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MAJOR ARTICLE

Prevalence of *Mycoplasma genitalium* Infection and Macrolide and Fluoroquinolone Resistance Mutations Among US Air Force Service Members With HIV, 2016–2020

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Background. *Mycoplasma genitalium* (MG) infection is a public health concern due to antimicrobial resistance (AMR). Data are limited on repeat MG infection and AMR among US Air Force service members with HIV.

Methods. US Air Force service members seeking HIV care were screened for MG infection during the surveillance period (16 May 2016–16 March 2020). Baseline and repeat MG prevalence rates were estimated. An extended Cox proportional hazards regression model evaluated characteristics associated with repeat MG infection. MG-positive rectal samples were tested for macrolide or fluoroquinolone resistance.

Results. Among 299 male patients from a total of 308 patients followed during the surveillance period, baseline prevalence of MG infection was 19.7% (n = 59); among the 101 patients who screened positive for MG at any time during the surveillance period, repeat MG was 35% (n = 36). Characteristics independently associated with increased risk of repeat infection were sexually transmitted infection history vs none (adjusted hazard ratio [aHR], 2.33; 95% CI, 1.26–4.31), a sexually transmitted infection coinfection vs no positive test result in the medical records (aHR, 5.13; 95% CI, 2.78–9.49), and a new HIV diagnosis (<1 vs ≥1 year; aHR, 2.63; 95% CI, 1.45–3.73). AMR in MG-positive rectal specimens was 88% (43/49) indicating macrolide resistance, 18% (10/56) quinolone resistance, and 18% (10/56) both.

Conclusions. Macrolide and fluoroquinolone resistance mutations were common. Testing for co-occurring MG infection and AMR mutations may be warranted in guiding treatment for sexually transmitted infections such as chlamydia or gonorrhea detected at HIV diagnosis.

Keywords. antimicrobial resistance; human immunodeficiency virus; repeat infection; epidemiology; *Mycoplasma genitalium*.

BACKGROUND

Mycoplasma genitalium (MG) is a sexually transmitted infection (STI) that has been linked to nongonococcal urethritis, cervicitis, pelvic inflammatory disease, infertility, and preterm birth [1–4]. The prevalence of MG infection varies by country and by the population studied. In the United States and Britain, the prevalence among men and women in the general population has been low, ranging from 1.0% to 1.7%, whereas in less developed countries the summary prevalence was 3.9% [5–8]. In clinic-based studies, the prevalence ranged from 28.7% among men with urethritis in the United States, 20.8% among asymptomatic women with bacterial vaginosis enrolled from 10 US cities, 16.6% among attendees of sexual health clinics in 6 US cities, and 16.7% among users of HIV preexposure prophylaxis in high-income countries [9–12]. HIV infection can increase susceptibility to STIs and potentially complicate treatment due to compromised immune function. As compared with uninfected men, men with HIV bear a reportedly 6-fold higher overall burden of MG, although this association was not observed for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* [13].

The emergence of macrolide- and fluoroquinolone-resistant MG strains in the United States posed a challenge to the established treatment regimen utilizing azithromycin (first line) and

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moxifloxacin (second line). Consequently, the national treatment guidelines were revised in 2021 [14, 15]. Macrolide resistance leading to treatment failure has been associated with 5 single-nucleotide mutations within the V region of the *23S* rRNA gene at positions 2058 and 2059 (*Escherichia coli* numbering) [16]. Resistance to fluoroquinolones has been linked to amino acid substitutions in the quinolone resistance-determining region of the *gyrA* and *parC* genes. A decline in moxifloxacin efficacy from 100% to 89% has been reported due to resistance associated with mutations found in *gyrA/B* and *parC/E* genes [17].

Historically, STI has posed a significant infectious disease threat to US military personnel, potentially amplifying the problem of MG infection [18]. While some studies have investigated the prevalence of MG among US military personnel with and without HIV, reports focusing on antimicrobial resistance, repeat infection, and risk factors are absent [19–22]. Treatment failure due to antimicrobial-resistant MG and subsequent reinfection, especially in an immunocompromised population, could present a problem in patient management and disease control that deserves attention. This study sought to describe the prevalence of repeat MG infection among US Air Force service members with HIV, identify associated factors, and evaluate the prevalence of macrolide- and quinolone-associated resistance exhibited by MG isolates from follow-up of those who sought specialty care for HIV while in service.

METHODS

Patient Population

This study analyzed prospectively collected data from biannual/annual mandatory medical assessments of US Air Force active duty personnel with HIV who traveled for specialty care at a infectious disease clinic in San Antonio, Texas (May 2016–March 2020). During these evaluations, for patients undergoing routine testing for *C trachomatis* and *N gonorrhoeae* and urinalysis, a nurse practitioner collected a urine specimen aliquot and additional extragenital swabs (pharyngeal and rectal) for MG and *Trichomonas vaginalis* testing and completed a standardized risk assessment for demographic, STI, and sexual risk history. Collection of specimens and risk information has been previously detailed [21]. The extra pharyngeal and rectal swabs and urine specimen aliquot were placed in transport tubes (Hologic) shipped for nucleic acid amplification testing (Aptima MG analyte-specific reagents [research use only] and Aptima *T vaginalis*; Hologic) to the HIV Diagnostics and Reference Laboratory (Walter Reed Institute of Research).

Patient Consent Statement

The Division of Human Subject Protection of the Walter Reed Army Institute of Research and the Public Health Review Board of the Defense Centers of Public Health–Aberdeen (formerly, Army Public Health Center) determined that the project was a public health activity not requiring informed consent.

