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Distribution of Macrolide Resistant *Mycoplasma genitalium* in Urogenital Tract Specimens From Women Enrolled in a US Clinical Study Cohort

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Background. This study evaluated the distribution of macrolide-resistant *Mycoplasma genitalium* in multiple urogenital specimens collected from women enrolled in a prospective multicenter US clinical study.

Methods. Four female urogenital specimens (vaginal swab, urine, endocervical swab, ectocervical brush/spatula) collected from each subject were tested using a transcription-mediated amplification (TMA) assay for *M. genitalium*. TMA-positive specimens were evaluated by reverse transcription–polymerase chain reaction and bidirectional Sanger sequencing of *M. genitalium* 23S rRNA to identify the presence of macrolide-resistance–mediating mutations (MRMs) at base positions 2058/2059.

Results. Of 140 women with ≥1 TMA-positive specimens, 128 (91.4%) yielded *M. genitalium* 23S rRNA sequence. MRMs were found in 52% of vaginal specimens, 46.3% of urine specimens, 37.8% of endocervical specimens, and 46% of ectocervical specimens. There were 44 unique specimen type/sequence phenotype combinations of *M. genitalium* infection. Most (81; 63.3%) women had single specimen-sequence phenotype (macrolide-susceptible, MRM, or both) infections, while 24 (18.8%) women had multiple specimen-sequence phenotype concordant infections, and 23 (17.9%) women had multiple specimen-sequence phenotype discordant infections. The sensitivity for any single specimen type to detect overall urogenital tract macrolide-resistant *M. genitalium* infection status was 96.3% for vaginal swab samples, 82.6% for urine samples, 70.8% for endocervical swab samples, and 82.1% for ectocervical brush/spatula liquid Pap samples.

Conclusions. The distribution of *M. genitalium* infections in female urogenital tract specimens is highly complex, with multiple phenotypic combinations of the organism infecting a significant proportion of women at different anatomic specimen collection sites. Vaginal swab sampling yielded the highest sensitivity for identifying women with macrolide-resistant *M. genitalium* urogenital tract infections.

Keywords. *Mycoplasma genitalium*; macrolide-resistance mutations; urogenital infection; sexually transmitted infection.

Mycoplasma genitalium is a sexually transmitted bacterial pathogen that confers increased risk for adverse reproductive and sexual health outcomes, including nongonococcal urethritis in men [1] and preterm birth, infertility, vaginitis, cervicitis, and pelvic inflammatory disease in women [2–4]. Diagnosis of infection has been difficult since *M. genitalium* has fastidious culture requirements and slow growth in vitro. Nucleic acid amplification tests (NAATs) are now the primary tools for detecting the organism in clinical specimens obtained from infected persons.

In the research setting, use of NAATs has provided an understanding of the epidemiology of *M. genitalium* infection, revealing prevalence estimates often similar to or exceeding those of *Chlamydia trachomatis* infections in low-risk populations [5] as well as in persons at higher risk for contracting a sexually transmitted infection (STI) [6–9]. The recent advent of clinically validated, in vitro, diagnostic NAATs provides for sensitive methods for detecting *M. genitalium* in specimens collected from various urogenital sites in symptomatic women, including urine samples as well as swab specimens from cervical and vaginal epithelia [10–12].

Since *Mycoplasma* spp. lack a cell wall, bacteriostatic antimicrobial agents form the basis for treating *M. genitalium* infections. The semisynthetic macrolide azithromycin has been the first-line treatment for *M. genitalium* infection due to its advantageous pharmacokinetic properties for treating intracellular bacterial infections [13], but resistance to the drug has been increasing steadily due to drug-induced mutations in *M. genitalium* ribosomal RNA (rRNA), specifically at base positions 2058 and 2059 (*Escherichia coli* numbering) of the 23S subunit [14, 15]. To avoid treatment failure and selection of

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resistant strains in vivo, Centers for Disease Control and Prevention (CDC) treatment guidelines now recommend testing *M. genitalium* NAAT–positive specimens from symptomatic patients for the presence of these macrolide-resistance–mediating mutations (MRMs) prior to initiation of treatment [16].

