Clinical Characteristics Associated With *Mycoplasma genitalium* Infection Among Women at High Risk of HIV and Other STI in Uganda

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Background: *Mycoplasma genitalium* is a common infection of the genitourinary tract, but its pathogenic effects have not been well described, especially in women. The increasing evidence that *M. genitalium* is associated with HIV infection calls for an urgent consensus on how best to control this infection. The aim of this study was to describe symptoms and signs associated with *M. genitalium* infection among high-risk women in Uganda.

Methods: A cohort of 1027 female sex workers was recruited in Kampala in 2008. At enrollment, HIV testing was performed, genital specimens were tested for other sexually transmitted infection, and urogenital symptoms and signs were recorded. Endocervical swabs were tested for *M. genitalium* using a commercial Real-TM PCR assay (Sacace Biotechnologies, Como, Italy). The associations of clinical signs and symptoms with prevalent *M. genitalium* were investigated using multivariable logistic regression models.

Results: Reported dysuria and presence of mucopurulent vaginal discharge were significantly associated with *M. genitalium* infection (OR: 1.85, 95% confidence interval: 1.13–3.03 and OR: 1.55, 95% confidence interval: 1.06–2.29, respectively). There was little evidence for an association with cervicitis or with pelvic inflammatory disease.

Conclusions: In this specific population, we found evidence that symptoms of urethritis and mucopurulent vaginal discharge were associated with *M. genitalium* infection. This supports earlier studies showing that *M. genitalium* may lead to clinically relevant genitourinary disorders and should be treated. In the absence of sensitive screening tests, further work is needed to validate clinical findings as possible indicators of *M. genitalium* infection to guide a possible syndromic approach for its control.

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Mycoplasma genitalium is a sexually transmitted infection (STI) increasingly reported in both high- and low-risk populations worldwide. Compared with other STI, there are little data on the prevalence of *M. genitalium*.[1] In our recent cross-sectional study among female sex workers in Uganda, we estimated the prevalence of *M. genitalium* at 14% (95% confidence interval [CI]: 12%–17%).[2] The infection was more prevalent in younger age groups, in HIV-positive women, and in those coinfected with *Neisseria gonorrhoeae* (NG) and *Trichomonas vaginalis* (TV).

A systematic review of epidemiologic studies found that *M. genitalium* infection is associated with HIV infection, especially in sub-Saharan Africa.[3] This is calling for an urgent consensus on whether and how best to control and treat this novel STI, particularly in areas with a substantial burden of HIV infection. Unfortunately, more than 2 decades after it was first isolated, little is certain about the pathogenicity of *M. genitalium*, especially in women. Although the bacterium is now recognized as an important cause of nongonococcal nonchlamydial urethritis in men, the role of *M. genitalium* in upper and lower genitourinary tract diseases in women remains inconclusive.[4] Also the choice of antibiotics to treat *M. genitalium* infection is not established. Tetracycline and the older quinolone derivatives fail to eliminate the infection, and treatment with azithromycin seemed more promising until it transpired that the bacterium may have developed resistance to this antibiotic.[5] Satisfactory treatment outcomes were obtained with moxifloxacin, but this drug is not affordable in most low-income countries.[6,7]

In most settings in Africa, STI diagnosis is based exclusively on symptoms and signs. So far, few studies from sub-Saharan Africa have presented data on *M. genitalium* infection.[8–10] The aim of our study was to describe symptoms and signs associated with this infection among high-risk women in Uganda and to investigate whether the clinical pattern varied by HIV status. Such data are urgently needed to understand the pathogenicity of the bacterium and to decide whether to include *M. genitalium* treatment into established STI syndromic guidelines.

METHODS

Study Population and Clinical Procedures

Between April 2008 and May 2009, 1027 women who engaged in sex work and/or were employed in entertainment facilities, were recruited from red-light areas in southern Kampala.

The recruitment process has been described in detail previously.[11] Consenting participants were interviewed about their sociodemographic characteristics, risk behavior, and presence of current symptoms of STI, if any. An experienced clinician or midwife performed a gynecological examination including the use of a speculum. All reported symptoms and observed signs were recorded on standardized data entry forms.

