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BRIEF REPORT

High Prevalence of Multidrug-Resistant *Mycoplasma genitalium* in Human Immunodeficiency Virus-Infected Men Who Have Sex With Men in Alabama

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We tested for *Mycoplasma genitalium* in 157 HIV-infected men. Urogenital and rectal prevalence were 10.8% and 6.4%. Macrolide resistance mutations were detected in 70.6% and 80% of urogenital and rectal samples, and fluoroquinolone resistance mutations in 26.7% and 40%, respectively.

Keywords. *Mycoplasma genitalium*; multidrug resistance; men who have sex with men (MSM); macrolide resistance; fluoroquinolone resistance.

Mycoplasma genitalium is a sexually transmitted bacterial pathogen that causes up to 20% of nongonococcal urethritis and has been detected in 12% of men who have sex with men (MSM) with proctitis [1, 2]. Many *M. genitalium* infections are asymptomatic, and surveillance is limited by the lack of a Food and Drug Administration (FDA)–approved diagnostic test. The US Centers for Disease Control and Prevention (CDC) recommends single-dose azithromycin (1 g) as first-line *M. genitalium* therapy and moxifloxacin (400 mg/d for 7, 10, or 14 days) as second-line therapy for cases of suspected treatment failure [1]. Efficacy of *M. genitalium* treatment is threatened by rapid emergence of antimicrobial resistance [3].

The most common mutations that confer macrolide resistance in *M. genitalium* are A2071G (*Escherichia coli* numbering 2058) and A2072G (*E. coli* numbering 2059) in the 23S ribosomal RNA (rRNA) gene, known as macrolide resistance–mediating mutations (MRMMs). Fluoroquinolone resistance in *M. genitalium* can be mediated by mutations in quinolone resistance–determining regions (QRDRs) of topoisomerase IV and DNA gyrase genes (mostly *parC* and *gyrA*, but also *parE* and *gyrB*). Data linking QRDR mutations to treatment failures are limited, compared with the proven association with MRMMs [4].

MSM with human immunodeficiency virus (HIV) infection have elevated rates of *M. genitalium* infection compared with HIV-uninfected MSM but little is known about infection prevalence and antimicrobial resistance patterns in HIV-infected MSM in the United States [2]. Because surveillance data inform *M. genitalium* screening and treatment recommendations, we evaluated the prevalence of *M. genitalium* infection and antimicrobial resistance among HIV-infected MSM in Alabama.

METHODS

This is a substudy of a sexually transmitted infection (STI) study that enrolled HIV-infected MSM from a HIV primary care clinic in Birmingham, Alabama. MSM in active care were eligible if they were ≥19 years old, reported receptive anal intercourse in the past 30 days, and had no exposure to antibiotics in the past 30 days (except for trimethoprim-sulfamethoxazole). Participants were asked about STI symptoms (dysuria, penile/rectal discharge, proctitis, testicular pain/swelling), STI history, number of sex partners, and condom use in the past 30 days. CD4 cell count and HIV load were abstracted from the medical record for the date closest to and within 6 months of the visit. Nucleic acid amplification testing had been performed for chlamydia and gonorrhea at urogenital and rectal sites. For this substudy, archived self-collected rectal swab and first-catch urine samples were tested for *M. genitalium* and the presence of *M. genitalium*–associated macrolide and fluoroquinolone resistance mutations. The study was approved by the University of Alabama at Birmingham (UAB) Institutional Review Board.

Statistical Analyses

M. genitalium infection prevalence was calculated as the number of real-time polymerase chain reaction (PCR)–positive infections per number of PCR tests. For categorical variables, χ^2 or Fisher exact tests were used to compute *P* values. Because continuous variables were nonnormally distributed, a nonparametric 1-way analysis of variance was used, and *P* values were calculated using the Kruskal-Wallis test. Significance was set at $P < .05$, and analyses were performed with SAS software (version 9.4).