Antimicrobial Resistance Sequencing

Sample aliquots remaining from urine and extragenital specimens, which were shipped in transport tubes and tested for MG and *T vaginalis*, were frozen at −80 °C until 10 December 2020, when all available aliquots were transferred to the Viral Sequencing Core laboratory (Walter Reed Institute of Research) for sequencing. The transport tube contained buffer that lysed target cells and released target ribosomal RNA, thus preventing degradation during storage. Due to the higher proportion of positive rectal samples, these were prioritized for sequencing. While methods for urine and pharyngeal samples were established, resource limitations necessitated focusing on rectal samples for this study. Consequently, corresponding methods and results presented here pertain solely to rectal samples.

The main gene targets for macrolide (*23S*) and fluoroquinolone (*parC* and *gyrA*) were selected for investigation, with other targets around the *L4* and *L22* proteins of the ribosomal complex known to confer macrolide resistance in other Mollucites, such as *Mycoplasma pneumoniae* [16, 23, 24].

For available volume from all rectal swab samples, DNA isolation was performed with the NucleoSpin DNA Stool Mini Kit for DNA (Macherey-Nagel Inc) with an additional wash with ST4 buffer as recommended by the supplier. Nucleic acids were amplified via a nested polymerase chain reaction (PCR) strategy with Titanium Taq (Takara Bio USA Inc) for each region. The amplification primers were as shown in Table 1. The primer sequences were obtained from the literature for regions *23S*, *L4* and *L22*, *parC*, and *gyr* or designed for MG strain 75956 (GenBank: CP145105) as a template for alternative amplification primer sites proximal to target regions [16, 23, 25]. Candidate primers were further subjected to a BLASTn search to maximize specificity to MG by excluding primers that had any similarity to other pathogens or human genes matched in the BLASTn search.

PCR cycling conditions for the various amplifications included standard denaturation of 95 °C for 30 seconds and 68 °C for a 2-minute extension for 30 cycles. The annealing conditions varied per each region as follows for 30 seconds: *23S* region, 60 °C for first round, 63 °C for second round; *parC*, 55 °C for first round, 58 °C for second round; *L4*, 63 °C for first round, 55 °C for second round. *Gyrase* and *L22* annealing conditions were 60 °C for both rounds of PCR. Samples that failed to amplify were then subjected to a postextraction genome amplification with Genomiphi (Cytiva) prior to PCR. PCR cycling conditions for the various amplifications include standard denaturation of 95 °C for 30 seconds and 68 °C for a 2-minute extension for 30 cycles. The annealing conditions varied

Table 1. Primers Used for Sequencing of *Mycoplasma genitalium* in the Study

Primer	Sequence (5′ → 3′)	Citation
MG 23S-1stF	GAAGGTTAAAGAAGGAGGTTAGCAAT	In house
MG 23S-1stR	TATAACGGCTCTTACTTTCCTTTAACAG	In house
MG 23S-2682r	CGGTCCTCTCGTACTAGAAGCAAAG	Jensen 2008 [16]
MG 23S-1986f	GTGTAACCATCTCTTGACTGTCTCGG	Jensen 2008 [16]
MG_L4_1st F	CCCAAAAATTGAAGAAGAGAAAACT	In house
MG_L4_1st R	ACCCCATAGATAATTTCAAACGCTAG	In house
MG L4-4f	AAGTAATGGCTAAACTTAAAGTAATCC	Jensen 2008 [16]
MG L4+1r	TTTAAGAGTATGTTGGTTACATCCATAG	Jensen 2008 [16]
MG L22+55f_1st F	CCTTCAGTGTGCATAACGGTA	In house
MG L22+55f_1st R	AGGTTACGGATCTTCTCATCTTG	In house
MG L22+55f	ATGGTAGGTCATAAGTTGGGTGAGTTT	Jensen 2008 [16]
MG L22+55r	AGTTCTTATTAATGCCAAACCTTAAGCC	Jensen 2008 [16]
MG-PARC-A_1st	GGCAATTGAAGAAGTCTTTGCAG	In house
MG-PARC-B_1st	GCTATCCCACTCGCACCATT	In house
MG-PARC-A	TGGGCTTAAAACCCACCACT	Shimada 2010 [23]
MG-PARC-B	ATGCGTTACACAGAAACCCG	Shimada 2010 [23]
ParC_2nd RD_FWD	GCTTTACCTGATCTAAGAGATGGG	In house
ParC_2nd RD_R	CATACAAGCTTAAGCGGGTTTC	In house
MG gyrBA_F_1st	GGTTAGAACATTGTTAAAAGTTACTGTTGA	In house
MG gyrBA_R_1st	AGCTGCTGGTAGAACAGTTGGTT	In house
MG-GYRA-A	CGTCGTGTTCTTTATGGTGC	Shimada 2010 [23]
MG-GYRA-B	ATAACGYYGTGCAGCAGGTC	Shimada 2010 [23]
gyrktF	GCTCGTGCTTTACCTGATGCTAGA	Tagg 2013 [25]
gyrktR	AACGTTGTGCAGCAGGTCT	Tagg 2013 [25]

per each region as follows for 30 seconds: *23S* region, 60 °C for first round, 63 °C for second round; *parC*, 55 °C for first round, 58 °C for second round; *L4*, 63 °C for first round, 55 °C for second round. *Gyrase* and *L22* annealing conditions were 60 °C for both rounds of PCR.

Sanger sequencing was performed on the amplicon of the targeted regions directly on both strands with Big Dye terminator reaction kits and an ABI_3730xl genetic analyzer (Thermo Fisher). DNA sequences were assembled with Sequencher software version 3.1 (Genecodes Inc). The obtained sequences were aligned to reference sequence for MG strain M2321 (GenBank: CP003770.1).

