Published in final edited form as:

Int J STD AIDS. 2011 March ; 22(3): 155–159. doi:10.1258/ijsa.2010.010320.

***Mycoplasma genitalium* infection among HIV-positive women: prevalence, risk factors and association with vaginal shedding**

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Summary:

This study examined the prevalence and factors associated with *Mycoplasma genitalium* (MG) infection among HIV-positive women and the association between MG and vaginal HIV-1 RNA shedding. HIV-positive women attending an outpatient clinic in New Orleans, Louisiana, USA, from 2002 to 2005 were examined for a battery of sexually transmitted infections (STIs) and underwent a behavioural survey. A selected subset had a measurement of vaginal shedding analysed. Of the 324 HIV-positive women, 32 (9.9%) were infected with MG. HIV-positive women with MG were more likely to be co-infected with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* and to have had 1 male sexual partners in the last month. In the subset ($n = 164$), no differences were found in the presence of detectable vaginal HIV-1 RNA between women infected and not infected with MG (30.8% versus 34.8% shedding; $P = 0.69$). While MG was a common co-STI in this sample of HIV-positive women, it was not associated with vaginal HIV shedding.

Keywords

Mycoplasma genitalium; HIV-infected women; vaginal shedding

INTRODUCTION

Mycoplasma genitalium (MG) is a sexually transmitted organism¹ causing non-gonococcal urethritis in men² and is associated with cervicitis,³⁻⁸ pelvic inflammatory disease^{9,10} and infertility¹¹ in women. Cross-sectional studies have shown a strong association between MG infection and HIV infection,^{12,13} but whether this association is causal or co-incidental needs further evaluation. Studies among HIV-1-positive women have found prevalence rates of MG between 7.1% and 33.0%.^{9,14-16}

The potential for increased HIV transmission in the presence of other sexually transmitted infections (STIs) has been shown in several studies,¹⁷⁻¹⁹ but less is known about the influence of MG on HIV vaginal shedding. MG has been shown to increase HIV replication in peripheral blood mononuclear cells *in vitro*²⁰ and a study by Manhart *et al.*¹⁶ found an association between high MG organism burdens and cervical shedding of HIV-1 DNA among Kenyan women. However, to date, no studies have been published on this topic among women in the USA, where antiretroviral therapy (ART) is more widely available.

The purpose of this study was to describe the prevalence and factors associated with MG infection among women with HIV from a USA urban population, and examine the association between MG infection and vaginal HIV-1 RNA shedding.

MATERIALS AND METHODS

Participants

HIV-1-infected women were recruited from the HIV Outpatient Program (HOP) in New Orleans, Louisiana, USA, from June 2002 to January 2005 to take part in a cross-sectional screening study to investigate multiple issues Surrounding STIs, including infection with MG. Women had to be aged at least 18 years, scheduled to undergo a gynaecological examination, and provide informed consent to be eligible.

A subset of women enrolled in the cross-sectional study were also enrolled into a cohort study to examine the relationship between *Trichomonas vaginalis* and vaginal HIV-1 RNA detection; these methods have been published elsewhere.²¹ In brief, women who tested positive for *T. vaginalis* and were negative at follow-up visits were matched on antiretroviral status and date of enrolment to women who tested negative at all time points. Subset participants were followed at baseline, one month and three months, and had a measure of vaginal HIV viral load analysed at each time point. Women were excluded from the subset if they had been treated with metronidazole in the past two weeks, and/or had a medical contraindication to taking metronidazole. Women who presented with menstrual bleeding at the time of the pelvic exam were rescheduled for a later gynaecological visit.

For the purposes of the current study, data from participants in the cross-sectional study were used to examine the prevalence and risk factors for MG infection. Baseline data from subset participants were used to examine the association between MG infection and vaginal shedding.

Specimen collection

Clinical examinations for the cross-sectional study were performed by the patient's medical provider using a standard protocol. Non-lubricated speculums were utilized for the pelvic examinations. Following speculum insertion, Dacron swabs were used to obtain vaginal specimens for various tests. The first swab was placed into the vaginal vault and rolled thrice on the vaginal wall and stored for quantification of HIV-1 RNA levels, as described below. Subsequent swabs were placed into the posterior fornix to obtain vaginal secretions for additional testing (MG polymerase chain reaction [PCR], Gram stain preparation and *T. vaginalis* culture). Endocervical specimens were collected for Pap smear and MG tests. Specimens were always collected in the same order and were not randomized. Urine specimens were also collected for *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and MG.

