Prevalence and Antimicrobial Resistance of Mycoplasma genitalium Infection Among Women Living With Human Immunodeficiency Virus in South Africa: A Prospective Cohort Study

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Prevalence and antimicrobial resistance of *Mycoplasma genitalium* infection among women living with HIV in South Africa: a prospective cohort study

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ABSTRACT

This prospective cohort study of 622 women living with HIV from Johannesburg (2012) detected MG in 7.4%(95%CI:5.5-9.7, 46/622), with detection more likely with lower CD4 counts(AOR 1.02 per 10 cells/μL decrease, 95%CI:1.00-1.03) and higher plasma HIV-1 RNA(AOR 1.15 per log copies/mL increase, 95%CI:1.03-1.27). No mutations for macrolide/quinolone resistance was detected.

Keywords: *Mycoplasma genitalium*, women, HIV, sexually transmitted infections, antimicrobial resistance

BACKGROUND

Mycoplasma genitalium(MG) is a sexually transmitted pathogen associated with non-gonococcal urethritis in males,[1] cervicitis and pelvic inflammatory disease(PID).[2] There is an association of MG with HIV acquisition,[3] but it is unclear how HIV affects MG epidemiology and whether MG has a role in HIV progression. If left undetected, inadequately treated MG may contribute to reproductive morbidity in women and development of antimicrobial resistance(AMR).[4] MG has a high propensity for developing AMR, particularly to macrolides used for syndromic sexually transmitted infection(STI) management in low- and middle-income countries(LMIC).[5]

This study aims to determine the prevalence, persistence/reinfections and risk factors associated with MG in a cohort of women living with HIV (WLHIV) in South Africa. We report MG-AMR profile for both macrolides and fluoroquinolones.

METHODS

Study population

Cervical specimens were collected as part of an evaluation study of cervical cancer screening strategies in Burkina Faso and South Africa, as previously described.[6] Inclusion criteria were WLHIV, aged 25-50 years, and resided in Ouagadougou or Johannesburg. This analysis focuses on WLHIV recruited in South Africa, as MG rates were found to be low in Burkina Faso(4/615). WLHIV were recruited from primary health care centres and surrounding communities in Johannesburg from December 2011 to October 2012.

Specimen collection and laboratory analysis

For all women at baseline and endline, a cervical swab was collected to detect *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, MG, and *Trichomonas vaginalis* using a validated in-house real-time multiplex polymerase chain reaction(mPCR) assay. For the endline, not all samples could be tested for cervical PCR, and we only report MG testing for those found positive at baseline. Specimens that tested MG positive with the in-house mPCR assay were confirmed with the commercial Sacace MG real-time PCR assay(Sacace Biotechnologies, Como, Italy). All real-time PCR assays were performed using the Rotor-Gene platform(Qiagen, Hilden, Germany). A vaginal smear was Gram-stained for the diagnosis of bacterial vaginosis(Nugent's score of at least 7) and *Candida* spp.

Venous blood was taken to perform herpes simplex virus type 2(HSV-2) serology(Kalon IgG2 ELISA, Kalon Diagnostics, Guilford, UK), *Treponema pallidum* hemagglutination and rapid plasma reagin(Omega Diagnostics, Cape Town, South Africa), HIV-1 plasma viral load(Cobas Taqman, Roche Diagnostics, Johannesburg, South Africa) and $CD4^+$ T-lymphocyte count(FACScount, Becton-Dickinson, Franklin Lakes, New Jersey, USA).

MG-AMR testing

Macrolide resistance associated mutations in region V of the 23S rRNA gene of MG(positions A2058 and A2059 using *Escherichia coli* numbering) was determined.[7] Mutations in the region corresponding to the quinolone resistance determining region(QRDR) of the DNA gyrase(*gyrA* only) and the topoisomerase IV genes(*parC* only) were analysed.[8] To visualize PCR products, 1µl of PCR products, including a 1000bp marker were run on an Agilent 2100 Bioanalyzer(Agilent Technologies, Santa Clara, USA). The PCR products were purified using the MSB® Spin PCRapace kit(Invitek, Berlin, Germany). After purification of the PCR products, cycle sequencing reactions were performed in both directions using the same primers that were used for amplification. The cycle sequencing

reactions were performed using the ABI Prism Big Dye terminator reaction kit version 3.1(Applied Biosystems, Foster City, CA) and analysed on a 16-capillary ABI 3130xl Genetic Analyzer(Applied Biosystems, Foster City, CA). Contigs obtained from initial sequencing were aligned using Sequencher version 5.4.6(Gene Codes Corporation, MI, USA) and the FASTA sequences obtained were aligned(MEGA 7.0.20) and compared with MG reference strains on GenBank(G37, M2288, M2321, M6282 and M6320).