For macrolide resistance, nucleic acid changes were evaluated at position 2058 or 2059 for the *23S* gene [16]. For fluoroquinolone resistance, mutations at amino acid positions AA 84, 87, or 97 Y of the *parC* gene (topoisomerase) were examined [23].

Statistical Analysis

The proportion of patients with baseline prevalence, incident, repeat, and macrolide/fluoroquinolone-resistant MG was described during follow-up in the surveillance period. Baseline prevalence was assessed at the first visit in the surveillance period. Patients who initially tested negative (baseline) or during follow-up visits but subsequently tested positive were considered to have acquired an incident infection. Repeat infection was classified as a positive test result followed by another positive test result for the same patient at 1 or more subsequent visits within the surveillance period. To be considered a repeat infection, the positive test result had to occur >30 days after treatment for the previous infection. Only 1 positive test result per patient per visit was counted as an infection, irrespective of the collection site. During the surveillance period, patients were evaluated for MG at every biannual/annual visit and, if diagnosed, received appropriate treatment.

Descriptive statistical analyses were used to explore differences in demographic, behavioral, and laboratory characteristics at entry for patients with single vs repeat infection via the chi-square, Fisher exact, or Kruskal-Wallis test. All 95% CIs were 2-tailed, and statistical significance was assessed at a $P < .05$. The Andersen-Gill extension of the Cox proportional hazards regression model was fit to the data to evaluate baseline and time-varying patient characteristics associated with repeat MG. While the Cox model analyzes the hazard of an event occurring, the counting process approach introduced by Andersen and Gill can handle recurrent or multiple events within a subject. This allows for the inclusion of those who experience multiple or repeated events over time (eg, multiple MG infections in the same patient) [26]. We investigated the relationship between individual characteristics and repeat infection via univariate Andersen and Gill models and estimated unadjusted hazard ratios (HRs). Characteristics significantly associated with repeat infection ($P < .05$) were then incorporated into a multivariate model to account for the potential

confounding effects of other factors and to estimate adjusted HRs. All patient characteristics were self-reported except for STI coinfection status, which was extracted from electronic medical records. All data management and analyses were performed with SAS version 9.2 (SAS Institute).

RESULTS

Over a nearly 4-year period (16 May 2016–16 March 2020), 2417 specimens were collected and tested from 308 patients with HIV who were receiving infectious disease specialty care. Analysis in this report was restricted to male patients (n = 299, 97%), since only 9 patients were female. Among females who were excluded, 1 patient's rectal MG specimen was identified with macrolide resistance (*23S* A2059G, *L22* bp81G>R). *T vaginalis* infections were excluded from analysis since only 8 infections were identified among men in the surveillance period.

Male patients were followed a median 1.8 years (IQR, 0.5–2.8) and tested a median 9 times (IQR, 6–12), accounting for each specimen type. Most patients (n = 198, 66%) had no diagnosis of MG infection in the surveillance period, whereas almost a quarter (n = 65, 22%) were diagnosed with infection only once (Figure 1). The follow-up time for patients with 1 infection (median, 2.0 years; IQR, 0.6–2.7) was similar to that of patients with multiple infections (median, 2.1 years; IQR, 1.1–3.0). The difference was not statistically significant ($P > .05$). Baseline prevalence of MG was 19.7% (59/299; 95% CI, 15.5%–24.5%) and was highest for rectal specimens (13.9%, 41/294), followed by urine (6.7%, 18/268) and pharyngeal (1.7%, 5/296) specimens. Out of 240 patients initially testing negative for MG, 193 had a follow-up visit, during which 42 were diagnosed with MG infection, resulting in an incidence rate of 10.8 per 100 person-years (95% CI, 7.8–14.7). Among an overall 101 (33.8%) patients diagnosed with MG infection at any time during the surveillance period, 36 (35%) were detected with MG repeatedly up to 5 times, for a repeat diagnosis rate of 47 events per 100 person-years (95% CI, 33.7–64.8). Repeat infections were identified a median 11.9 months apart (IQR, 6.9–12.6). Figure 2 depicts the pattern of repeat infection observed during the 46-month surveillance time frame.

Most male patients were 19 to 39 years of age (90%), enlisted (84%), and single (57%; Table 2). A higher proportion (25%) of patients with repeat infections reported prior STI symptoms at entry as compared with those with a single infection (12%; Table 2). In univariate analysis, 3 characteristics were significantly associated ($P < .05$) with an increased risk of repeat infection (Table 3): self-report of prior STI (vs none; HR, 4.00; 95% CI, 1.87–8.56), having an STI coinfection (vs no documented positive test result; HR, 5.58; 95% CI, 2.41–12.9), and a recent diagnosis of HIV (<1 vs ≥1 year; HR, 3.56; 95% CI, 1.72–7.37). Although the risk decreased after adjustment in multivariate analysis, the 3 characteristics remained independently associated with an increased risk of repeat infection: STI history vs none (adjusted HR [aHR], 2.33; 95% CI 1.45–3.73), presence of STI coinfection vs no documented positive

