At baseline, women were interviewed about their sociodemographic characteristics, sexual risk behavior, alcohol use (using the CAGE questionnaire [24]), intravaginal practices, reproductive health history and symptoms of STI. Blood was collected for HIV, herpes simplex virus type 2 (HSV-2) and syphilis testing. A speculum examination was performed and 2 endocervical specimens were collected using thin polyester (Dacron) swabs, 1 for the diagnosis of gonococcal and chlamydial infection and 1 to be stored for a later diagnosis of *M. genitalium*. One high vaginal swab was used to collect a specimen to be inoculated for culture of *Trichomonas vaginalis*, another to prepare a slide for the detection of bacterial vaginosis and of *Candida* infection. Women with symptomatic STIs were treated syndromically on the spot. Women with asymptomatic STIs were treated as soon as laboratory results became available. Because the diagnosis of *M. genitalium* was made on stored specimens up to 2 years after specimen collection, it was not possible to specifically treat for *M. genitalium* at the time of the survey.

Informed consent was obtained from all study participants. The study was approved by the Science and Ethics Committee of the Ugandan Virus Research Institute and by the Uganda National Committee for Science and Technology.

Laboratory Methods

For the detection of *M. genitalium*, the endocervical specimen was inserted in a buffer solution, using Cobas Amplicor specimen transport medium collection tubes (Roche Diagnostic Systems Inc., Branchburg, NJ). The samples were stored at 4°C until transport to the Medical Research Council (MRC)/UVRI laboratories in Entebbe within 12 hours of collection. The specimens were kept at −20°C until sample collection from all enrolled women was completed. They were then sent on dry ice to the Sexually Transmitted Infections Reference Centre at the National Institute for Communicable Diseases in Johannesburg, South Africa, for PCR testing.

Real-time polymerase chain reaction for M. genitalium

Genomic DNA was extracted from endocervical specimens using an X-tractor Gene automated DNA extractor (Corbett Life Science, Concorde, Australia). A total volume of 200 μL of each specimen was extracted and eluted to a final volume of 150 μL. For the first 200 DNA specimens, we performed an in-house real-time PCR for the qualitative detection of *M. genitalium* as well as a commercially available *M. genitalium* Real-TM assay (Sacace Biotechnologies, Como, Italy) [25]. The primers and probe for the in-house real-time PCR were designed at the Centers for Disease Control and Prevention and used to amplify the *pdhD* gene (coding for dihydrolipoamide dehydrogenase) in *M. genitalium*. The following primers and probe were used (nucleotides [nt] corresponding to the sequence with GenBank number L43967.2): forward primer, MG-041 (5′-CGG ATC AAG ACC AAG ATA CTT AAC TTT-3′; nt 329417-329391); reverse primer, MG-042 (5′-AGC TTG GGT TGA GTC AAT GAT AAA C-3′; nt 329336-329360); and a JOE-labeled probe, MG-048 (5′-[AminoC6+JOE] CCA GGG TTT GAA AAA GCA CAA CAA GCT G [BHQ1a]-3′; nt 329389-329362). The primers were synthesized at the University of Cape Town, South Africa, and the probe was synthesized at Eurofins MWG Operon, Germany. The commercially available *M. genitalium* Real-TM assay targeted the DNA gyrase subunit B of *M. genitalium*. Due to a 100% concordance between the in-house and commercial *M. genitalium* assay results, we decided to test the remaining 825 specimens exclusively with the commercial assay. For both assays, the DNA was amplified using a real-time PCR platform (Rotor-Gene 3000/6000, Corbett Life Science) and detected using the specific fluorescent reporter dye probes of the 2 assays. The commercial assay included an internal control that served as an amplification control for each individually processed specimen and to identify possible inhibition reactions. *M. genitalium* G37 genomic DNA (ATCC 33530D) as well as the *M. genitalium* positive control included in the commercial assay were used as

a positive control. The sequences of the primers and probes used in the commercial assay are proprietary. The potential cross-reactivity and the analytical sensitivity of the kit, as well as the analytical specificity of the primers and probes, were tested and validated by Sacace Biotechnologies. Both assays were internally validated at the Sexually Transmitted Infections Reference Centre in Johannesburg, South Africa.