Statistical analysis

Logistic regression analysis was used to explore patient characteristics associated with MG detection. We identified variables with p value <0.20, and examined each variable independently in separate regression models, adjusted for age, education, and income. We present adjusted odds ratios(AORs) alongside 95% confidence intervals(CI). Data were analyzed using STATA(StataCorp. 2013).

RESULTS

623 WLHIV were recruited, and 622 were included in this analysis as one woman was not tested for MG. MG was detected in 7.4%(95%CI:5.5-9.7), *Trichomonas vaginalis* in 16.2%(95%CI:13.4-19.4), *Chlamydia trachomatis* in 5.0%(95%CI:3.4-7.0), *Neisseria gonorrhoeae* in 2.3%(95%CI:1.2-3.7), BV in 41.6%(95%CI:37.6-45.6), and *Candida* spp. in 8.5%(95%CI:6.4-11.0). Overall, MG monoinfection was found in 16 women(2.6%), MG with STI/RTI coinfection in 30 women(4.8%), no MG but another STI/RTI in 343 women(55.1%) and no MG and no STI/RTI in 233 women(37.5%).

Table 1 shows that compared to women with no MG, women with MG were significantly younger(mean age 31.8 years vs. 35.2, p<0.001), had more lifetime sexual partners(4.3 vs. 3.7, p<0.001), were more likely to have active syphilis(4.4% vs. 0.9%, p=0.032) and more likely to have a lower median CD4 count(387 vs. 433 cell/μL, p=0.018) and higher median plasma HIV-1 RNA levels(5170 vs. 470 copies/mL, p=0.003). In the multivariable model, detection of MG was more likely in women with lower CD4 counts(AOR 1.02 per 10 cells/L decrease) and higher plasma HIV-1 RNA(AOR 1.15 per log copies/mL increase). There was a significant difference between HIV-1 plasma viral loads in women with MG detected(median=5170 copies/mL, IQR:155-4910) compared to those who did not(median=470 copies/mL, IQR:44-7820, p=0.003).

MG-AMR

Macrolide and quinolone resistance testing were conducted for 43 out of the 46 MG-positive specimens at baseline as three specimens tested negative for MG when retested in January 2018. The following number of specimens produced full readable sequences for the respective genes: 23S rRNA(macrolide resistance): 43/43(100%), *gyrA*(QRDR): 26/43(60.5%) and *parC*(QRDR): 43/43(100%). Macrolide resistance-associated mutations were not detected in any of the specimens analysed(0/43). Mutations in the QRDR of the *gyrA* gene were not detected in any of the specimens that could be sequenced(0/26) while mutations in the QRDR of the *parC* gene were detected in 19/43(44.2%) specimens. The most common mutation in *parC* was C184T(18/19; 94.7%) resulting in the amino acid alteration P62S. The G284T mutation in *parC* was the only additional mutation that resulted in an amino acid change(S95I, 2/19, 10.5%).

For the follow-up, 41 specimens from returning women were tested for RNaseP(human internal control) to assess DNA adequacy for PCR and all 41 were valid. We tested for MG using both an in-

house real-time mPCR and the commercial Sacace MG real-time PCR. A total of 12/41(29.3%) specimens tested MG positive with both assays. There were no macrolide resistance-associated mutations nor mutations in the *gyrA* gene. Five out of 12 specimens had *parC* mutations: the *parC* sequence profiles of the follow-up specimens corresponded to the sequence profiles of the baseline specimens in all but one specimen.

Overall, 15/46 women received antibiotics within 3 months of the baseline visit: one woman had azithromycin and doxycycline, and 14 women only had doxycycline, which was the recommended treatment for genital discharge syndrome in South Africa at the time of this study. Between the first and final MG test at a median 16 months later(IQR, 15.6-16.8), 23/46 women received doxycycline 100mg twice daily for 7 days, three women received azithromycin 1g single dose, two women received both doxycycline and azithromycin, and no one received any quinolones. In total, 18 women did not receive any antibiotics that has activity against MG between the first and final MG test. For the 12 women with detectable MG at follow-up, six (50%) had previously been treated with doxycycline and three (25%) with azithromycin. For 29 women who had undetectable MG on follow-up, 13 (45%) women were treated with doxycycline and one (3%) with azithromycin.