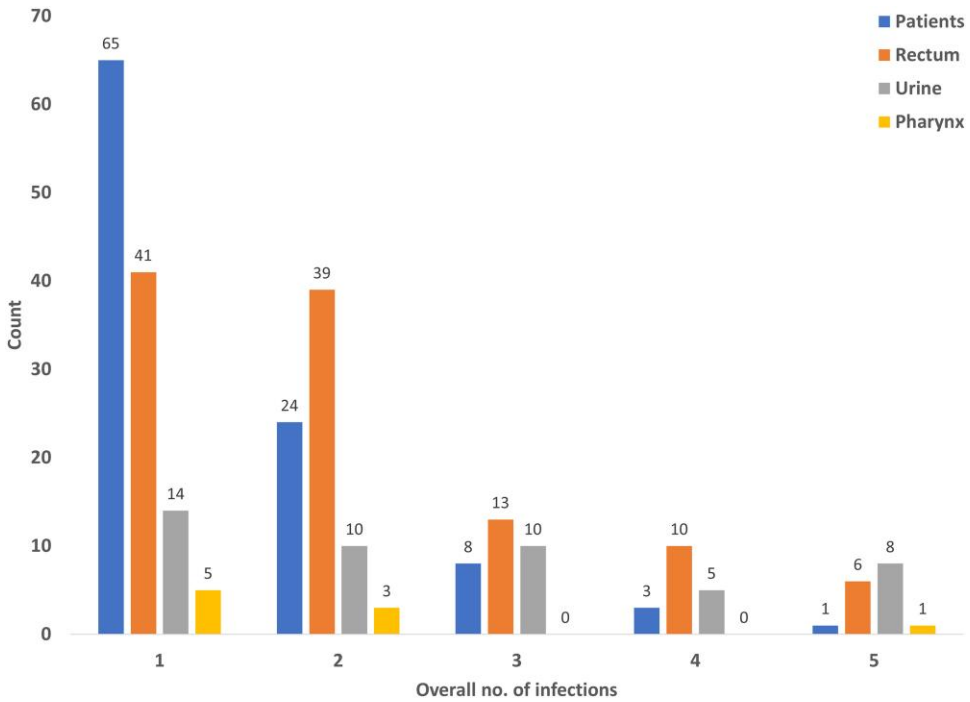

Figure 1. Distribution of *Mycoplasma genitalium* infections, overall and by specimen source, among airmen with HIV, 16 May 2016 through 16 March 2020.

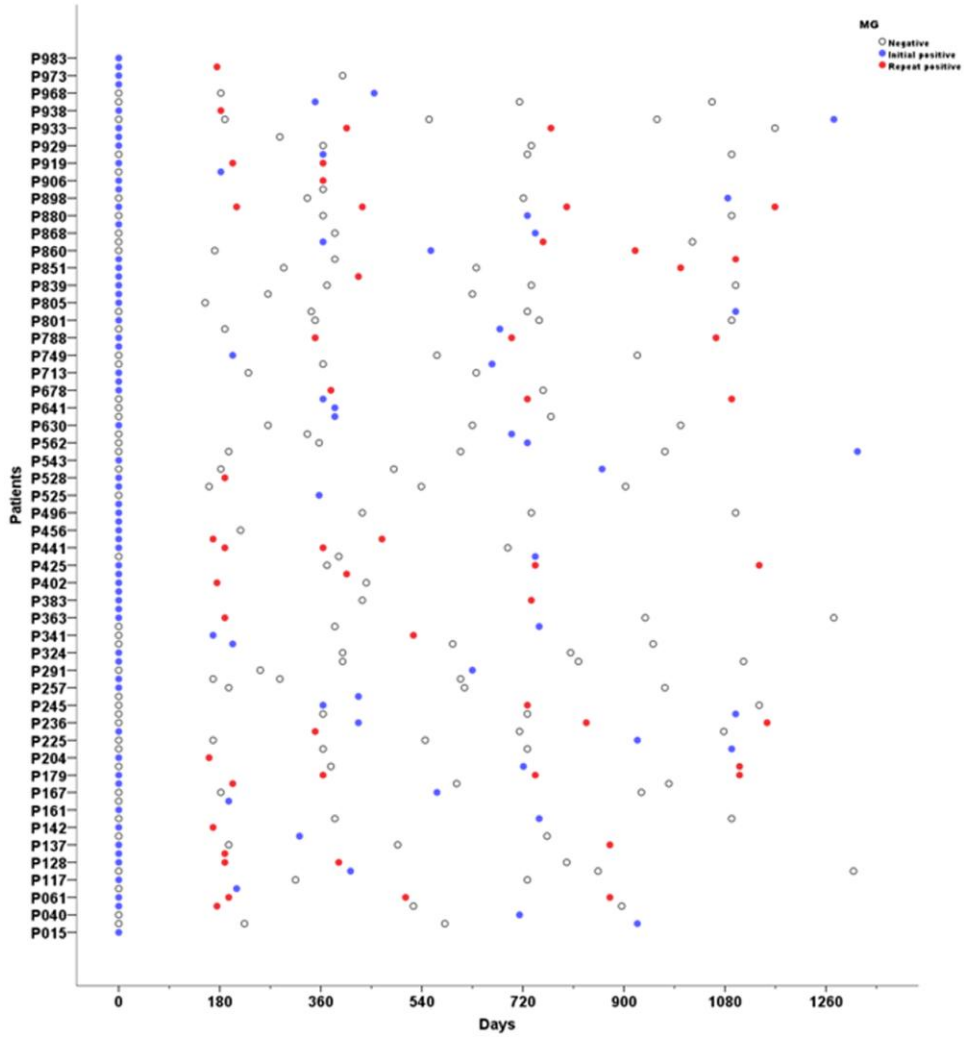

Figure 2. *Mycoplasma genitalium* (MG) infections among airmen with HIV, 16 May 2016 through 16 March 2020. This chart visualizes the progression of MG test results over time for each patient who tested positive during the study period.

test result (aHR, 5.13; 95% CI, 2.78–9.49), and <1 year since HIV diagnosis vs ≥1 year (aHR, 2.33; 95% CI, 1.45–3.73).