Specimens were used for laboratory diagnosis of: *T. vaginalis* by InPouch culture (Biomed Diagnostics, White City, OR, USA), and for *N. gonorrhoeae* and *C. trachomatis* using the ProbeTec strand displacement DNA amplification method (Becton Dickinson and Company, Sparks, MD, USA), MG by PCR as described below, bacterial vaginosis (BV) by Gram stain testing using Nugent score criteria, and vulvovaginal candidiasis by culture. Genital odour, vaginal discharge, vaginal petechiae and erythema were observed by the clinical provider.

Determination of MG status

MG PGR was performed as described previously in detail²² on endocervical and vaginal swabs and a urine specimen, although here amplicons were detected by dot blot rather than

Southern blot analysis. Details of the methods for the dot blot assay are available from the authors (DHM) on request. Positive PCR results (referred to here as the initial test) were confirmed by a repeat assay using the original extracted DNA sample. MG infection was defined as: (1) being positive on any one of the three initial tests (i.e. positive on initial cervical, vaginal or urine test) *and* positive on the confirmatory test, or (2) being positive on any two of the three initial tests, irrespective of confirmatory test results. Women had to be negative on all three initial tests to be considered MG negative.

Determination of HIV RNA levels for subset subjects

Vaginal secretions were collected for analysis of HIV RNA levels on Dacron swabs as described above and were immediately placed in 1 mL of sterile saline and put on ice. Samples were transported to the laboratory, processed and stored at -70°C within three hours of collection. Upon receipt in the laboratory, the tube was vortexed, the swab was removed and the sample was centrifuged at 400g for 10 minutes to separate cells from the supernatant. The cell-free fraction was stored at -70°C . Blood samples collected in ethylenediaminetetraacetic anticoagulant were received and processed in the laboratory within three hours of collection. The sample was centrifuged at 400g for 10 minutes and the plasma removed and stored frozen in 1-mL aliquots at -70°C .

To quantify the amount of HIV-1 RNA found in vaginal secretions and plasma, samples from subjects in the sub-study were thawed and analysed with the Roche Amplicor™ HIV-1 Monitor Assay, utilizing the ultra-sensitive protocol. Virus contained in the sample was purified by high-speed centrifugation, followed by lysis and PCR amplification as directed by the manufacturer's protocol. This protocol can quantitate as few as 50 copies of HIV-1 RNA per sample. HIV RNA levels were reported as copies per vaginal swab or milliliter of plasma. Undetectable/not quantifiable samples were reported as <50 copies. In addition, CD4 lymphocyte counts were reported as cells/mm³ of blood.

Behavioural data

Participants in the cross-sectional study were also interviewed for behavioural information. These interviews were conducted using computer-assisted self-administered interviews or computer-assisted personal interviews, depending on the woman's preference, and were conducted either before or after the clinical examination, depending on the clinic flow. The interviews elicited information on various sociodemographic, behavioural and additional clinical characteristics of the women.

Statistical analyses

Age was assessed as a dichotomous variable and was categorized by the median. Baseline characteristics were calculated as frequencies for the cross-sectional study and the subset, and percentages were compared across the two samples using chi-square or Fisher's exact test. Using data from the cross-sectional study, baseline associations between MG status and selected sociodemographic, clinical and behavioural data were examined. Chi-square or Fisher's exact tests were conducted as appropriate. Factors associated with MG at the bivariate level with a P value ≤ 0.10 were considered for multivariate analysis using logistic regression.

For women in the subset, detectable vaginal HIV-1 RNA was treated as a binary outcome (present or absent) because only 34.2% of women had detectable levels. Therefore, HIV shedding was defined as present (detectable) when HIV-1 RNA was >50 copies/swab, and absent (or undetectable) when HIV-1 RNA was ≤ 50 copies/swab. Baseline associations between shedding status and the selected sociodemographic, clinical and behavioural data were also examined using Chi-square or Fisher's exact tests as appropriate. Factors

associated with shedding at the bivariate level with a P value > 0.10 were considered for multivariate analysis using logistic regression. All analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Characteristics of participants

A total of 397 HIV-positive women were screened as part of the cross-sectional study, with 324 having complete testing information to determine MG status. The prevalence of MG infection was 9.9% (32/324). Of those women with MG ($n = 32$), 87.5% tested positive on the initial vaginal test ($n = 28$), 71.9% tested positive on the initial urine test ($n = 23$) and 46.9% tested positive on the initial cervical test ($n = 15$).