Detection of *Mycoplasma genitalium* and Macrolide Resistance

A modified real-time PCR assay targeting the 23S rRNA gene was used to detect *M. genitalium* directly from clinical specimens and simultaneously identify mutations known to confer macrolide resistance [5]. The sensor probe was modified to improve the signal: LC-Red 705-AACGGGACGGAAAGACCCCG-phosphate and

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the probes were synthesized by Technische Informationsbibliothek. PCR was carried out on a LightCycler 480 II (Roche) using a LightCycler 480 Probes Master kit. The reaction volume was 20 µL, containing 0.375 µmol/L of forward primer, 0.5 µmol/L of reverse primer, 0.2 µmol/L of each probe, 0.4 U of LightCycler Uracil-DNA Glycosylase, and 2 µL of template DNA. A touchdown program was used for the cycling conditions: after two sequential 10-minute pre-incubations (first at 40°C and then at 95°C), there were 10 cycles of preamplification at 95°C (10 seconds), 60°C (15 seconds; ramp rate, 1.1°C/s), and 72°C (15 seconds), followed by 45 cycles of amplification at 95°C (10 seconds); 60°C to 55°C (10 seconds; 1°C per step; ramp rate, 2.2°C/s), and 72°C (15 seconds). A melting analysis was then performed at 95°C (10 seconds), 50°C (40 seconds), and 75°C (ramp rate, 0.11°C/s; 5 acquisitions per 1°C), and equipment was cooled down to 40°C (30 seconds).

Nested Polymerase Chain Reaction

Four nested traditional PCRs were used to detect mutations in *gyrA*, *gyrB*, *parC,* and *parE* genes using published and newly designed primers [4]. PCR products were sequenced by the UAB Heflin Center for Genomic Science and analyzed with CLC Main Workbench 7.8.1.

RESULTS

Demographic and Clinical Characteristics

The study included 157 HIV-infected MSM enrolled between December 2014 and November 2016. Their median age was 34 years (interquartile range, 29–46 years), 42.7% reported >1 sex partner in the past month, 96% had sex with men only, and 75% used condoms inconsistently or not at all. The majority of subjects (61%) were practicing receptive and insertive anal intercourse, and 77% had a history of STI. The median CD4 cell count was in the normal range (565 cells/mL; interquartile range 415–787 cells/mL), and 85.3% of subjects had an HIV load <200 copies/mL.

Mycoplasma genitalium Infection Prevalence

The prevalence of *M. genitalium* infection was 17.2% (27 of 157): 10.8% at the urogenital and 6.4% at the rectal site. Two in 3 men (66%; 104 of 157) were African American, but neither race nor CD4 cell count <350/µL was associated with *M. genitalium* infection. The bacterial load ranged from 2 to 32 700 genome copies (GCs)/µL, with a median of 72 GCs/µL. There was no significant different in organism load by site (49.1 GCs/µL for urogenital vs 91.5 GCs/µL for rectal samples; $P = .47$). Only 11.8% (2 of 17) with urogenital infection and 20% (2 of 10) with rectal infection reported STI symptoms. The prevalence of urogenital and rectal chlamydia in study subjects was 2.5% and 14.6%, respectively, and the prevalence of urogenital and rectal gonorrhea, 0% and 7%.

Resistance Testing

MRMMs were detected in 20 of 27 *M. genitalium*–positive samples (74.1%); all had the typical 23S rRNA mutations (2058 or 2059, *E. coli* numbering). One subject had an additional A2029 (*E. coli* numbering 2016) insertion with unknown function. MRMMs were present in 80% of rectal samples (8 of 10), and 70.6% of urogenital samples (12 of 17). Eight samples (29.6%) had typical *parC* fluoroquinolone-resistance mutations (S83I or S83R); of these, 3 carried an additional *parC* mutation (P62S), and 1 had a *gyrB* mutation (F475S) of unknown clinical significance. QRDR mutations were present in 40% of rectal samples tested (4 of 10) and 26.7% of urogenital samples (4 of 15). QRDR testing failed in 2 urogenital samples, which may have been due to an insufficient *M. genitalium* template. Six of 25 subjects (24%) with *M. genitalium* had evidence of multidrug resistance with MRMMs and QRDR mutations, including 3 of 10 rectal samples (30%) and 3 of 15 urogenital samples (20%) (Figure 1).

DISCUSSION

In this study, 1 in 6 HIV-infected MSM in Birmingham, Alabama, had *M. genitalium* infection at urogenital or rectal sites. Of notable concern, most *M. genitalium*–positive specimens carried mutations associated with macrolide or fluoroquinolone resistance, and 30% of rectal specimens harbored mutations associated with resistance to both drug classes. This finding has the potential to limit the efficacy of both first- and second-line CDC–recommended therapies for *M. genitalium*. Some people in the United States may be infected with *M. genitalium,* for which there is no known effective treatment, and this raises concern about the potential spread of a multidrug resistant pathogen.