Full study procedures have been described previously.[2,11] Briefly, 2 endocervical specimens were collected, one for the diagnosis of gonococcal and chlamydial infection and another to be stored for a later diagnosis of *M. genitalium*. One swab was collected from the posterior fornix of the vagina to be inoculated for culture of TV, another from the lateral vaginal walls to prepare a slide for the detection of bacterial vaginosis (BV) and of *Candida* infection. Blood was collected for HIV, herpes simplex virus type 2 (HSV2), and syphilis testing. Women with symptomatic STIs were treated syndromically, whereas women with asymptomatic STIs were treated when laboratory results became available. As the diagnosis of *M. genitalium* was made on stored specimens up to 2 years after specimen collection, women were not specifically treated for *M. genitalium*.

Clinical Definitions

Cervicitis was defined as presence of mucopurulent endocervical discharge on clinical examination; a vaginal infection was defined as presence of profuse and/or mucopurulent or white vaginal discharge. Women presenting with vaginal infection and/or cervicitis were diagnosed as having vaginal discharge syndrome. NG and CT were considered as pathogens responsible for cervicitis; TV, *Candida albicans*, and BV as possible causes of vaginal infections. Pelvic inflammatory disease (PID) was defined as reported lower abdominal pain and/or dyspareunia confirmed by bimanual palpation.

Laboratory Methods

For the detection of *M. genitalium*, the endocervical specimen was inserted in a buffer solution, using Cobas Amplicor STM collection tubes (Roche Diagnostic Systems Inc., Branchburg, NJ). The samples were stored at 4°C at the clinic until transport to the 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 and then finally sent on dry ice to the Centre for HIV and Sexually Transmitted Infections, National Institute for Communicable Diseases, National Health Laboratory Service in Johannesburg for polymerase chain reaction (PCR) testing. Genomic DNA was extracted from endocervical specimens using an X-tractor Gene automated DNA extractor (Corbett Life Science, Concorde, Australia) and tested for *M. genitalium* using a commercially available Real-TM assay (Sacace Biotechnologies, Como, Italy) targeting the DNA gyrase subunit B of *M. genitalium*. Further details of the PCR test have been reported previously.[2]

Laboratory testing for all other infections was performed at the central laboratories of the MRC/UVRI Uganda Unit in Entebbe. NG *and Chlamydia trachomatis* were diagnosed on endocervical specimens using the Amplicor PCR test (Roche Diagnostic Systems Inc.) and TV was detected using the commercial culture kit (InPouch TV, BioMed Diagnostics, White City, OR). Microscopy on a gram-stained vaginal specimen was performed to diagnose BV (using the Nugent's criteria) and *Candida* infection. Serum samples were tested for antibodies against HIV-1 (Abbott Determine HIV-1/2 with confirmation by 2 independent ELISA tests Vironostika Uniform II plus O, Murex HIV 1.2.O), for HSV2 (IgG ELISA test, Kalon Biologicals Ltd, Surrey, United Kingdom) and to diagnose syphilis infection (RPR Biotec and TPHA Biotec). Active serological syphilis was defined by a positive RPR test confirmed by a positive TPHA result.

Data Analysis

Data were double entered in Access and analyzed using STATA 11.0 (Stata Inc., College Station, TX). Associations of clinical signs and symptoms with *M. genitalium* infection and coinfections were first assessed using a χ^2 statistic. Then, each symptom and sign was considered in turn as the outcome (independent variable) and *M. genitalium* as the exposure (dependent variable). Age as well as other coinfections (HIV, HSV2, active syphilis, NG, CT, TV, *C. albicans*, and BV) were considered as potential confounders. All associations were analyzed using logistic regression to estimate odds ratios (ORs) and 95% CIs. *P* values were obtained using likelihood ratio tests.

First, the univariable association between each clinical symptom/sign and *M. genitalium* infection was assessed. Then, for each clinical characteristic, a separate multivariable analysis was done to adjust for potential confounders, retaining only factors that acted as confounders (changing the crude OR by 10% or greater). Finally, a likelihood ratio test was carried out to assess whether each adjusted association was modified by HIV infection status ($P < 0.10$).