Evidence from research studies [8, 17, 18] and formal clinical validation studies [11, 12] for *M. genitalium* NAATs shows that vaginal swab specimens have superior sensitivity compared with urine or cervical sampling for detecting *M. genitalium* urogenital infections in women. However, little is known about the distribution of *M. genitalium* infection at different anatomical sites of the urogenital tract of individuals, nor what the optimal specimen type is for ascertaining the overall urogenital tract MRM status of an infected person. To address these questions, we used a sensitive NAAT RNA sequencing assay to investigate MRM distribution and sensitivity of detection of *M. genitalium* MRMs in sets of specimens collected at multiple urogenital anatomic sites, from women enrolled in a prospective clinical study encompassing a broad geographical region of the United States.

METHODS

Clinical Study Cohort

The urogenital specimens analyzed in this study were obtained from women who participated in a prospective multicenter clinical study investigating the prevalence of urogenital tract STIs. Details of this study enrollment have been described previously [7]. Briefly, following institutional review board approval, 1368 women were enrolled between January 2013 and July 2014 from diverse clinical practice types (adolescent gynecology, family medicine, family planning, adult obstetrics-gynecology practices, and public health and hospital-based STD [sexually transmitted disease] clinics) from 7 different geographic locations in the United States (1 Northeast, 1 mid-Atlantic, 1 Southeast, 3 Midwest, 1 Southwest). After obtaining informed consent, 4 urogenital specimens were obtained from each participant, in the following order: self-collected urine, clinician-collected vaginal swab, and clinician-collected speculum-assisted endocervical swab, each placed into a separate Aptima specimen transport tube, and a clinician-collected speculum-assisted ectocervical brush/spatula sample placed into ThinPrep Preservcyt medium (all from Hologic, Inc, San Diego, CA, USA). Women with a complete specimen collection set (n = 515, ages 14–70 years, 36.5% asymptomatic) were tested with an NAAT for *M. genitalium,* and women with 1 or more positive specimens (n = 140) were included for subsequent sequencing analysis. The prevalence of macrolide-resistant *M. genitalium* in this cohort categorized by demographic status (race, age range, symptomatic status) was reported previously [7].

Laboratory Procedures

Urogenital specimens were tested for *M. genitalium* using the Aptima Mycoplasma genitalium assay, a transcription-mediated amplification (TMA) NAAT targeting 16S rRNA (Hologic, Inc), as previously described [7, 8]. For sequencing analysis, 23S RNA from a 0.4-mL sample from TMA-positive specimens was subsequently extracted into 40 µL nuclease-free water using Aptima target capture reagent on a KingFisher platform (ThermoFisher Scientific, Carlsbad, CA, USA) from which 5 µL was subjected to reverse transcription and nested polymerase chain reaction (RT-PCR) followed by bidirectional PCR Sanger sequencing, using custom primer designs and PCR cycling conditions targeting the 1870–2250 base region of *M. genitalium* 23S rRNA (*E. coli* numbering), as described previously [7]. Sequencing results analysis was performed using Geneious v10 software (Biomatters, Auckland, New Zealand). Sequence data exhibiting a Phred quality score of 20 or better (≥99% base call accuracy) were used for analysis of results [19]. All laboratory testing was conducted during 2014–2015.

Statistical Analyses

Subjects were considered infected if they had 1 or more specimens with positive results. The prevalence of infection was calculated using the infected status standard. All tests for 95% confidence intervals (CIs) were 2-tailed and performed at the .05 significance level, using the efficient score method. Calculation of odds ratios (ORs) and tests for significance were performed as described previously [20], with *P* values of less than .05 being considered significant.

RESULTS

Sensitivity of RT-PCR Sanger Sequencing for Detecting *M. genitalium* 23S rRNA

A sensitive nested RT-PCR Sanger sequencing assay system was used to analyze *M. genitalium*–positive female urogenital specimens to determine the specimen-specific prevalence and distribution of 23S rRNA mutations at base positions 2058 and 2059 that confer macrolide antibiotic resistance to the organism [14]. Specimens from 140 TMA-positive subjects were sequenced. Compared with reference sequence obtained from *M. genitalium* strain G37 (GenBank accession NC_000908.2), all sequences obtained had 100% base identity (non-MRM bases) with *M. genitalium* strain 23S rRNA (data not shown). Table 1 shows that the sensitivity of sequencing compared with TMA by subject age category was 91.4% for all women ages 14 to 47, 92.9% for ages 14–17, 91.3% for ages 18–20, 92.2% for ages 21–30, 85.7% for ages 31–40, and 100% for ages 41–47 years. Percent-positive sequencing yields were 91.1% for Black women, 91.7% for White women, and 90.3% and 96.3% for symptomatic and asymptomatic women, respectively. Sequencing detected *M. genitalium* 23S rRNA in 96.2% of vaginal swab specimens, 83.7% of urine specimens, 95.7%