Laboratory testing for all other infections was performed at the central laboratories of the MRC/UVRI Uganda Unit in Entebbe. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were diagnosed on endocervical specimens using the Amplicor PCR test (Roche Diagnostic Systems Inc.). *T. vaginalis* was detected using a commercial culture kit (InPouch TV, BioMed Diagnostics, White City, OR). Microscopy on a Gram-stained vaginal specimen was done to diagnose bacterial vaginosis (using the Nugent criteria) and *Candida* infection. Serum specimens were processed to detect antibodies against HIV-1 (using Abbott Determine HIV-1/2 rapid assay with confirmation by 2 independent enzyme-linked immunosorbent assay [ELISA] tests [Vironostika Uniform II plus O, Murex HIV 1.2.O] and against HSV-2 (using immunoglobin G ELISA test, Kalon Biological Ltd, United Kingdom) and to diagnose syphilis infection (using Biotec rapid plasma reagin [RPR] and Treponema pallidum hemagglutination [TPHA] test kits). Active syphilis was defined as having both RPR and TPHA tests positive; an RPR titer of $<$1:8 was considered as low-titer active syphilis, and a RPR titer of $\geq$1:8 as high-titer active syphilis.

Statistical Analysis

Data were double entered in Access and analyzed using STATA 11.0 (Stata Inc., College Station, TX). Factors independently associated with prevalent *M. genitalium* infection were analyzed using logistic regression to estimate OR and 95% CI. *P* values were obtained using likelihood ratio tests. The univariable associations of *M. genitalium* with sociodemographic and behavioral characteristics, reproductive health factors, and HIV and other STIs were assessed, and all factors associated with the outcome at $P < .10$ in univariable analyses were included in a multivariable model. The final model included only those factors that remained or became independently associated with *M. genitalium* infection ($P < .05$) after adjustment for all other factors.

RESULTS

Details of the study population have been published previously [23]. Briefly, 1027 women likely to be involved in high-risk sexual behavior in Kampala were enrolled between April 2008 and May 2009. The median age of the study population at baseline was 26 years (interquartile range, 22–30 years). The majority of women (70%) were formerly married (widowed, divorced, or separated) and 90% had received no more than primary school education. At enrollment, 96% reported engaging in sex work. Baseline HIV prevalence was 37%, more than 50% of the women were diagnosed with 1 or more other curable STIs, and 56% with bacterial vaginosis.

Among the 1025 women with results from endocervical samples, prevalence of *M. genitalium* was 14% (95% CI, 12%–17%), *N. gonorrhoeae* 13% (95% CI, 11%–15%), and *C. trachomatis* 9% (95% CI, 7%–11%). Among the 148 *M. genitalium*–infected women, 105 (71%) had *M. genitalium* infection only, while 26 (17%) were coinfected with *N. gonorrhoeae*, 12 (9%) with *C. trachomatis*, and 5 (3%) with both pathogens. Overall, 176 (17%) women were diagnosed with *T. vaginalis*, 36 (20%) of them being coinfected with *M. genitalium*.

Factors Associated With *M. genitalium* Infection

Univariable analysis

Prevalence of *M. genitalium* infection was higher in younger age groups, decreasing from 19% among those younger than 25 years to 7% among those 35 years or older (*P*-trend $<$.01; Table 1 and Figure 1). There was little evidence that *M. genitalium* was associated with level of education or marital status. Women who had been pregnant but not had a live birth were at higher risk than those who had had at least 1 live birth (OR, 2.96; 95% CI, 1.46–5.99). Prevalence of *M. genitalium* infection was lower in women using injectable contraceptives (Depo-Provera) than in those not using any hormonal contraceptives (OR, 0.54; 95% CI, .33–.89); this was not the case for women using oral contraceptives. There was little evidence of an association with other behavioral factors, including number of lifetime partners, type of and condom use with last sexual partner, number of sexual partners in the last month, reported condom use with paying clients, alcohol use, and vaginal cleansing (Table 1).