Demographic and behavioural information was available on 309 women (Table 1), with a median age of 35 years (range 18–57 years). The majority of women were African American (83.8%, $n = 259$), not married or cohabitating (80.6%, $n = 249$), unemployed (74.4%, $n = 230$) and had a high-school education or less (77.0%, $n = 238$). Most women reported douching (67.0%, $n = 207$), using a condom at last vaginal intercourse (70.6%, $n = 218$) and having one or more male sexual partners in the last four weeks (66.3%, $n = 205$). Of the 324 women with known MG status, 55.6% were on ART ($n = 180$).

Other infections were common in this group of HIV-positive women, with an overall prevalence rate of 25.7% for *T. vaginalis* (79/308), 3.8% for *C. trachomatis* (12/320), 0.9% for *N. gonorrhoeae* (3/320), 49.4% for BV (157/318) and 29.9% for candidiasis (95/318). According to the provider assessment ($n = 324$), 11.4% of the women had unusual genital odour ($n = 37$), 5.9% had vaginal erythema ($n = 19$) and 2.5% had vaginal petechiae ($n = 8$). Most women had scant to no vaginal discharge (60.8%, $n = 197$).

Factors associated with MG infection

Table 2 presents sociodemographic, clinical and behavioural characteristics of the women by MG infection status. In bivariate analysis, HIV-positive women with MG were more likely to be younger than 36 years ($P = 0.03$), and to be co-infected with *C. trachomatis* ($P < 0.01$), *N. gonorrhoeae* ($P = 0.03$) and *T. vaginalis* ($P = 0.03$), compared with MG-negative women. MG infected women were also more likely to have had one or more male sexual partners in the last four weeks ($P = 0.02$), and to have abnormal consistency of vaginal discharge on examination ($P < 0.01$). Although not statistically significant, MG infected women were more likely to douche ($P = 0.07$) and have moderate to copious amounts of vaginal discharge ($P = 0.08$). When all factors found to be significant in the bivariate analysis were examined in multivariate analysis, factors that remained statistically significant were: one or more recent sexual partners and co-infection with *C. trachomatis* and *N. gonorrhoeae*.

MG infection and vaginal shedding of HIV among subset subjects

A total of 164 women from the subset had information available on MG status and vaginal HIV shedding. The characteristics of the subset were similar to those of women in the cross-sectional study (Table 1) except for the rates of *T. vaginalis* and MG were higher in the subset. Of the women in the subset, 15.9% were positive for MG ($n = 26$), 48.2% had a plasma viral load $> 10,000$ copies ($n = 79$), 27.3% had a CD4 count $< 200/\text{mm}^3$ ($n = 38$; total $n = 139$) and 50.3% were on ART ($n = 82$). Only 34.2% ($n = 56$) of the women had detectable vaginal HIV-1 RNA (i.e. shedding). There were no differences in the presence of detectable vaginal HIV-1 RNA between women infected with MG (30.8% shedding) and

women not infected with MG (34.8% shedding; $P = 0.69$). Also, no differences in plasma viral load, CD4 count, BV status or ART status were detected by MG infection status.

In bivariate analysis of the subset sample of women ($n = 164$), factors that were associated with vaginal shedding of HIV included *T. vaginalis* infection ($P = 0.04$), candidiasis ($P = 0.02$), plasma viral load $> 10,000$ copies ($P < 0.01$) and not being on ART ($P < 0.01$). Also, BV was associated with vaginal shedding at borderline significance ($P = 0.08$). All other factors examined in Table 2 were not found to be associated with vaginal shedding of HIV. When factors found to be significant in the bivariate analysis were examined in multivariate analysis, BV, high plasma viral load and not being on ART all remained positively associated with shedding (Table 3).