TABLE 1. Prevalence of *Mycoplasma genitalium* (MG) Infection and Its Association With Other HIV and Other STI/RTI Among Women at High Risk in Kampala, Uganda

	All n	MG+ n (%)	OR (95% CI)
	N = 972	n = 137 (14)	
HIV			$P = 0.01$
Negative	610	73 (12)	1
Positive	362	64 (18)	1.58 (1.10–2.27)
Neisseria gonorrhoeae			$P = 0.002$
Negative	846	107 (13)	1
Positive	126	30 (24)	2.16 (1.37–3.41)
Chlamydia trachomatis			$P = 0.52$
Negative	887	123 (14)	1
Positive	85	14 (16)	1.22 (0.67–2.24)
Trichomonas vaginalis			$P = 0.02$
Negative	802	103 (13)	1
Positive	170	34 (20)	1.70 (1.10–2.61)
Candida albicans			$P = 0.04$
Negative	862	128 (15)	1
Positive	110	9 (8)	0.51 (0.25–1.04)
Bacterial vaginosis			$P = 0.005$
Negative	433	46 (11)	1
Positive	539	91 (17)	1.71 (1.17–2.50)
HSV2 serology			$P = 0.28$
Negative	196	23 (12)	1
Positive	776	114 (15)	1.30 (0.80–2.09)
Active syphilis (TPHA+RPR+)			$P = 0.56$
No	879	122 (14)	1
Yes	93	15 (16)	1.19 (0.66–2.14)

TABLE 2. Description of Clinical Symptoms and Signs Among Women Without Any STI, Women Negative for *M. genitalium* But Positive for Any Other STI, Women Positive for *M. genitalium* in Single Infection and Women Positive for *M. genitalium* in Coinfection With Other STI

	All N = 972	MG− NG−CT−TV− CA−BV−* n = 249	MG− NG/CT/TV/ CA/BV +† n = 586	MG+ NG−CT−TV− CA−BV−‡ n = 33	MG+ NG/CT/TV/ CA/BV +§ n = 104	*P*
Clinical symptoms						
Dysuria	110 (11)	25 (10)	61 (10)	4 (12)	20 (19)	0.06
Vaginal discharge	392 (40)	80 (32)	252 (43)	13 (39)	47 (45)	0.02
Low abdominal pain and/or dyspareunie	370 (38)	85 (34)	225 (38)	11 (33)	49 (47)	0.13
Any of above symptoms	584 (60)	128 (51)	366 (62)	20 (61)	70 (67)	0.009
Clinical examination						
Whitish vaginal discharge	328 (34)	78 (31)	215 (37)	7 (21)	28 (27)	0.06
Mucopurulent vaginal discharge	257 (26)	38 (15)	172 (29)	10 (30)	37 (36)	<0.01
Profuse vaginal discharge	587 (60)	121 (49)	385 (66)	18 (55)	63 (61)	<0.01
Profuse and/or abnormal colored vaginal discharge	609 (63)	125 (50)	400 (68)	18 (55)	66 (63)	<0.01
Mucopurulent endocervical discharge	226 (23)	34 (14)	155 (26)	6 (18)	31 (30)	<0.01
Confirmed VDS	617 (63)	127 (51)	406 (69)	18 (54)	66 (63)	<0.01
Confirmed PID	215 (22)	47 (19)	130 (22)	8 (24)	30 (29)	0.23

*Testing negative for *M. genitalium*, *N. gonorrhoeae*, *C. trachomatis*, *T. vaginalis*, *C. albicans*, and bacterial vaginosis.
†Testing negative for *M. genitalium* and testing positive for *N. gonorrhoeae* and/or *C. trachomatis* and/or *T. vaginalis* and/or *C. albicans* and/or bacterial vaginosis.
‡Testing positive for *M. genitalium* and testing negative for *M. genitalium*, *N. gonorrhoeae*, *C. trachomatis*, *T. vaginalis*, *C. albicans*, and bacterial vaginosis.
§Testing positive for *M. genitalium* and testing positive for *N. gonorrhoeae* and/or *C. trachomatis* and/or *T. vaginalis* and/or *C. albicans* and/or bacterial vaginosis.

Ethical Considerations

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 the Uganda National Committee for Science and Technology.

RESULTS

Characteristics of the Study Population

Details of the study population have been published previously.[11] Briefly, out of the 1027 enrolled women, 96% reported engagement in sex work and 4% were employed in an entertainment facility. The median age of the women was 26 years (interquartile range, 22–30 years), and 90% had received no more than primary school education. The majority (70%) was formerly married (widowed, divorced, or separated), 8% were currently married or living as married, and 22% were single.