Prevalence of *M. genitalium* infection was significantly higher among HIV-positive women than HIV-negative women (18% vs 12%; OR, 1.52; 95% CI, 1.07–2.17; Table 1 and Figure 1) and was also associated with *N. gonorrhoeae*, *T. vaginalis*, and bacterial vaginosis ($P = .003$, $P = .02$, and $P = .03$, respectively) but not with *C. trachomatis*. Women infected with *Candida albicans* had a lower prevalence of *M. genitalium* infection than women without (8% vs 15%; OR, 0.49; 95% CI, .24–.98). There was little evidence of association with HSV-2 infection or syphilis.

Multivariable analyses

On multivariable analyses, *M. genitalium* infection remained independently associated with younger age (*P*-trend $<$.01) and use of injectable contraceptives (adjusted OR for use of Depo-Provera 0.53; 95% CI, .32–.87; Table 1). *M. genitalium* prevalence also remained associated with reproductive history: women who got pregnant but never had a live birth were more likely to be infected with *M. genitalium* than women with at

Table 1. Sociodemographic, Sexual Behavior, and Reproductive Health Characteristics and Biological Factors Associated with *M. genitalium* (MG) Infections Among Female Sex Workers in Kampala, Uganda (Univariable and Multivariable Analysis)

	N	MG+ n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
	1025	148 (14)		
Sociodemographic characteristics				
Age, years			*P*-trend <.01	*P*-trend <.01
<25	411	77 (19)	1	1
25–34	505	63 (12)	0.62 (0.43–0.89)	0.61 (0.41–0.90)
35+	109	8 (7)	0.34 (0.16–0.74)	0.32 (0.15–0.71)
Education, highest level obtained			*P* = .50	
Primary not completed	503	78 (15)	1	…
Primary completed	417	58 (14)	0.88 (0.61–1.27)	…
Higher than primary	105	12 (11)	0.70 (0.37–1.34)	…
Current marital status			*P* = .42	
Formerly married	715	101 (14)	1	…
Currently married	82	16 (20)	1.47 (0.82–2.65)	…
Single	228	31 (14)	0.96 (0.62–1.48)	…
Having a regular partner			*P* = .30	
No	53	5 (9)	1	…
Yes	890	127 (14)	1.60 (0.62–4.09)	…
Reproductive health				
Reproductive history[b]			*P* = .006	*P* = .11
≥1 live birth	918	120 (13)	1	1
Pregnant, but no live births	39	12 (31)	2.96 (1.46–5.99)	2.25 (1.04–4.88)
Never pregnant	60	13 (22)	1.84 (0.97–3.50)	1.42 (0.72–2.80)
Termination of pregnancy (intended or unintended)[c]			*P* = .85	
None	464	66 (14)	1	…
At least 1	560	82 (15)	1.03 (0.73–1.47)	…
Lifetime no. of stillbirths[c]			*P* = .32	
None	942	133 (14)	1	…
At least 1	82	15 (18)	1.36 (0.76–2.45)	…
Use of hormonal contraceptives[d]			*P* = .006	*P* = .003
None	603	95 (16)	1	1
Oral contraceptives	104	22 (21)	1.43 (0.85–2.41)	1.56 (0.92–2.66)
Injectable contraceptives	239	22 (9)	0.54 (0.33–0.89)	0.53 (0.32–0.87)
Risk behavior				
No. of lifetime sexual partners			*P* = .34	
<10	128	24 (19)	1	…
≥10	218	29 (13)	0.66 (0.37–1.20)	…
Don't remember	679	95 (14)	0.70 (0.43–1.16)	…
Type of last sexual partner			*P* = .12	
Regular	486	79 (16)	1	
Casual	539	69 (13)	0.76 (0.53–1.07)	–
Condom use with last sexual partner			*P* = .10	
Yes	677	89 (13)	1	…
No	348	59 (17)	1.35 (0.94–1.93)	…
No. of sexual partners in the last month			*P* = .94	
≤1	172	24 (14)	1	…
2–9	284	44 (15)	1.13 (0.66–1.94)	…
≥10	496	69 (14)	1.00 (0.60–1.64)	…
Don't remember	73	11 (15)	1.09 (0.51–2.37)	…
Condom use with paying clients in the last month[e]			*P* = .91	