DISCUSSION

To our knowledge, this is the first prevalence study of MG among HIV-positive women in the USA. Of interest, the prevalence of MG among this group of HIV-positive women was 9.9%, which was more than 2.5 times the prevalence of *C. trachomatis* (3.8%) and nearly 11 times more than the prevalence of *N. gonorrhoeae* (0.9%). Infection with *T. vaginalis* and BV were more common than MG. The prevalence of MG in young sexually active populations, as are found in STD clinics, is usually about the same as *C. trachomatis* and both are more common than *N. gonorrhoeae*;^{4,23} although a recent study found a high prevalence of MG (19.2%) among a female STD clinic population in Baltimore, Maryland, USA, where *C. trachomatis* and *N. gonorrhoeae* prevalence was 11.1% and 4.6%, respectively.⁸ Systematic screening for MG, in addition to *C. trachomatis* and *N. gonorrhoeae*, may be necessary in the future for high-risk groups of women including HIV-positive women;^{24,25} however, more research is needed on the causal relationship between MG and female reproductive tract disease.

In this study, co-infections with *C. trachomatis* and *N. gonorrhoeae*, and having had one or more recent sexual partners were all more common among MG infected women than non-infected women in multivariate analysis. Manhart *et al.*¹⁶ studied HIV-positive women in Kenya and found *C. trachomatis*, one or more sexual partners and younger age to be associated with MG at the bivariate level, but did not find *N. gonorrhoeae* to be associated. A previous study has shown non-statistically significant trends for *T. vaginalis* infection to be more common among MG-positive women compared with MG-negative women in an adolescent population.²³ We found no association between MG infection and ART status, high plasma viral load, and low CD4 count, indicating that HIV disease progression does not appear to be associated with MG infection. Other studies have shown an association between douching and MG infection;^{3,26} however, we found this relationship to be of only borderline significance ($P = 0.07$) among our group of HIV-positive women.

To assure accurate diagnosis, MG testing in our study was sampled from three anatomical sites: cervical, vaginal and urine; with the vaginal specimen being the most sensitive. This observation is in accordance with that of Wroblewski *et al.*²⁷ who have shown that a vaginal swab specimen is more sensitive than endocervical and urine specimens for the detection of MG in non-HIV-infected women using two different MG nucleic acid amplification methods.

Our subset analysis found no difference in detectable vaginal HIV-1 RNA shedding by MG infection status. Manhart *et al.*¹⁶ also found little difference in cervical HIV-1 DNA shedding by MG infection status (19% shedding in women with MG versus 15% shedding in women without MG, $P = 0.31$). However, Manhart *et al.* further assessed MG infection by quantitative organism burden and found a significant association between high MG

organism burdens and cervical shedding after adjustment. Our study was not designed to address MG organism burdens, thus this nuance may have been missed. Furthermore, by design, women with *T. vaginalis* were oversampled in the subset analysis and since *T. vaginalis* has been associated with vaginal shedding,^{21,28} it is possible that there was a masking of effect of MG and vaginal shedding. However, factors which we did find to be associated with vaginal shedding of HIV at the bivariate and multivariate levels are congruent with findings from other studies,^{21,29-33} suggesting that selection bias did not play a large factor in our findings.

The limitations of our main study include the cross-sectional design. The limitations of the subset analysis include the small sample size, selection criteria and the lack of quantitative MG organism data. Future studies using prospective, longitudinal designs should assess genital HIV shedding in women pre- and post-treatment for MG to determine if treatment for MG reduces shedding of HIV. Despite these potential limitations, our study is the first extensive description of MG among HIV-positive women in the USA. While the rate of MG in this population was high and MG was associated with other STIs, specifically *C. trachomatis* and *N. gonorrhoeae*, it was not associated with an increased risk for vaginal shedding of HIV in a subset of women. Given the high rate of MG among HIV-infected women, studies specifically designed to prospectively examine the association of MG and HIV vaginal shedding, with larger sample sizes and more thorough measurements of potential confounders, are needed.

Acknowledgments

We wish to thank Mary Welsh, Catherine Cammarata, Denise Diodene and Judy Burnett for the performance of the laboratory assays. This study was supported by grants from the Louisiana Board of Regents Health Excellence Fund and the Centers for Disease Control and Prevention RA1/CCR622272 and MAID U19 A161972.