DISCUSSION

The evidence is accruing for the public health importance of MG and its impact on reproductive morbidity. We extend the literature by reporting the prevalence and AMR associated with MG among WLHIV in South Africa. We found that MG was as common as other STIs. Nearly a third of women with detectable MG at baseline had detectable MG after a median of 16 months. We detected no mutations associated with macrolide or quinolone resistance but with the change in 2015 of guidelines for syndromic management for genital discharge and PID syndromes in replacing

doxycycline with azithromycin as the first-line antibiotic, macrolide resistance selection pressure may become considerable. This may result in a rapid rise of macrolide resistance as demonstrated in countries where macrolides are frequently used.[9] Ongoing monitoring of MG and its AMR profile is urgently needed.

Whilst MG may influence HIV acquisition,[10] little is known of reciprocal interactions. We found that MG was associated with increased HIV-1 plasma viral load and lower CD4+ cell count, which may affect the clearance of STIs. It may be possible that MG persisted because of uncontrolled HIV replication and immunosuppression, or, alternatively, MG may increase HIV replication, preventing rapid or full CD4 cell recovery. Previous studies have shown that

In conclusion, MG is as common as other cervical STIs among WLHIV in South Africa. We document no macrolide/quinolone resistance-associated mutations in 2012 for a group at high risk for STIs. However, MG-AMR needs ongoing monitoring given the inclusion of macrolides into national guidelines for syndromic management for genital discharge and PID syndromes since 2015.

Contributors

PM, SDM, & MS conceived and planned the study; AC, SD, HK and PM coordinated the study, participant recruitment and management: MPM, MND and EM performed the lab testing and QC for MG and other STIs, JJO analysed the data and drafted the manuscript, and all authors revised and approved the final manuscript.

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Conflict of interest

The authors have no conflicts of interest to disclose.

Ethical approval

Ethical clearance for the HARP study was obtained from the Ministry of Health of Burkina Faso, the University of Witwatersrand in Johannesburg, South Africa, and the London School of Hygiene & Tropical Medicine, including for various sub-studies. Written informed consent was obtained at the screening visit and all women provided a second written informed consent at the enrolment visit for enrolment and follow-up over 16 months.

REFERENCES

1. Taylor-Robinson D, Jensen JS. Mycoplasma genitalium: from Chrysalis to multicolored butterfly. Clin Microbiol Rev **2011**; 24(3): 498-514.

2. Lis R, Rowhani-Rahbar A, Manhart LE. Mycoplasma genitalium infection and female reproductive tract disease: a meta-analysis. Clin Infect Dis **2015**; 61(3): 418-26.

3. Vandepitte J, Weiss HA, Bukenya J, et al. Association between Mycoplasma genitalium infection and HIV acquisition among female sex workers in Uganda: evidence from a nested case-control study. Sex Transm Infect **2014**; 90(7): 545-9.

4. Unemo M, Jensen JS. Antimicrobial-resistant sexually transmitted infections: gonorrhoea and Mycoplasma genitalium. Nat Rev Urol **2017**; 14(3): 139-52.

5. Lau A, Bradshaw CS, Lewis D, et al. The Efficacy of Azithromycin for the Treatment of Genital Mycoplasma genitalium: A Systematic Review and Meta-analysis. Clin Infect Dis **2015**; 61(9): 1389-99.

6. Kelly HA, Sawadogo B, Chikandiwa A, et al. Epidemiology of high-risk human papillomavirus and cervical lesions in African women living with HIV/AIDS: effect of anti-retroviral therapy. AIDS **2017**; 31(2): 273-85.

7. Jensen JS, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin treatment failure in Mycoplasma genitalium-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. Clin Infect Dis **2008**; 47(12): 1546-53.

8. Shimada Y, Deguchi T, Nakane K, et al. Emergence of clinical strains of Mycoplasma genitalium harbouring alterations in ParC associated with fluoroquinolone resistance. Int J Antimicrob Agents **2010**; 36(3): 255-8.