Table 1 continued.

	N	MG+ n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Consistent	540	76 (14)	1	…
Not consistent	363	52 (14)	1.02 (0.70–1.50)	…
Alcohol use			*P* = .12	
Not using	223	26 (12)	1	…
Nonproblem drinker	230	28 (12)	1.05 (0.59–1.85)	…
Problem drinker	572	94 (16)	1.49 (0.94–2.37)	…
Vaginal cleansing in last 3 months			*P* = .35	
Cleansing without soap	379	60 (16)	1	…
Cleansing with soap	582	82 (14)	0.87 (0.61–1.25)	…
No cleansing	64	6 (9)	0.55 (0.23–1.33)	…
Inserting substances in vagina in last 3 months			*P* = .63	
No	455	63 (14)	1	…
Yes	570	85 (15)	1.09 (0.77–1.55)	…
Biological factors				
HIV			*P* = .02	*P* = .01
Neg	643	80 (12)	1	1
Pos	382	68 (18)	1.52 (1.07–2.17)	1.64 (1.12–2.41)
HSV-2			*P* = .20	
Neg	205	24 (12)	1	…
Pos	820	124 (15)	1.34 (0.84–2.14)	…
Syphilis[f]			*P* = .43	
No infection (RPR–, TPHA–)	806	110 (14)	1	…
Past infection (RPR–, TPHA+)	113	19 (17)	1.28 (0.75–2.18)	…
Low-titer active (RPR titer <1:8)	74	15 (20)	1.61 (0.88–2.94)	…
High-titer active (RPR titer ≥ 1:8)	29	4 (14)	1.01 (0.34–2.96)	…
Trichomonas vaginalis			*P* = .02	*P* = .06
Neg	849	112 (13)	1	1
Pos	176	36 (20)	1.69 (1.12–2.57)	1.56 (1.00–2.44)
Bacterial vaginosis			*P* = .03	*P* = .35
Neg	353	40 (11)	1	1
Indeterminate	100	11 (11)	0.97 (0.48–1.96)	0.91 (0.44–1.90)
Pos	572	97 (17)	1.60 (1.08–2.37)	1.30 (0.85–1.98)
Candida infection			*P* = .03	*P* = .02
Neg	913	139 (15)	1	1
Pos	112	9 (8)	0.49 (0.24–0.98)	0.41 (0.18–0.91)
Neisseria gonorrhoeae			*P* = .003	*P* = .02
Neg	892	117 (13)	1	1
Pos	133	31 (23)	2.01 (1.29–3.15)	1.84 (1.13–2.98)
Chlamydia trachomatis			*P* = .26	
Neg	933	131 (14)	1	…
Pos	92	17 (18)	1.39 (0.79–2.42)	…

Abbreviations: OR, odds ratio; CI, confidence interval; HIV, human immunodeficiency virus; Neg, negative; Pos, positive; RPR, rapid plasma reagin; TPHA, Treponema pallidum hemagglutination.

[a] Adjusted for all variables that remained significantly associated with *M. genitalium* ($P < .05$) in the final model (age, use of hormonal contraceptives, *N. gonorrhoeae*, *Candida* infection, and HIV).

[b] Eight missing results.

[c] One missing result.

[d] Among nonpregnant women.