Table 1. Sensitivity of *Mycobacterium genitalium* rRNA Sequencing, by Female Subject Demographic Status and Urogenital Specimen Type

Category	No. of 23S rRNA Sequencing Results Obtained/No. of TMA Positives (%, 95% CI)
Age (years)	
14–17	13/14 (92.9, 68.5–98.7)
18–20	42/46 (91.3, 79.7–96.6)
21–30	59/64 (92.2, 83.0–96.6)
31–40	12/14 (85.7, 60.1–96)
41–47	2/2 (100, 34.2–1)
All (14–47)	128/140 (91.4, 85.6–95.0)
Race[a]	
American Indian or Alaska Native	1/1 (100, 20.7–1)
Black or African American	102/112 (91.1, 84.3–95.1)
White	22/24 (91.7, 74.2–97.7)
Unknown	3/3 (100, 43.9–1)
Symptomatic status[b]	
Symptomatic	102/113 (90.3, 83.4–94.5)
Asymptomatic	26/27 (96.3, 81.7–99.3)
Urogenital specimen type	
Vaginal swab	50/52 (96.2, 87.0–98.9)
Urine	41/49 (83.7, 71.0–91.5)[c]
Endocervical swab	45/47 (95.7, 85.7–98.8)[d]
Ectocervical brush/spatula	50/60 (83.3, 72.0–90.7)[e]

Abbreviations: CI, confidence interval; OR, odds ratio; rRNA, ribosomal RNA; TMA, transcription-mediated amplification.

[a]Self-identified status. For ethnicity, 139 of 140 self-identified as non-Hispanic.

[b]Clinician diagnosis.

[c]OR: .87 (95% CI: .49–1.54; $P = .632$) vs vaginal swab.

[d]OR: .99 (95% CI: .57–1.75; $P = .988$) vs vaginal swab.

[e]OR: .87 (95% CI: .51–1.49; $P = .604$) vs vaginal swab.

of endocervical swab specimens, and 83.3% of ectocervical brush/spatula specimens.

Accounting of 23S rRNA sequence results by specimen type and macrolide-resistance sequence phenotype are shown in Table 2. From the total of 560 urogenital specimens evaluated from the 140 *M. genitalium* TMA–positive women, 352 (62.9%) specimens were TMA-negative and were not sequenced. An additional 22 (3.9%) specimens were TMA-positive but negative by sequencing, resulting in 89.4% (186/208) of TMA-positive specimens that yielded a valid sequencing result. These included 101 specimens with macrolide-susceptible wild-type (WT; A2058 or A2059) sequences, 67 specimens with MRM sequences (A2058C/G/T and/or A2059G), and 18 specimens with both WT and MRM (WT/MRM) sequences. Compared with TMA NAAT results, urine and ectocervical specimens had higher proportions of sequencing false-negative results (16.3% and 16.7%, respectively) compared with vaginal swab and endocervical swab samples (3.8% and 4.3%, respectively). The false-negative sequencing rates in ectocervical samples compared with vaginal samples were not significantly different (OR: 1.154; 95% CI: .672, 1.98; $P = .604$).

Distribution of *M. genitalium* 23S rRNA Phenotypes in Urogenital Specimens

Table 3 shows the distribution of *M. genitalium* 23S rRNA sequence phenotypes in the 4 urogenital specimens obtained from women with *M. genitalium*–positive TMA results. Among all 128 women with 23S rRNA sequencing results, there were 44 unique sequence phenotype/specimen type combinations of *M. genitalium* infection. The majority (81; 63.3%) had single urogenital specimen infections, either a WT infection (n = 45), an MRM infection (n = 28), or a mixed WT/MRM infection (n = 8). Twenty-four (18.8%) women had multiple specimen infections with concordant sequence phenotypes, in which the same combination of sequence phenotypes (either single or multiple sequence phenotypes) were found in 2 or more urogenital specimens. There were also 23 (17.9%) women who had multiple specimen-discordant sequence phenotype infections, in which the sequence phenotype combination differed between specimens collected at different urogenital sites. One woman had 3 *M. genitalium* 23S sequence phenotypes (WT/A2058G/A2059G) in both vaginal and urine specimens and a WT/A2059G sequence phenotype in the ectocervical specimen. This group of discordant sequence phenotype results included 17 specimens from 15 women that had WT sequence phenotype in 1 specimen but MRM sequence phenotype in 1 or more other urogenital specimens from the same person.