Out of 109 rectal swab specimens from 77 patients with rectal infections, sequencing for antimicrobial resistance failed on 16 swabs from 13 patients. Among the sequenced specimens (56 from 43 patients), 88% (43/49) indicated markers for macrolide resistance, 18% (10/56) for quinolone resistance, and 18% (10/56) for resistance to both. The most common macrolide-associated mutations that were detected were A2058G (n = 21) and A2059G (n = 18; Table 4). Other uncommon mutations were A2058R, A2058T, A2059C (n = 2), and A2059R (n = 1). Quinolone-associated mutations detected for *parC* were AA84 S to I (n = 6) and S to R (n = 1) and AA87 D to N (n = 3). Among 43 patients whose samples were sequenced, more than half (58%) had repeat infections. A higher proportion of samples with resistance were from repeat infections, especially for macrolides (repeat vs single infection, 79% vs 67%). Patient characteristics did not differ significantly by age, grade, marital status, and education for those whose samples were tested for resistance as compared with patients who were MG positive but not tested ($P \geq .05$).

DISCUSSION

The prevalence of MG infection was 18.6% among the first 102 US Air Force service members with HIV in a prior analysis of this enhanced surveillance project. In this follow-up analysis, which included an additional 197 male service members with HIV, a similar baseline prevalence of MG was identified (19.7%). The incidence rate was 10.8 cases per 100 person-years among service members who tested negative at baseline. Macrolide or quinolone resistance was commonly identified

Table 2. Sociodemographic, Clinical, and Sexual Behavior Characteristics of 299 Male Air Force Service Members With HIV, Overall and by *Mycoplasma genitalium* Positivity, 2016–2020

	Overall (n = 299)		Single Infection (n = 65)		Repeat Infection (n = 36)		
Characteristic at Entry	No.	%	No.	%	No.	%	*P* Value
Age							
Median, IQR	30	25–35	29	26–35	27	24–33	
19–29	144	48	33	51	20	56	.5196
30–39	126	42	29	45	13	36	
40–49	25	8	3	5	2	6	
50–59	3	1	…	…	1	3	
≥60	1	0	…	…	…	…	
Grade							.4791
E1–E4	93	31	19	29	14	39	
E5–E9	157	53	36	55	13	36	
Officer	26	9	5	8	7	19	
Not indicated	23	8	5	8	2	6	
Education level attained							.4099
Some high school/high school	35	12	9	14	5	14	
Some college/technical school	108	36	21	32	11	31	
College degree	58	19	13	20	10	28	
Undergraduate degree	38	13	10	15	4	11	
Graduate degree	31	10	7	11	5	14	
Not indicated	29	9	5	8	1	3	
Marital status							.0354
Single	169	57	46	71	23	64	
Live-in partner	6	2	1	2	2	6	
Married	60	20	8	12	5	14	
Separated	13	4	1	2	2	6	
Divorced	20	7	4	6	3	8	
Not indicated	31	10	5	8	1	3	
Race							.4792
Black	136	46	30	46	17	47	
White	95	32	21	32	14	39	
Other	68	22	14	22	5	14	
Prior symptoms							.0103
Yes	46	15	8	12	9	25	
No	173	58	28	43	19	53	
Not indicated	80	27	29	45	8	22	
Symptoms at visit							.0002
Yes	5	2	3	5	…	…	
No	58	19	25	38	7	19	
Not indicated	236	79	37	57	29	81	
Prior STI							.507
Yes	39	13	8	12	6	17	
No	231	78	51	78	29	81	
Not indicated/not applicable	29	10	6	9	1	3	
STI coinfection							<.0001
No positive result in medical records	280	94	52	80	30	83	
Yes	19	6	13	20	6	17	
Chlamydia trachomatis	9	47	7	54	2	33	
Neisseria gonorrhoea	6	32	3	23	3	50	
Syphilis	3	16	2	15	1	17	
Chlamydia trachomatis/syphilis	1	5	1	8	0	0	
Type of sexual contact, last visit							.4589
Men only	153	51	36	55	23	64	
Women only	37	12	8	12	1	3	
Both men and women	4	1	1	2	…	…	
No sexual contact	34	11	8	12	1	3	

Table 2. Continued

Characteristic at Entry	Overall (n = 299)		Single Infection (n = 65)		Repeat Infection (n = 36)		*P* Value
	No.	%	No.	%	No.	%	
Not indicated	71	23	12	18	11	31	
Sexual contact, past year							.0207
Men only	184	62	45	69	30	83	
Women only	50	17	9	14	3	8	
Both men and women	14	5	2	3	1	3	
No sexual contact	22	7	2	3	1	3	
Not indicated	29	9	7	11	1	3	
Positive MG result at entry							<.0001
Yes	100	34	64	98	36	100	
No/not applicable	199	67	1	2	…	…	
Years from HIV diagnosis							.0182
Median, IQR	1.8	0.13–5.57	1.11	0.11–5.49	0.4	0.09–2.65	
<1	129	43	32	49	21	58	
≥1	163	55	33	51	15	42	
Missing	7	2	…	…	…	…	
Years in service							.0593
Median, IQR	8	5–14	9	5–14	5.5	3–11.5	
<9	144	48	31	48	22	61	
≥9	135	45	32	49	14	39	
Missing	20	7	2	3	…	…	

Abbreviations: MG, *Mycoplasma genitalium*; STI, sexually transmitted infection.

(88% and 18%, respectively) among almost half the patients with MG-positive specimens that were sequenced.

Although studies of MG infection among men with HIV are limited, estimates in this analysis are comparable to those from other developed countries. The prevalence and incidence of MG were 20.3% and 19.5 cases per 100 person-years, respectively, among 148 men who have sex with men (MSM) with primary HIV screened in a prospective study in Zurich, Switzerland, and 21% among 48 MSM with HIV who sought care at STI or HIV clinics for proctitis in Melbourne, Australia; incidence was not assessed in the latter study [27, 28].