[e] Among 903 women having paid sex in the last month.

[f] Three missing results.

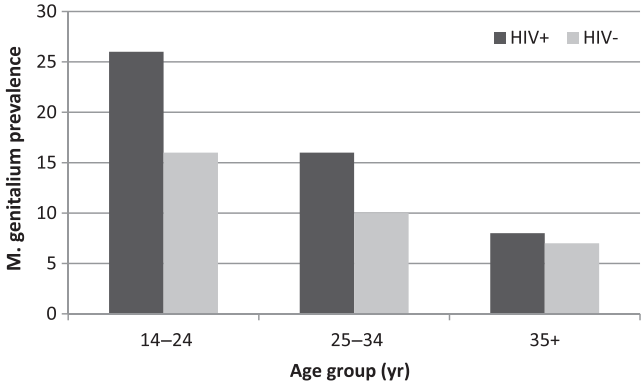

Figure 1. Prevalence of *M. genitalium* by age and HIV status.- Abbreviation: HIV, human immunodeficiency virus.

least 1 life birth (adjusted OR, 2.25; 95% CI, 1.04–4.88). *M. genitalium* remained associated with HIV and gonococcal infection (adjusted OR, 1.64; 95% CI, 1.12–2.41; adjusted OR, 1.84; 95% CI, 1.13–2.98, respectively); with *Candida* infection (adjusted OR, 0.41; 95% CI, .18–.91) and *T. vaginalis* (adjusted OR, 1.56; 95% CI, 1.00–2.44).

DISCUSSION

M. genitalium infection was detected at baseline in 14% of female sex workers enrolled in a cohort in Kampala, Uganda. The prevalence of *M. genitalium* infection was higher than the prevalence of gonorrhea (13%) and of chlamydial infection (9%). There are few comparable studies of *M. genitalium* in sub–Saharan Africa. The prevalence of *M. genitalium*, *N. gonorrhoeae*, and *C. trachomatis* among sex workers in West Africa was 26%, 16%, and 3%, respectively; in Kenya, prevalence was 16%, 8%, and 6%, respectively [17, 26]. A general population survey in Tanzania found that 3%, 2%, and 0.1% of women were infected with *M. genitalium*, *C. trachomatis*, and *N. gonorrhoeae*, respectively; and in Guinea-Bissau, the prevalence of *M. genitalium* among pregnant women was 6% [27, 28]. In high-income countries, the reported prevalence of *M. genitalium* is between 1% and 3% among women in the general population [29–32] and between 4% and 8% among female STD clinic attendees [19, 33–35], although higher infection rates were detected in sex workers, adolescents, and nonwhite women attending STD clinics (between 13% and 38%) [9, 36–40]. Most of the African studies, including ours, reported a higher prevalence of *M. genitalium* than of *C. trachomatis* infection, both in high- and low-risk women. This is in contrast with the studies in other regions where *M. genitalium* infection seems to be less prevalent than that of *C. trachomatis*. Furthermore, in the Kenyan sex workers cohort, the incidence of *M. genitalium* was also higher than that of *C. trachomatis* (23/100 and 14/100 person years, respectively) [26].

A plausible explanation for the higher prevalence of *M. genitalium* in Africa is that the high prevalence of HIV infection across the continent increases the susceptibility of many individuals for *M. genitalium*, as suggested by the observations from the Kenyan cohort. However, it is likely that other factors also play a role. For example, the reservoir of latent persistent *M. genitalium* infections in men and women may be larger than expected. Because *M. genitalium* appears to have milder symptoms and signs compared with *N. gonorrhoeae* and *C. trachomatis* infections [16, 41], even in symptomatic infections, women may less often seek care for *M. genitalium* than for other STIs. Furthermore, the doses of doxycycline and other antibiotics usually given as part of the syndromic treatment of nongonococcal urethritis, cervicitis, or pelvic inflammatory disease were found to be clinically effective but did not eradicate *M. genitalium* [42–45]. Alternatively, *M. genitalium* may simply be more strongly associated with HIV than *C. trachomatis*.

