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Lack of Association Between the S83I ParC Mutation in *Mycoplasma genitalium* and Treatment Outcomes Among Men Who Have Sex With Men with Nongonococcal Urethritis

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Abstract

From February 2015 to October 2017, among 20 men who have sex with men with *Mycoplasma genitalium*-associated nongonococcal urethritis, 15% had macrolide resistance and S83I ParC mutations. Azithromycin followed by moxifloxacin cleared *Mycoplasma genitalium* in 2 of 2 with and 11 of 13 without S83I mutations. Dual failures were cleared after doxycycline. S83I mutations were not associated with moxifloxacin failure.

Mycoplasma genitalium (MG) is associated with nongonococcal urethritis (NGU) in men,¹ several reproductive tract sequelae in women,² and risk for human immunodeficiency virus (HIV) infection.³ Although MG prevalence varies by geographic region and risk group, men who have sex with men (MSM) are among those at highest risk.⁴ Traditionally, azithromycin (AZM) has been recommended as first-line therapy for MG-associated NGU (MG-NGU), and moxifloxacin (MOX) is recommended when AZM treatment fails. However, the high prevalence of macrolide resistance (range, 7–100%)^{5,6} and increasing reports of fluoroquinolone (range, 2–47%)^{7,8} and dual drug (range, 1–33%)^{9,10} resistance have generated substantial concern over our continuing ability to treat MG infections.

Molecular tools to detect resistance-associated mutations at the time of diagnostic testing now permit resistance-guided therapy, which has yielded promising results.¹¹ However, this

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is currently limited to detection of macrolide resistance. The greater variety of mutations in the quinolone resistance determining region of the *parC* and *gyrA* genes and uncertainty over which are most relevant has, thus far, prevented implementation of this approach for fluoroquinolones. To address this, we evaluated the role of resistance-associated mutations in treatment outcomes among MSM with MG-NGU enrolled in a cohort study.

MATERIALS AND METHODS

From February 2015 to October 2017, we recruited people with NGU attending the Public Health-Seattle and King County STD Clinic who were age 16 years or older, assigned male sex at birth, and had no female sex partners in the past year. Patients were ineligible if they had no sex in the past 60 days, antibiotics in the past 30 days, urethral contact to *Neisseria gonorrhoeae* (GC), no contact information, or no freezer at home for storing urine specimens for microbiota analyses (not reported here).

Enrolled participants attended 5 in-clinic visits at 3-week intervals, at which the clinician collected a urethral swab specimen for Gram staining and first-void urine. We defined NGU as greater than or equal to 5 polymorphonuclear leukocytes (PMNs) per high-power field (HPF) plus either urethral symptoms or visible urethral discharge on examination in the absence of GC. NGU was treated with AZM (1 g). Participants reported sociobehavioral data on computer-assisted self-interviews and daily antibiotics, urethral symptoms, and sexual activity on weekly Web-based diaries.

Prior to implementing routine MG testing in April 2016, we stored specimens at -80°C and tested them retrospectively for MG. Upon receiving retrospective MG test results, we asked participants with positive results to undergo an additional post-study test and provided MOX (400 mg daily, 10–14 days)¹² if MG-positive. Starting in April 2016, we performed MG tests within 10 days of each visit. Participants still received AZM for NGU at enrollment, but those with MG were recalled and provided MOX.

We tested urine for GC and *Chlamydia trachomatis* using the Aptima Combo 2 assay and MG using the Aptima assay with analyte-specific reagents (Hologic, Marlborough, Massachusetts). We performed DNA extraction from MG-positive specimens in Aptima transport medium using a MagNA Pure 96 Instrument (Roche, Pleasanton, California) with large volume (1 mL) universal pathogen extraction protocol and elution in 50 μL . We estimated organism load using real-time quantitative polymerase chain reaction with a limit of detection of less than 5 genome equivalents (geq) per reaction.^{13,14} Macrolide resistance mutations (MRM) and *ParC* mutations were detected with PyroMark Q96 sequencing¹⁴ and conventional Sanger sequencing,¹⁵ respectively. *GyrA* mutations were not sought because they have never been associated with MOX treatment failure without the simultaneous presence of *ParC* mutations.¹⁶

We compared treatment outcomes by pretreatment resistance mutation profile using Fisher's exact tests. We defined microbiologic cure as an MG-negative test after treatment. We defined clinical cure as either (1) less than 5 PMNs/HPF, or (2) neither symptoms nor visible discharge after treatment. We used linear regression with robust standard errors to estimate

the association between pretreatment resistance mutation profile and log₁₀ organism load. We compared treatment outcomes by pretreatment organism load using Wilcoxon rank-sum tests.