Recent diagnosis of HIV was associated with repeat MG infection in this analysis, suggesting a link to a weakened immune system. In 1 review, MG prevalence was highest among patients with AIDS (43.7%), with a markedly lower burden among asymptomatic persons with HIV (27.3%) and uninfected individuals (11.3%) [29]. In other studies, MG infection has been bidirectionally associated with HIV, found to increase shedding of HIV DNA, and thought to facilitate HIV transmission in women by compromising the epithelial lining of the urogenital tract and to increase susceptibility of peripheral blood mononuclear cells to HIV [29–32]. The role of MG infection in men is unclear, although it is postulated that inflammation of the rectal mucosa due to MG infection may increase the presence of HIV-susceptible cells in men without HIV and facilitate HIV acquisition [27].

In this analysis, self-reported STI history and STI coinfection were associated with repeat MG infection. Coinfection with other STIs or infection with a single STI increases the risk of a subsequent STI and the risk of HIV [30, 33, 34]. Furthermore, these and factors such as recent macrolide use and male-to-male sexual contact have been associated with macrolide/fluoroquinolone-associated resistance mutations [34–37]. Moreover, MSM with recurrent STIs had a higher prevalence of MG infections and antibiotic resistance-associated mutations. It has been suggested that the higher prevalence of mutations may be due to an increased chance of antimicrobial selection pressure from treatment of co-occurring STIs, such as chlamydia or gonorrhea, in populations at high risk of STI, such as MSM [34]. Single-dose azithromycin was part of the recommended treatment for chlamydia or gonorrhea infection before a change in guidelines in 2021 to treating nonpregnant individuals with doxycycline for 7 days [15, 38].

Resistance to macrolides or fluoroquinolones in this analysis were 88% and 18%, respectively, among 43% of patients with MG whose samples were sequenced. These estimates are comparable to the reported prevalence (68.4%–90.5%) among high-risk populations, such as clinic attendees seeking care for nongonococcal urethritis, cervicitis, or proctitis and MSM seeking STI testing [34, 39–41]. Globally, macrolide and fluoroquinolone resistance in MG increased at least 5-fold in a decade. The overall prevalence of macrolide resistance across 21 countries increased from 2010 (10%) to 2016–2017 (51.4%), whereas the prevalence of resistance associated with fluoroquinolone (7.7%) did not vary markedly over time [42].

Table 3. Unadjusted and Adjusted Hazard Ratios for Male Air Force Service Members With HIV With Specimens Repeatedly Positive for *Mycoplasma genitalium*, 2016–2020

	Unadjusted			Adjusted		
Characteristic: Comparison	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age: 19–29 vs ≥30 y						
At entry	1.36	.65–2.86	.41			
At sample collection	1.24	.60–2.57	.56			
Race: White vs Black/other	1.98	1.00–3.91	.05			
Pay grade vs E1–E4						
E5–E9	0.52	.23–1.17	.11			
Officer	1.23	.36–4.19	.75			
Highest education attained vs high school or less						
Some college/technical school	0.35	.11–1.07	.07			
College degree	0.48	.15–1.56	.22			
Undergraduate degree	0.37	.08–1.67	.20			
Graduate degree	0.71	.20–2.52	.59			
Marital status: single vs ever married	1.16	.51–2.65	.73			
Sexually transmitted infection						
Symptoms since last visit: yes vs no	1.86	.75–4.64	.18			
History: yes vs no	4.00	1.87–8.56	.0004	2.33	1.26–4.31	.007
Coinfection: yes vs no positive result in medical records	5.58	2.41–12.9	<.0001	5.13	2.78–9.49	<.0001
Since the last visit						
Type of sexual partner: men vs women/men and women/no sex	1.46	.61–3.53	.40			
No. of sexual partners: ≥1 vs none						
Male	1.87	.24–14.87	.55			
New male	1.47	.67–3.22	.34			
Female	0.16	.02–1.00	.05			
In the past year						
Type of sexual partner: men vs women/men and women/no sex	3.68	.75–18.03	.11			
No. of new sexual partners: ≥1 vs none						
Male	1.18	.55–2.52	.68			
Female	0.22	.04–1.33	.10			
Years since HIV diagnosis at study entry: <1 vs ≥1	3.56	1.72–7.37	.0006	2.33	1.45–3.73	.0004
Years in service: <9 vs ≥9						
At study entry	1.69	.80–3.56	.17			
At sample collection	1.59	.78–3.25	.20			
Sensitivity: resistant vs sensitive						
Macrolide	1.12	.30–4.17	.87			
Quinolone	1.25	.42–3.70	.69			

Comparatively, the prevalence of macrolide resistance in the Americas increased even more from 0% to 67.3% in the same period. Similarly, the prevalence of fluoroquinolone resistance in the Americas region of the World Health Organization was higher at 10.1% for the 2013–2017 period. These data emphasize the alarming rise of macrolide and fluoroquinolone resistance in MG, particularly among patients with MG, mirroring trends observed in high-risk populations.

A limitation of this analysis was that samples from less than half of patients with detected MG were sequenced due to resource limitations or sample unavailability. However, the preliminary finding—specifically, a higher proportion of repeat infection samples having resistance in combination with the association of STI history and coinfection with repeat MG—suggests that treatment of prior or concurrent bacterial STIs may play a role in development of resistance in MG strains. Alternatively, resistant MG strains may have been sexually transmitted from an infected sexual partner.