For the current study, 53 menstruating women and 2 women without endocervical samples at enrollment were excluded. Among the remaining 972 women, the prevalence of *M. genitalium* was 14% (95% CI: 12%–16%). Thirty-seven percent of the participants tested HIV-positive, 13% were diagnosed with NG, 9% with *C. trachomatis*, 18% with TV infection, and 55% with BV. *M. genitalium* was significantly more prevalent in HIV-positive than in HIV-negative women (18% vs. 12%, $P = 0.01$), in women infected with NG (24% vs. 13%, $P = 0.002$), with TV (20% vs. 13%, $P = 0.02$), and with BV (17% vs. 11%, $P = 0.005$) (Table 1).

Clinical signs and symptoms are shown in Table 2. The majority (60%) of women reported at least 1 symptom suggestive of a genitourinary tract infection at enrollment visit: 40% of the women complained of vaginal discharge, 11% of dysuria, and 38% of symptoms suggestive of PID. The prevalence of dysuria was the highest among women with *M. genitalium* in single or in coinfection, whereas vaginal discharge was most often reported by women with another STI than *M. genitalium*.

On clinical examination, 63% presented with signs suggestive of vaginal infection and 23% with endocervical mucopus. vaginal discharge syndrome and PID were diagnosed in 63% and 23% of the women, respectively. Women with *M. genitalium* infection presented more often with mucopurulent vaginal discharge, less frequent with profuse vaginal discharge and less frequent with mucopurulent endocervical discharge than those coinfected with another pathogen.

Clinical Characteristics Associated With *M. genitalium* Infection

On univariable analysis, reported dysuria was significantly associated with *M. genitalium* infection (OR: 1.85, 95% CI: 1.13–3.03), as was mucopurulent vaginal discharge (OR: 1.55, 95% CI: 1.06–2.29) (Table 2). Presence of a white vaginal discharge was less common in those with *M. genitalium* infection than those without (OR: 0.63; 95% CI: 0.42–0.96). There was no evidence of an association between other symptoms, cervical/vaginal signs or diagnosed STI syndromes, and *M. genitalium* infection (Table 3).

The crude ORs of the associations between each clinical characteristic and *M. genitalium* infection did not change by 10% or more after adjusting for age or any of the other STI (no confounding); therefore, no adjusted odd ratios could be presented. In addition, none of the associations varied by HIV status (*P* value for effect modification >0.10, data not shown).

CONCLUSIONS

This study examined the clinical correlates of *M. genitalium* infection among a population of women with a high prevalence of HIV and other STI in Uganda.

TABLE 3. Clinical Characteristics Associated With Prevalent *M. genitalium* Infection Among 972 Women at High Risk in Kampala, Uganda

	All N (%)	MG+ n (%)	MG− n (%)	OR (95% CI)
	N = 972	n = 137	n = 835	
Clinical symptoms				
Pain on passing urine				$P = 0.02$
No	862 (89)	113 (82)	749 (90)	1
Yes	110 (11)	24 (18)	86 (10)	1.85 (1.13–3.03)
Vaginal discharge				$P = 0.37$
No	580 (60)	77 (56)	503 (60)	1
Yes	392 (40)	60 (44)	332 (40)	1.18 (0.82–1.70)
Lower abdominal pain and/or pain during sexual intercourse				$P = 0.14$
No	602 (62)	77 (56)	525 (63)	1
Yes	370 (38)	60 (44)	310 (37)	1.32 (0.92–1.90)
Any symptoms				$P = 0.14$
No	388 (40)	47 (34)	341 (41)	1
Yes	584 (60)	90 (66)	494 (59)	1.32 (0.91–1.93)
Clinical examination				
Amount of vaginal discharge			$P = 0.74$	
Normal	385 (40)	56 (41)	329 (39)	1
Profuse	587 (60)	81 (59)	506 (61)	0.94 (0.65–1.36)
Whitish vaginal discharge				$P = 0.03$
No	644 (66)	102 (74)	542 (65)	1
Yes	328 (34)	35 (26)	293 (35)	0.63 (0.42–0.96)
Mucopurulent vaginal discharge			$P = 0.03$	
No	715 (74)	90 (66)	625 (75)	1
Yes	257 (26)	47 (34)	210 (25)	1.55 (1.06–2.29)
Vaginal infection*				$P = 0.73$
No	363 (37)	53 (39)	310 (37)	1
Yes	609 (63)	84 (61)	525 (63)	0.94 (0.65–1.36)
Mucopurulent endocervical discharge		$P = 0.27$		
No	746 (77)	100 (73)	646 (77)	1
Yes	226 (23)	37 (27)	189 (23)	1.26 (0.84–1.91)
Confirmed VDS†				$P = 0.57$
No	355 (37)	53 (39)	302 (36)	1
Yes	617 (63)	84 (61)	533 (64)	0.90 (0.62–1.30)
Confirmed PID‡				$P = 0.09$
No	757 (78)	99 (72)	658 (79)	1
Yes	215 (22)	38 (28)	177 (21)	1.43 (0.95–2.15)
Confirmed GUD§				$P = 0.39$
No	898 (92)	124 (91)	774 (93)	1
Yes	74 (8)	13 (9)	61 (7)	1.33 (0.71–2.49)