The University of Washington Human Subjects Division approved all study procedures. Participants provided written informed consent. We compensated participants US \$360 through a tiered schedule. We used Stata 13 (StataCorp, College Station, TX), 2-sided tests, and alpha = 0.05.

RESULTS

Twenty (22%) of 92 participants had MG-NGU at enrollment. Among these, 12 (60%) were non-Hispanic white, median age was 30.5 years (interquartile range, 26.5–42.5), and all selfidentified as cisgender men. Two (10%) were living with HIV. At enrollment (i.e., pretreatment), 18 men (90%) had MRM, and 3 (15%) had the S83I ParC fluoroquinolone resistance-associated mutation (Table 1). Five men (25%) had other ParC mutations not previously associated with fluoroquinolone resistance. All with ParC mutations also had MRM.

Treatment Outcomes

AZM Alone—In the absence of routine MG testing, 5 men (25%) were only managed syndromically (participants 1–5, Figure 1). Median time from specimen collection to receipt of MG test results was 162 days (interquartile range, 118.5–174.25). Pretreatment organism loads were low (range, not detectable to 3643 geq/5 μ L template). None received MOX during follow-up. Although clinical cure occurred in 4 men (80%), MG was eradicated in only 2 (40%), 1 (participant 2) with and 1 (participant 1) without MRM. In 3 men (participants 3–5), MG was persistently detected at each study visit, or for 88 to 90 days after enrollment. Two men (participants 3 and 4) returned for a post-study test; both had MG, but treatment outcomes were not available. All 3 men with persistent infections had MRM; 1 (participant 5) also had the S83I mutation. Despite not meeting our NGU definition, each had either symptoms or 5 PMNs/HPF or greater at several subsequent visits.

AZM Followed by MOX—MG test results were available during follow-up for 15 men (participants 6–20). They received initial presumptive AZM followed by MOX after their MG-positive test. Pretreatment organism loads ranged from 6 to 289,549 geq/5 μ L template. Clinical and microbiologic cure occurred in 12 men (80%). All 12 had MRM; 2 (participants 19 and 20) also had the S83I mutation. One man with MRM but no ParC mutations (participant 12) experienced persistent NGU at week 3 despite eradication of MG. This resolved by week 6 without additional therapy.

Two men (13%) were potential dual treatment failures (participants 6 and 7). Although participant 7 had MRM, neither had ParC mutations. Participant 6 reported insertive oral sex and condomless insertive anal sex between enrollment and week 3 and, thus, could have been reinfected. However, participant 7 did not report any urethral sexual exposures between enrollment and week 6. Both received doxycycline (DOX) (100 mg twice daily, 7 days), after which MG was not detected.

Organism Load and Resistance-associated Mutations

Among men with no resistance-associated mutations, MRM only, and both MRM and S83I, pretreatment organism load ranged from 3,643 to 289,54aa9 geq/5 μ L; not detectable to 40,350 geq/5 μ L; and 50 to 1,371 geq/5 μ L template, respectively. Men with MRM alone had 0.01-fold lower median pretreatment organism load than the 2 men with no resistance-associated mutations (95% confidence interval, 0.0002–0.55; $P=0.03$); men with MRM and S83I also had 0.01-fold lower median organism load (95% confidence interval, 0.0002–0.83; $P=0.04$). Nevertheless, there was wide variation, and some men with MRM had higher organism loads than men without MRM. Treatment failure after AZM alone was not associated with pretreatment organism load. However, pretreatment organism load was somewhat higher among the 2 men with treatment failure after sequential AZM and MOX than among those with microbiologic cure (median, 147,781 vs. 1368 geq/5 μ L template; $P=0.09$).

DISCUSSION

Among MSM with MG-NGU in Seattle, 90% had MRM, and 15% had both MRM and the S83I ParC mutation. The efficacy of AZM was low, and treatment failures correlated with detection of MRM. In contrast, AZM followed by MOX had relatively high efficacy, but there was no association between the S83I mutation and MOX failure. DOX was effective in 2 cases of possible dual AZM and MOX failure.