In this initial study evaluating resistant strains of MG among US military service members with HIV, prevalence of macrolide and/or fluoroquinolone resistance was commonly found in rectal specimens, especially for repeat positive samples. New diagnosis of HIV, STI history, and/or co-occurrence was associated with the detection of repeat MG. Further investigation is needed to fully understand the complex interplay of factors contributing to repeat MG diagnosis in men with HIV. This includes exploring the mechanisms by which STI coinfection may influence resistance development and the transmission dynamics of resistant MG strains. Determining the utility of testing for co-occurring MG and its resistance profile

Table 4. Rectal Samples Positive for *Mycoplasma genitalium* and Sequenced for Macrolide and/or Fluoroquinolone Resistance Mutations Among Air Force Service Members With HIV and Single or Repeat Infection, 2016–2020

	Single Infection (n = 18)		Repeat Infection (n = 38)	
	No.	%	No.	%
Main macrolide resistance, rRNA *23S*				
Not tested	2	11	6	16
Sensitive	4	22	2	5
Resistant	12	67	30	79
A2058G	5		15	
A2058R	1		0	
A2058T	1		0	
A2059G	5		13	
A2059C	0		2	
A2059R	1		0	
Quinolone resistance, *parC*				
Not tested	1	6	0	0
Sensitive	15	83	30	79
Resistant	2	11	8	21
S84I	1		5	
S84R	1		0	
D87N	0		3	
Macrolide and quinolone resistance				
23S	10	56	22	58
23S and *parC*	2	11	8	21
Other macrolide targets[a]				
L4 amino acid change				
Not tested	9		23	
N172S	2		1	
A172T	1		0	
A114V	0		1	
T204A	0		1	
L210F	1		0	
K66E	0		1	
H69R	1		5	
None	4		6	
L22 amino acid change				
Not tested	10		30	
S100F	0		1	
None	8		7	

[a]Additional macrolide resistance genes center on the *L4* and *L22* proteins of the ribosomal complex. These mutations have been associated with resistance in other species of bacteria, though not directly with *M genitalium*. *L4* protein has shown low-level macrolide resistance in Mollicutes such as *M pneumoniae* [24]. All other amino acid changes in *L4* and *L22* are noted but have not to date shown resistance in the literature.

alongside common STIs, such as chlamydia or gonorrhea, at HIV diagnosis may be valuable for guiding more effective treatment strategies.

Notes

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Data availability. Sequences described here have been submitted to GenBank, a publicly available database of genetic sequences, under accession numbers PP910697–PP910751 for *parC* and PP937693–PP937741 for *23S* rRNA.

Disclaimer. Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting true views of the Department of the Army or the Department of Defense, Ministry of Health (Jordan), or the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70-25.