*Diagnosed as abnormal amount and/or color of vaginal discharge.
†Vaginal discharge syndrome.
‡Pelvic inflammatory disease.
§Genital ulcer disease.

As the diagnosis of female urethritis is not routinely based on laboratory tests for the clinical management of STI in Uganda, reported dysuria was the only available indicator suggestive of urethritis in our study. The association of dysuria with *M. genitalium* infection remained after controlling for other STI and despite the fact that *M. genitalium* was not sampled from urine. Besides, dysuria was not associated with any of the other STI under investigation (data not shown). Our study is the first in Africa reporting such an association in women. Studies conducted among female STD clinic attendees in Western countries failed to find a significant association of symptomatically defined urethritis with *M. genitalium*.[12–15] However, 3 large Scandinavian studies diagnosing urethritis microscopically from urethral smears also found a significant association with *M. genitalium*.[13,16,17] Considering all the available data, it seems that *M. genitalium* may indeed cause urethritis in women, but further confirmatory studies using both clinical and laboratory methods are needed.

In our study, vaginal infection, defined as presence of profuse vaginal discharge and/or a change in the color (to whitish, yellow, or green) as found on speculum examination, was not associated with *M. genitalium* infection. This is most likely because presence of an abnormal amount of vaginal discharge on itself, regardless of the color, was not associated with *M. genitalium.* However, a significantly higher prevalence of *M. genitalium* was detected among women with yellow/green (mucopurulent) vaginal discharge and a significantly lower prevalence among

those with whitish vaginal discharge, and these associations were not confounded by the presence of any other reproductive tract infections. No other clinical studies have documented similar detailed clinical findings.

The findings of Manhart et al that an elevated amount of PMNL (polymorphic mononuclear leucocytes) in vaginal smears was associated with *M. genitalium* ($P = 0.04$) supports the hypothesis that *M. genitalium* infection may indeed be a cause of vaginal infections.[18]

We diagnosed cervicitis if women presented with mucopurulent endocervical discharge on speculum examination. Our study did not find evidence of an association between *M. genitalium* and cervicitis. Results from other studies using a similar clinical definition of cervicitis were inconsistent; significant associations were reported by 2 studies conducted among female sex workers (adjusted OR: 1.6, 95% CI: 1.0–2.7 in West-Africa and OR: 2.2, 95% CI: 1.2–4 in Kenya),[8,10] 2 of the 4 studies conducted in women attending STD clinics[12,14,18,19] and 2 of the 3 studies conducted in the general population.[20–22] Also studies defining cervicitis based on microscopic findings (>10 PMNL in cervical smears) reported inconsistent results; 3 of the 4 studies found evidence for an association between *M. genitalium* and cervicitis.[12,18,23,24] The clinical definition of cervicitis has a lower sensitivity compared with a definition based on objective microscopic findings, which calls again for further validation work.

Clinical PID defined as lower abdominal pain and/or dyspareunia confirmed by pain on bimanual palpation was not associated with *M. genitalium* in our study. Our results on clinical PID were in line with those reported from 2 community-based surveys[15,25] and from the Kenyan sex workers cohort study[9] but contrasted with the results from 2 case-control studies that found evidence of an association between clinical PID and *M. genitalium*[26,27] and 2 studies confirming that *M. genitalium* was significantly more prevalent in histologically confirmed endometritis.[28,29]

In conclusion, *M. genitalium* infection is prevalent in women at high risk of HIV/STI in Uganda. Based on clinical symptoms and signs, our study showed evidence that *M. genitalium* is associated with urethritis and with vaginal infections, but not with cervical infections or PID. Our observations support earlier studies showing that *M. genitalium* infection may lead to clinically relevant genitourinary disorders and should be treated. In the absence of sensitive screening tests, further work is needed to validate clinical findings as possible indicators for *M. genitalium* infection in different populations to guide a possible syndromic approach for its management.

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