There has been increasing clinical awareness and public health concern about failure of azithromycin treatment for *M. genitalium* infection [1]. Azithromycin treatment efficacy fell precipitously from 87% to 40% in 3 randomized controlled trials published between 2009 and 2013 [3]. A 2017 study of a 5-day azithromycin regimen showed no benefit in microbiologic cure rates compared with single-dose therapy [6]. The prevalence of MRMMs among adults with urogenital *M. genitalium* is as high as 51% in the United States and 52%–72% in Australia [6–8]. Other treatment options are limited because *M. genitalium* lacks a cell wall (thus inherent resistance to β-lactams), and cure rates with doxycycline are low (31%–45%) [3].

Moxifloxacin has favorable potency against *M. genitalium* compared with other available quinolones, but a 2017 meta-analysis demonstrated a decrease in moxifloxacin efficacy for *M. genitalium* infection from 100% to 89% since 2010 [9]. Reported global frequencies of QRDR mutations in urogenital *M. genitalium* infection include 18.6% in the Asia-Pacific region, 15% in Australia, and 6% in Russia and France [3, 10–12].

Our study fills an important knowledge gap, because to our knowledge there are no published reports on the prevalence of fluoroquinolone resistance in *M. genitalium* strains in the United States, and studies focusing on rectal *M. genitalium* infections in HIV-infected MSM are sparse. Other strengths of

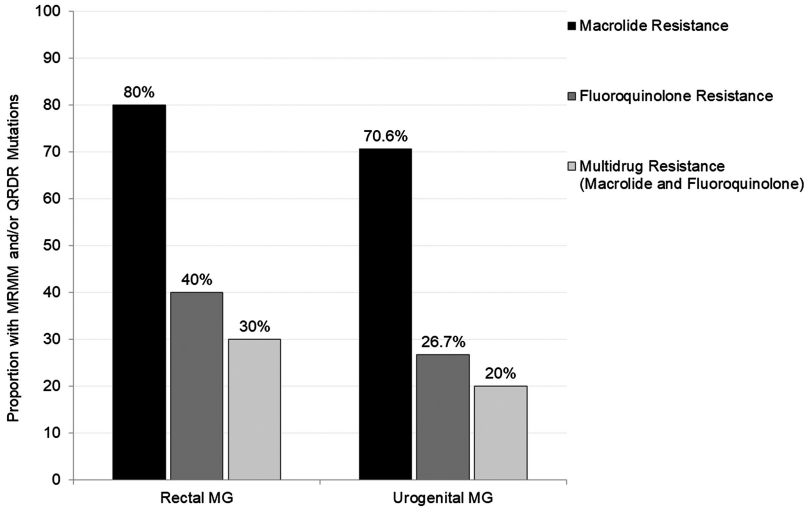

Figure 1. Proportion of *Mycoplasma genitalium* samples with macrolide and/or fluoroquinolone resistance mutations by site (n = 27); samples were obtained from human immunodeficiency virus–infected men who have sex with men. Abbreviations: MRMMs, macrolide resistance–mediating mutations; QRDR, quinolone resistance–determining region.

our study include use of a novel, sensitive real-time PCR that can simultaneously detect *M. genitalium* and macrolide resistance mutations on clinical samples.

One limitation of our study is the sample size. In addition, the presence of mutations associated with quinolone resistance may not necessarily correlate with microbiologic and/or clinical failure. Given the sensitivity of PCR and low *M. genitalium* load noted in some samples, some infections detected may have been clinically insignificant. More studies of *M. genitalium* are needed to determine the natural history of infection and whether the novel mutations noted in this study are associated with treatment failure.

Surveillance for *M. genitalium* and drug resistance in the United States is warranted, but lack of a FDA–approved testing to detect infection and resistance is a significant barrier. Some reference laboratories offer in-house *M. genitalium* PCR assays. Because 85% of *M. genitalium*–infected MSM in our study were asymptomatic, a policy of routine screening at exposure sites among high-risk MSM may be reasonable but not feasible until testing is more readily available and effective treatment options defined.

In conclusion, novel diagnostic methods facilitated the diagnosis of multidrug-resistant *M. genitalium* infection, which was highly prevalent in rectal and urogenital sites among HIV-infected MSM in Alabama. Studies of new *M. genitalium* treatment options should be prioritized, given the rapid development of antimicrobial resistance.

Notes

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