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References

1. Lis R, Rowhani-Rahbar A, Manhart LE. *Mycoplasma genitalium* infection and female reproductive tract disease: a meta-analysis. Clin Infect Dis **2015**; 61:418–26.
2. Taylor-Robinson D, Jensen JS. *Mycoplasma genitalium*: from chrysalis to multicolored butterfly. Clin Microbiol Rev **2011**; 24:498–514.
3. Horner PJ, Martin DH. *Mycoplasma genitalium* infection in men. J Infect Dis **2017**; 216:S396–405.
4. Jensen JS, Cusini M, Gomberg M, Moi H, Wilson J, Unemo M. 2021 European guideline on the management of *Mycoplasma genitalium* infections. J Eur Acad Dermatol Venereol **2022**; 36:641–50.
5. Sonnenberg P, Ison CA, Clifton S, et al. Epidemiology of *Mycoplasma genitalium* in British men and women aged 16–44 years: evidence from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3). Int J Epidemiol **2015**; 44: 1982–94.
6. Torrone EA, Kruszon-Moran D, Philips C, et al. Prevalence of urogenital *Mycoplasma genitalium* infection, United States, 2017 to 2018. Sex Transm Dis **2021**; 48:e160–2.
7. Manhart LE, Gaydos CA, Taylor SN, et al. Characteristics of *Mycoplasma genitalium* urogenital infections in a diverse patient sample from the United States: results from the Aptima *Mycoplasma genitalium* Evaluation Study (AMES). J Clin Microbiol **2020**; 58:e00165-20.
8. Baumann L, Cina M, Egli-Gany D, et al. Prevalence of *Mycoplasma genitalium* in different population groups: systematic review and meta-analysis. Sex Transm Infect **2018**; 94:255–62.
9. Sokoll PR, Migliavaca CB, Siebert U, Schmid D, Arvandi M. Prevalence of *Mycoplasma genitalium* infection among HIV PrEP users: a systematic review and meta-analysis. Sex Transm Infect **2023**; 99:351–9.
10. Bachmann LH, Kirkcaldy RD, Geisler WM, et al. Prevalence of *Mycoplasma genitalium* infection, antimicrobial resistance mutations, and symptom resolution following treatment of urethritis. Clin Infect Dis **2020**; 71:e624–32.
11. Seña AC, Lee JY, Schwebke J, et al. A silent epidemic: the prevalence, incidence and persistence of *Mycoplasma genitalium* among young, asymptomatic high-risk women in the United States. Clin Infect Dis **2018**; 67:73–9.
12. Manhart LE, Leipertz G, Soge OO, et al. *Mycoplasma genitalium* in the US (MyGeniUS): surveillance data from sexual health clinics in 4 US regions. Clin Infect Dis **2023**; 77:1449–59.
13. Soni S, Alexander S, Verlander N, et al. The prevalence of urethral and rectal *Mycoplasma genitalium* and its associations in men who have sex with men attending a genitourinary medicine clinic. Sex Transm Infect **2010**; 86:21–4.
14. Workowski KA, Bolan GA; Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep **2015**; 64: 1–137.
15. Centers for Disease Control and Prevention. Sexually transmitted infections treatment guidelines, 2021: *Mycoplasma genitalium*. Available at: https://www.cdc.gov/std/treatment-guidelines/mycoplasmagenitalium.htm. Accessed 19 December 2023.
16. 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: 1546–53.
17. Li Y, Le WJ, Li S, Cao YP, Su XH. Meta-analysis of the efficacy of moxifloxacin in treating *Mycoplasma genitalium* infection. Int J STD AIDS **2017**; 28:1106–14.
18. Korzeniewski K, Juszczak D, Paul P. Sexually transmitted infections in the military environment. Int Marit Health **2020**; 71:207–12.
19. Horseman TS, Crecelius EM, Miller MA, et al. Prevalence and epidemiology of *Mycoplasma genitalium* in a Pacific-region military population. Sex Transm Dis **2021**; 48:578–82.
20. Romo ML, Moreland SC, Yates AM, et al. Prevalence of urogenital *Mycoplasma genitalium* infection at two US army medical facilities. Sex Transm Dis **2024**; 51:367–73.
21. Hakre S, Casimier RO, Danboise BA, et al. Enhanced sexually transmitted infection screening for *Mycoplasma genitalium* in human immunodeficiency virus–infected US Air Force personnel. Clin Infect Dis **2017**; 65:1585–8.
22. Baseman JB, Dallo SF, Tully JG, Rose DL. Isolation and characterization of *Mycoplasma genitalium* strains from the human respiratory tract. J Clin Microbiol **1988**; 26:2266–9.
23. 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**; 36:255–8.
24. Pereyre S, Guyot C, Renaudin H, Charron A, Bébéar C, Bébéar CM. In vitro selection and characterization of resistance to macrolides and related antibiotics in *Mycoplasma pneumoniae*. Antimicrob Agents Chemother **2004**; 48:460–5.
25. Tagg KA, Jeoffreys NJ, Couldwell DL, Donald JA, Gilbert GL. Fluoroquinolone and macrolide resistance-associated mutations in *Mycoplasma genitalium*. J Clin Microbiol **2013**; 51:2245–9.
26. Andersen PK, Gill RD. Cox's regression model for counting processes: a large sample study. Ann Stat **1982**; 10:1100–20.
27. Bissessor M, Tabrizi SN, Bradshaw CS, et al. The contribution of *Mycoplasma genitalium* to the aetiology of sexually acquired infectious proctitis in men who have sex with men. Clin Microbiol Infect **2016**; 22:260–5.
28. Ring A, Balakrishna S, Imkamp F, et al. High rates of asymptomatic *Mycoplasma genitalium* infections with high proportion of genotypic resistance to first-line macrolide treatment among men who have sex with men enrolled in the Zurich Primary HIV Infection Study. Open Forum Infect Dis **2022**; 9:ofac217.
29. Napierala Mavedzenge S, Weiss HA. Association of *Mycoplasma genitalium* and HIV infection: a systematic review and meta-analysis. AIDS **2009**; 23:611–20.
30. Asare K, Osman F, Ngcapu S, et al. Burden of sexually transmitted infections from acute HIV infection among women in South Africa: evidence from a prospective cohort study. Ann Epidemiol **2022**; 74:132–9.
31. Manhart LE, Mostad SB, Baeten JM, Astete SG, Mandaliya K, Totten PA. High *Mycoplasma genitalium* organism burden is associated with shedding of HIV-1 DNA from the cervix. J Infect Dis **2008**; 197:733–6.
32. Das K, De la Garza G, Siwak EB, Scofield VL, Dhandayuthapani S. *Mycoplasma genitalium* promotes epithelial crossing and peripheral blood mononuclear cell infection by HIV-1. Int J Infect Dis **2014**; 23:31–8.
33. Peterman TA, Tian LH, Metcalf CA, et al. High incidence of new sexually transmitted infections in the year following a sexually transmitted infection: a case for rescreening. Ann Intern Med **2006**; 145:564–72.
34. De Baetselier I, Vuylsteke B, Reyniers T, et al. Worryingly high prevalence of resistance-associated mutations to macrolides and fluoroquinolones in *Mycoplasma genitalium* among men who have sex with men with recurrent sexually transmitted infections. Int J STD AIDS **2022**; 33:385–90.
35. Li Y, Su X, Le W, et al. *Mycoplasma genitalium* in symptomatic male urethritis: macrolide use is associated with increased resistance. Clin Infect Dis **2020**; 70: 805–10.
36. de Salazar A, Barrientos-Durán A, Espadafor B, Fuentes-López A, Chueca N, Garcia F. Macrolide and fluoroquinolone resistance of *Mycoplasma genitalium* in southern Spain, 2018–2019. Sex Transm Infect **2021**; 97:8–10.
37. DiMarco DE, Urban MA, McGowan JP, et al. Mycoplasma genitalium management in adults. Baltimore (MD): Johns Hopkins University, **2022**.
38. Centers for Disease Control and Prevention. Sexually transmitted disease surveillance 2015. Atlanta (GA): US Department of Health and Human Services, **2016**.
39. Read TRH, Fairley CK, Murray GL, et al. Outcomes of resistance-guided sequential treatment of *Mycoplasma genitalium* infections: a prospective evaluation. Clin Infect Dis **2019**; 68:554–60.
40. Couldwell DL, Jalocon D, Power M, Jeoffreys NJ, Chen SC, Lewis DA. *Mycoplasma genitalium*: high prevalence of resistance to macrolides and frequent anorectal infection in men who have sex with men in western Sydney. Sex Transm Infect **2018**; 94:406–10.
41. Spornraft-Ragaller P, Dumke R. Prevalence and antibiotic resistance of rectal Mollicutes in HIV-infected men who have sex with men at the university hospital of Dresden, Germany. Infection **2020**; 48:259–65.
42. Machalek DA, Tao Y, Shilling H, et al. Prevalence of mutations associated with resistance to macrolides and fluoroquinolones in *Mycoplasma genitalium*: a systematic review and meta-analysis. Lancet Infect Dis **2020**; 20:1302–14.