Sensitivity of Individual Urogenital Specimen Types for Detecting Macrolide-Resistant *M. genitalium* Urogenital Tract Infections

The prevalence of *M. genitalium* MRMs by specimen type and the sensitivity of each specimen type for detecting a woman's overall MRM urogenital tract infection status (UIS) are shown in Table 4. Macrolide-resistance–mediating mutations were found in 52% of vaginal specimens, 46.3% of urine specimens, 37.8% of endocervical specimens, and 46% of ectocervical specimens. Vaginal swab specimens had the highest sensitivity (96.3%) for the detection of MRM UIS, followed by urine specimens (82.6%), ectocervical brush/spatula samples (82.1%), and endocervical swab specimens (70.8%). The difference between endocervical swab sensitivity and vaginal swab sensitivity was not statistically significant (OR: .736; 95% CI: .323–1.674; $P = .464$).

DISCUSSION

This study investigated the distribution of rRNA mutations conferring macrolide antibiotic resistance to *M. genitalium* in multiple urogenital specimens collected from women seeking care at a variety of clinical practice types and geographic regions in the United States. To our knowledge, this is the first report on the distribution of macrolide antibiotic–resistant *M. genitalium* infections in sets of clinical specimens collected from multiple anatomic sites from women. Using sensitive

Table 2. Accounting of 23S rRNA Sequence Results and Macrolide-Resistance–Mediating Mutation Status in Urogenital Specimens From 140 Women With *Mycoplasma genitalium* Infection

Sequencing Result Category	Specimen Type				Total *M. genitalium* Sequence Phenotypes No. (%)
	Vaginal Swab	Urine	Endocervical Swab	Ectocervical Brush/Spatula	
WT (A2058/A2059)	24	22	28	27	101 (18.0)
WT + MRM (A2058C/G/T; A2059G)	8	3	4	3	18 (3.2)
MRM (A2058C/G/T; A2059G)	18	16	13	20	67 (12.0)
Sequencing negative[a]	2	8	2	10	22 (3.9)
Not done[b]	88	91	93	80	352 (62.9)
Total	140	140	140	140	560 (100)

Abbreviations: MRM, macrolide-resistance–mediating mutation; rRNA, ribosomal RNA; RT-PCR, reverse transcription–polymerase chain reaction; TMA, transcription-mediated amplification; WT, wild-type.

[a]*M. genitalium* TMA positive, RT-PCR Sanger sequencing negative.

[b]*M. genitalium* TMA negative, sequencing not performed.

NAAT methods for *M. genitalium* detection and rRNA sequencing, we found a highly complex distribution of rRNA sequence phenotypes from the organism in specimens collected at 4 different urogenital sites (urinary tract, vaginal epithelium, endocervical canal, ectocervical epithelium), consisting of over 40 different combinations of specimen types and macrolide-susceptible and -resistant sequence phenotypic strains, including a variety of mixtures of sequence phenotypes within and between specimen types from the same person. Although much of this diversity resulted from women with a single or mixed sequence phenotype(s) in a single specimen type, almost one-fifth of the women with sequencing results had infections in which the sequence phenotypes found differed between specimen types. This latter group included 15 women (11.7% of 128 sequencing-positive women) who had macrolide-susceptible rRNA phenotype in 1 specimen but rRNA with macrolide-resistant phenotype in 1 or more companion specimens. We also found that the prevalence of macrolide-resistant rRNA phenotypes differed between each specimen type; vaginal swab specimens had a 38% (52%/37.8%) higher *M. genitalium* macrolide-resistance positivity rate than endocervical swab specimens, although this difference was not statistically significant.

The discovery of discordant sequence phenotype infections in different specimens collected from the same person, and variability in MRM prevalence among sets of urogenital specimens collected from women seeking care, has implications for understanding the epidemiology of *M. genitalium* antibiotic resistance in the US population, as well as assessment of the efficacy of treating *M. genitalium* infections using current clinical practice, and the selection and transmission of macrolide antibiotic–resistant strains of the organism in women at risk for infection.

First, the choice of specimen type used to study macrolide-resistant *M. genitalium* in females could affect the prevalence estimates obtained for this phenotype. Although based on a limited sample size, our results show that vaginal swab sampling yields the highest prevalence for macrolide-resistance status of the urogenital tract. Use of cervical or urine samples for studying *M. genitalium* macrolide-resistant infection rates could underestimate the true prevalence of this phenotype.