It is currently not clear whether the association between HIV and *M. genitalium* infections is the consequence of a causal relationship whereby HIV infection increases the risk of *M. genitalium* acquisition or vice versa, whether it merely reflects the confounding effect of sexual risk behavior or whether a combination of these 3 possible mechanisms is at work, as is the case with some other STIs. A recent meta-analysis found a strong association between *M. genitalium* and HIV in cross-sectional studies, with an even stronger association in the subgroup analysis, which included studies from sub–Saharan Africa, although the authors did report that only a few studies had adjusted for confounding factors [22]. Longitudinal studies are urgently needed in order to better understand the temporal relationship between *M. genitalium* and HIV. To date, only 1 longitudinal study reported a significant association between prevalent HIV and acquisition of new *M. genitalium* infections [26], and there are no published studies yet investigating whether prevalent *M. genitalium* infection enhances HIV acquisition or transmission.

Our study also assessed the sociodemographic, behavioral, and reproductive health characteristics associated with *M. genitalium* infection. The higher prevalence detected in younger women has also been reported in other studies [19, 32, 39], and may indicate that some immunity could be acquired after initial or serial infection, as is the case with chlamydial infections. However, immunological studies are needed to confirm this theory.

There was no evidence for an association between high-risk sexual behavior and *M. genitalium* infection, which was consistent with results from the West African sex workers study [17]. This may be partly explained by the nature of this specific study population. Studies conducted in the general population [29, 30, 31, 32] or among women attending STD clinics [19, 39], on the other hand, have reported contradicting results on the associations between inconsistent condom use, numbers of sexual partners, and the risk of *M. genitalium* infection.

Our data showed that injectable contraceptives may be protective against *M. genitalium* infection and that oral contraceptives were not associated with the infection. Previous studies have reported conflicting results on the correlation between hormonal contraception and *M. genitalium*. Consequently, this relationship needs further exploration [39, 46].

In our study, *M. genitalium* infection was associated with a history of having been pregnant but never having a live birth. Because women with current *M. genitalium* infection may have had other episodes of the infection before or may have had an untreated infection for a long time, it is plausible that past *M. genitalium* infections may have induced adverse pregnancy outcomes. To date, studies investigating the evidence for a relationship between *M. genitalium* and adverse pregnancy outcomes led to contradictory conclusions, and more research is needed [47].

To our knowledge, this is the first study reporting results on *M. genitalium* using the commercial *M. genitalium* Sacace test. A 100% concordance was found between the in-house real-time PCR and this commercial *M. genitalium* assay. The advantages of using a commercial test kit are low setup costs, built-in quality control, prevalidation by the manufacturer, ease of use, reduced susceptibility to contamination, and reduced interlaboratory variability. The disadvantages are the high cost per test, possible need for additional instrumentation, and inability of laboratories to control any aspect of the assay design.

The present study has several limitations. First, the cross-sectional design does not allow for investigation of temporal relationships between risk behavior, HIV/STI, and *M. genitalium*. Second, we had to rely on reported behaviors, which are sensitive to recall bias. Finally, our *M. genitalium* specimens were frozen for about 2 years until PCR testing in Johannesburg; this may have had a negative impact on the detection rate, as reported earlier in a Danish study [48].

In conclusion, we found that infection with *M. genitalium* was more prevalent than *N. gonorrhoeae* and *C. trachomatis* infections in Ugandan sex workers, and that in terms of risk factors, *M. genitalium* infection seems to behave like *C. trachomatis*, which is in line with other studies [29, 39]. The association of this emerging STI with HIV in cross-sectional studies urgently calls for further research to explore the potential role of *M. genitalium* in the acquisition and transmission of HIV infection. This missing information is needed in order to decide whether the treatment of *M. genitalium* should be incorporated into existing syndromic management or not, especially in high-risk population groups.

Notes

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