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Table 1Baseline characteristics of HIV-positive women in the cross-sectional study ($n = 324$) and subset ($n = 164$)

	Cross-sectional study ($n = 324$)	Subset for vaginal shedding analysis ($n = 164$)	<i>P</i> value
African American	83.8%	85.1%	0.72
Age ≥ 35 years	47.6%	46.0%	0.74
Douches	67.0%	67.1%	0.98
1 male sexual partner in last 4 weeks	66.3%	70.9%	0.32
On ART	55.6%	50.3%	0.27
<i>Mycoplasma genitalium</i>	9.9%	15.9%	0.05
<i>Chlamydia trachomatis</i>	3.8%	4.9%	0.55
<i>Neisseria gonorrhoeae</i>	0.9%	1.2%	1.00 [*]
<i>Trichomonas vaginalis</i>	25.7%	35.7%	0.02
Bacterial vaginosis	49.4%	53.1%	0.44
Candidiasis	29.9%	23.8%	0.16

ART = antiretroviral therapy

^{*}
Fisher's exact test

Table 2

Characteristics of HIV-positive women by *Mycoplasma genitalium* (MG) infection status ($n = 324$)

	MG-positive ($n = 32$) (%)	MG-negative ($n = 292$) (%)	<i>P</i> value	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
Demographic †					
African American	84.4	83.8	0.93		
Unemployed	65.6	75.5	0.23		
Not married/cohabitating	81.3	80.5	0.92		
High-school education or less	71.9	77.6	0.33		
Age ≥ 35 years	65.6	45.5	0.03	2.29 (1.06, 4.93)	1.34 (0.55, 3.29)
Behavioral †					
Douches	81.3	65.3	0.07	2.30 (0.91, 5.78)	2.41 (0.78, 7.39)
Condom used at last vaginal sex	78.1	70.2	0.35		
1 male sexual partner in last 4 weeks	87.1	65.9	0.02	3.49 (1.18, 10.27)	3.89 (1.11, 13.70)
Other infections					
<i>Chlamydia trachomatis</i> ‡	19.4	2.1	<0.01 [§]	11.32 (3.40, 37.70)	18.21 (4.22, 78.63)
<i>Neisseria gonorrhoeae</i> ‡	6.5	0.4	0.03 [§]	19.86 (1.75, 225.75)	47.59 (2.68, 846.19)
<i>Trichomonas vaginalis</i> **	41.9	23.8	0.03	2.31 (1.07, 4.96)	0.55 (0.19, 1.59)
Bacterial vaginosis ††	54.8	48.8	0.52		
Candidiasis ††	21.9	30.8	0.30		
HIV disease					
On ART	46.9	56.9	0.28		
Provider assessment					
Unusual genital odour	18.8	10.7	0.24 [§]		
Vaginal erythema	6.3	5.8	1.00 [§]		
Vaginal petechiae	3.1	2.4	0.57 [§]		
Amount of vaginal discharge: moderate to copious (versus scant to none)	53.1	37.5	0.08	1.89 (0.91, 3.94)	1.03 (0.36, 2.91)
Consistency of vaginal discharge: normal (versus purulent, curdy or milky/creamy)	29.0	56.6	<0.01	0.31 (0.14, 0.71)	0.34 (0.12, 1.02)
Colour of vaginal discharge: none or clear (versus white/grey, yellow, green, bloody)	15.6	26.6	0.18		

OR = odds ratio; CI = confidence interval; ART = antiretroviral therapy

* Adjusted for: age ≥ 35 years, douching, 1 male sexual partner in last four weeks, *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, amount of vaginal discharge: moderate to copious, consistency of vaginal discharge: normal† Total $n = 309$ ‡ Total $n = 320$

§ Fisher's exact test

** Total $n = 308$ †† Total $n = 318$

Table 3Multivariate analysis of the association with vaginal HIV-1 RNA shedding ($n = 164$)

Characteristic	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
<i>Mycoplasma genitalium</i>	0.83 (0.34, 2.06)	0.93 (0.31, 2.79)
<i>Trichomonas vaginalis</i>	2.00 (1.01, 3.96)	1.56 (0.65, 3.73)
Candidiasis	2.49 (1.18, 5.25)	1.84 (0.70, 4.82)
Bacterial vaginosis	1.79 (0.93, 3.47)	2.47 (1.03, 5.91)
Plasma viral load 10,000 copies/mL	3.90 (1.95, 7.78)	2.86 (1.20, 6.77)
Not on ART	4.98 (2.42, 10.24)	4.63 (1.90, 11.26)

OR = odds ratio; CI = confidence interval; ART = antiretroviral therapy