The 90% prevalence of MRM in our study is even higher than prior studies of MSM from North America^{17,18} and Europe^{10,19} and similar to observations from Australia.²⁰ Although the prevalence of the S83I mutation that we observed was also similar to other reports,²¹ the lack of association with MOX failure was surprising, given prior associations with treatment failure^{22–25} and high minimum inhibitory concentrations.^{26,27} However, 4 other MG cases with the S83I mutation have been cured with fluoroquinolones.^{8,24} These treatment successes despite the S83I mutation highlight the potential role of other factors, including decreased fitness, organism load,²⁸ and interactions with other ParC or GyrA mutations.^{25,27} Potential detection of non-viable nucleic acids with the highly sensitive Aptima assay, the length of time between completion of treatment and retesting, and spontaneous clearance may also have contributed. The lower median pretreatment organism loads with MRM may indicate decreased fitness and have contributed to spontaneous clearance.

Given the low efficacy of DOX,²⁹ we were surprised that it appeared to eradicate MG after suspected dual treatment failure. The persistently positive MG tests may reflect detection of nonviable nucleic acids rather than dual treatment failure. Alternatively, initial treatment with AZM and MOX may have reduced the organism load, similar to what has been observed after initial treatment with DOX under resistance-guided therapy.¹¹ Pristinamycin,²² spectinomycin,³⁰ and minocycline,²⁵ which have been used successfully in some dual treatment failures, are not all widely available. DOX may be another alternative for suspected dual treatment failures. Nonetheless, new antimicrobials are urgently needed.²⁹

Strengths of this study include the prospective design and frequent clinical evaluation. However, our sample size was small, and we could not estimate the frequency of “de novo”

mutations after specific antibiotics. Specimens were not tested for GyrA mutations, and these may play a role.

MRM in MG are now nearly ubiquitous in some populations. Persistent asymptomatic infection may be common after ineffective therapy. Resistance-guided therapy based on MRM is currently effective¹¹; however, more data are needed to identify appropriate predictors of fluoroquinolone treatment failure.

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Figure 1. Clinical presentation and management, detection of mutations, and organism load among MSM with MG-NGU. CT, Chlamydia trachomatis; DOX, doxycycline; ND, non-detectable; OTH, other antibiotic therapy; qPCR, quantitative polymerase chain reaction; Rx, antibiotic therapy; sC234T, silent C234T. *Reported condomless insertive anal sex and insertive oral sex (presumed condomless) between enroll and week 6 visits. †Ceftriaxone, provided with AZM for proctitis. ‡Ceftriaxone, provided with AZM for rectal gonorrhea. §Retreated with AZM because reexposed to untreated sex partner. ||Patient did not respond to our attempts to recall him to the clinic for a post-study MG test. ¶Reported condomless insertive anal sex and insertive oral sex (presumed condomless) between enroll and week 3 visits. Reported no urethral sexual exposures between enroll and week 6 visits. **No antibiotics per MD while we waited to see the next MG test result and discussed what third-line therapy to use. ††Nitrofurantoin for UTI, plus AZM for NGU.

TABLE 1.

Prevalence of Resistance-associated Mutations in Pretreatment Specimens and Summary of Treatment Outcomes Among MSM with MG-NGU

Mutation Profile	N = 20, n (Column %)	AZM, n (Row %)	MG Cleared After:		
			AZM + MOX, n (Row %)	AZM + MOX + DOX, n (Row %)	Unknown, n (Row %)
None	2 (10)	1 (50)	—	1 (50)	—
MRM only					
A2058G	5 (25)	1 (20)	3 (60)	—	1 (20)
A2059G	5 (25)		3 (60)	1 (20)	1 (20)
MRM and nonresistance ParC mutation					
A2058G and E72D	1 (5)	—	1 (100)	—	—
A2059G and P62S	2 (10)	—	2 (100)	—	—
A2059G and P62S/silent C234T	1 (5)	—	1 (100)	—	—
A2058G and S83N	1 (5)	—	1 (100)	—	—
MRM and S83I ParC mutation					
A2058G and S83I	1 (5)	—	1 (100)	—	—
A2058T and S83I	1 (5)	—	1 (100)	—	—
A2059G and S83I	1 (5)	—	—	—	1 (100)

DOX, doxycycline.