Second, single anatomic site sampling of the urogenital tract for diagnosis and characterization of *M. genitalium* infections has the potential to yield an inaccurate assessment of the macrolide-resistance status of women with *M. genitalium* infections, both before and after antibiotic treatment. A significant proportion of women in this study had a macrolide-susceptible *M. genitalium* sequence phenotype in 1 specimen but a macrolide-resistant sequence phenotype in 1 or more companion specimens collected at other urogenital sites. Treatment of these women with a macrolide antibiotic based on the macrolide-sensitive result would result in treatment failure and selection for the undetected resistant strain(s). We found vaginal swab sampling had the highest sensitivity (96.3%) for identifying the overall *M. genitalium* macrolide-resistant status of the female urogenital tract; the lower sensitivity of urine (urinary tract) and cervical sampling for detecting macrolide-resistant infections may increase the risk of mischaracterizing the true antibiotic-resistance status of the person being tested.

Finally, the complexity of *M. genitalium* 23S rRNA sequence phenotype combinations found in this cohort raises questions about the genesis of macrolide resistance in this organism and the dynamics of sexual transmission of *M. genitalium* between partners. Azithromycin has been the macrolide antibiotic recommended for initial treatment of *M. genitalium* and other bacterial STIs because the drug's large volume of distribution, long elimination half-life, and facile penetration into soft tissues allow drug concentrations at intracellular sites of

Table 3. Distribution of *Mycobacterium genitalium* 23S rRNA Phenotypes Detected in Female Urogenital Specimens

Urogenital Tract Infection Status and Number	*M. genitalium* 23S rRNA Phenotype by Specimen Type			
	Vaginal Swab	Urine	Endocervical Swab	Ectocervical Brush/Spatula
Single urogenital site (n = 81)				
16	WT	…	…	…
11	MRM	…	…	…
4	WT + MRM	…	…	…
9	…	WT	…	…
6	…	MRM	…	…
8	…	…	WT	…
6	…	…	MRM	…
3	…	…	WT + MRM	…
12	…	…	…	WT
5	…	…	…	MRM
1	…	…	…	WT + MRM
Multiple urogenital sites, sequence-concordant infections (n = 24)				
5	WT	…	WT	…
4	…	WT	WT	WT
3	…	WT	…	WT
2	…	…	WT	WT
2	A2058G	…	…	A2058G
1	A2058G	…	A2058G	…
1	…	A2058C	…	A2058C
1	A2059G	…	…	A2059G
1	A2059G	A2059G	…	…
1	A2058G	A2058G	A2058G	…
1	…	WT	WT	…
1	WT	WT	…	…
1	WT	…	…	WT
Multiple urogenital sites, sequence-discordant infections (n = 23)				
3	…	…	WT	A2059G
2	…	WT	…	A2059G
1	WT	WT/A2059G	…	WT
1	…	A2058T	…	WT
1	…	…	WT/A2059G	A2059G
1	…	A2058C	…	WT
1	…	WT	…	A2058G
1	WT/A2059G	A2058G	A2058G	…
1	WT/A2059G	…	WT	…
1	…	…	A2058G	A2058C
1	…	…	WT	A2058G
1	WT/A2058G/A2059G	WT/A2058G/A2059G	…	WT/A2059G
1	A2058G	…	A2059G	WT
1	…	WT	WT	A2058G
1	…	A2058G	…	A2059G
1	…	A2059G	…	WT
1	WT/A2058G	…	WT	…
1	…	A2059G	WT/A2059G	WT/A2059G
1	…	A2059G	A2058G	…
1	…	WT/A2059G	A2058G	A2059G

Abbreviations: MRM, 23S rRNA macrolide-resistance–mediating mutation (A2058C/G/T, A2059G); rRNA, ribosomal RNA; WT, wild-type (A2058, A2059).

infection to exceed the minimum inhibitory concentration (MIC) for the organism [21]. These pharmacokinetic characteristics have fostered a variety of dosing schedules of the drug for treating bacterial STIs, including historically a single 1-g dose for treating nongonococcal urethritis in men, the use of which was subsequently linked to the induction of macrolide resistance in *M. genitalium* and high rates of treatment failure [14, 22–24]. Our finding of multiple rRNA sequence

Table 4. Sensitivity of Individual Specimen Types for Detecting Macrolide-Resistant *Mycoplasma genitalium* Urogenital Tract Infections