9. Murray GL, Bradshaw CS, Bissessor M, et al. Increasing Macrolide and Fluoroquinolone Resistance in Mycoplasma genitalium. Emerg Infect Dis **2017**; 23(5): 809-12.

10. Napierala Mavedzenge S, Weiss HA. Association of Mycoplasma genitalium and HIV infection: a systematic review and meta-analysis. AIDS **2009**; 23(5): 611-20.

11. Taylor MM, Newman DR, Gonzalez J, Skinner J, Khurana R, Mickey T. HIV status and viral loads among men testing positive for rectal gonorrhoea and chlamydia, Maricopa County, Arizona, USA, 2011-2013. HIV Med **2015**; 16(4): 249-54.

12. Couldwell DL, Lewis DA. Mycoplasma genitalium infection: current treatment options, therapeutic failure, and resistance-associated mutations. Infect Drug Resist **2015**; 8: 147-61.

Table 1 Comparison of the demographics, sexual behaviours and laboratory results of *Mycoplasma genitalium* (MG) positive and MG negative women living with HIV in South Africa (n=622)

Variable	MG positive (N=46) % (n/N) or mean [SD]	MG negative (N=576) % (n/N) or mean [SD]	P value
Demographics			
Mean age (years) [± SD]	31.8 [± 4.9]	35.2 ± [6.1]	<0.001
Completed high school or above	41.3 (19/46)	43.0 (248/576)	0.817
Ever smoked	15.2 (7/46)	12.1 (70/576)	0.544
Not married	95.7 (44/46)	85.4 (492/576)	0.053
Income (Rand, R$)			
<1800	58.7 (27/46)	49.9 (288/576)	
1801-6000	41.3 (19/46)	43.8 (252/576)	
>6000	0 (0)	6.2 (36/576)	0.312
Sexual behaviours			
Age at first sex (years) [± SD]	17.6 [± 1.7]	17.8 [± 2.5]	0.369
Mean lifetime sexual partners [± SD]	4.3 [± 2.7]	3.7 [± 2.5]	<0.001
No sex in the last 3 months	19.6 (9/46)	17.5 (101/576)	0.728
Same sexual partner in the last 3 months	94.6 (35/37)	92.6 (440/475)	0.657
Condomless sex with regular sexual partner in the last 3 months	52.8 (19/36)	39.1 (182/464)	0.110
Condomless sex with casual sexual partner in the last 3 months	0 (0/2)	12.5 (2/16)	0.596
Laboratory findings			
N. gonorrhoeae	0 (0)	2.4 (14/576)	0.285

C. trachomatis	4.4 (2/46)	5.0 (29/576)	0.837
T. vaginalis	15.2 (7/46)	16.3 (94/576)	0.845
Bacterial vaginosis	47.8 (22/46)	41.0 (232/565)	0.371
Candida	10.9 (5/46)	8.5 (47/565)	0.551
Active syphilis (RPR+/TPHA+)	4.4 (2/46)	0.9 (5/572)	0.032
HSV-2 serology positive	100.0 (46/46)	94.8 (543/573)	0.112
HIV parameters			
Years since HIV diagnosis			0.853
<5 years	56.5 (26/46)	56.1 (323/576)	
5-9 years	34.8 (16/46)	32.6 (188/576)	
≥ 10 years	8.7 (4/46)	11.3 (65/576)	
Currently on ART	60.9 (28/46)	65.3 (377/576)	0.530
Median years on ART [IQR]	1.9 [0.8-3.0]	2.4 [0.9-4.2]	0.205
Self-reported adherence to ART			0.830
60-90%	85.7 (24/28)	84.2 (314/373)	
<60%	14.3 (4/28)	15.8 (59/373)	
Median CD4 count cells/μL [IQR]	387 [273-466]	433 [325-592]	0.018
Median HIV-1 RNA copies/mL [IQR]	5170 [155-49100]	470 [44-7820]	0.003
Undetectable plasma HIV-1 RNA (<40 copies/ml)	17.4 (8/46)	23.6 (136/576)	0.336

ART = antiretroviral therapy; CIN = cervical intraepithelial neoplasia; HSV-2 = herpes simplex virus type 2; IQR = interquartile range; MG = Mycoplasma genitalium; SD = standard deviation; RPR =rapid plasma reagin; TPHA = Treponema pallidum hemagglutination assa