Specimen Type	No.	Specimen Infection Status/Urogenital Tract Infection Status				Prevalence of MRM 23S rRNA Phenotype by Specimen Type, n/N (%; 95% CI)	Sensitivity for Detecting Macrolide Resistance in Urogenital Tract, n/N (%; 95% CI)
		MRM/MRM	MRM/WT	WT/MRM	WT/WT		
Vaginal swab	50	26	0	1	23	26/50 (52; 38.5–65.2)	26/27 (96.3; 81.7–99.3)
Urine	41	19	0	4	18	19/41 (46.3; 32.1–61.3)	19/23 (82.6; 62.9–93.0)
Endocervical swab	45	17	0	7	21	17/45 (37.8; 25.1–52.4)	17/24 (70.8; 50.8–85.1)[a]
Ectocervical brush/spatula	50	23	0	5	22	23/50 (46; 32.9–59.6)	23/28 (82.1; 64.4–92.1)

Abbreviations: CI, confidence interval; MRM, 23S rRNA macrolide-resistance–mediating mutation (A2058C/G/T, A2059G); OR, odds ratio; rRNA, ribosomal RNA; WT, wild-type (A2058, A2059).

[a]OR of .736 (95% CI: .323–1.674; $P = .464$) vs vaginal swab.

phenotypes of this bacterium in specimens collected at different female urogenital sites raises the possibility that subtle differences may exist in the exposure of organism to drug at different urogenital sites, perhaps due to the presence of polymorphonuclear leukocytes (PMNs) at sites of epithelial inflammation—azithromycin distribution into PMNs is known to yield intracellular azithromycin concentrations more than 1000-fold higher than in tissue interstitial fluid [13]—or due to suboptimal drug concentrations encountered during the pre– and post–steady-state phases of drug distribution with various dosing regimens. Whether the mosaic of MRM status infections we found in specimens from women presenting for care is the result of previous in situ exposures of prevalent WT *M. genitalium* to physical or temporal variances in azithromycin concentrations [13] or represents incident infections derived from repeated transmission events from sex partner(s) [25] will require further investigation.

Our results cannot be used to infer the tropism of *M. genitalium* for different epithelial environments in the female urogenital tract, since the specimen collection protocol used was not designed to address this question. It is possible that the routine clinical procedures used to collect specimens resulted in significant cross-sampling between anatomic sites, especially between vaginal and cervical samples. Hence, the data shown here reflect the scope and extent of diversity of *M. genitalium* rRNA phenotypes in clinical specimens obtained from sampling women using procedures that are in common clinical practice in the United States.

An additional limitation of this study is the slightly lower sensitivity achieved for sequencing of *M. genitalium*–positive urine and ectocervical (liquid Pap) specimens compared with vaginal and endocervical swab specimens. These false-negative sequencing results may have introduced a bias of unknown magnitude on the sensitivity estimates for detecting macrolide resistance in these specimen types. However, the differences in rates of results that were TMA-positive but negative by sequencing between specimen types were not significantly different, and the sensitivity of *M. genitalium* sequencing achieved in this study is similar to that reported previously [26–28]. We also did not collect specimens from the oropharynx or anus/rectum of the study participants, both of which have been shown to harbor *M. genitalium* infections [29, 30], nor did we investigate fluoroquinolone-resistance mutations in specimens with *M. genitalium* infection. The results shown here thus represent an incomplete picture of the total *M. genitalium* infection and antibiotic-resistance burden of the women in this cohort.

In summary, in this study we found complex patterns of macrolide-susceptible and -resistant infections of *M. genitalium* in urogenital specimens collected from women seeking care at a broad variety of clinical practices and geographic regions in the United States. The results indicate that specimen collection from a single urogenital anatomic site has the potential for underestimating the prevalence of macrolide resistance in women and may lead to inappropriate antibiotic treatment. Vaginal sampling yielded the highest sensitivity for determining urogenital tract macrolide-resistant infection status, a result that supports the use of this specimen type for diagnosis and management of *M. genitalium* urogenital infections in women.

Notes

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Potential conflicts of interest. D. G. and A. J. are scientists employed by Hologic, Inc, the sponsor and manufacturer of the tests used in the study. During the time the study was conducted, S. C. was an undergraduate student at Occidental College and a research intern at Hologic. D. G. reports US patents planned, issued or pending, and Hologic, Inc, stock and stock options. The authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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