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High Prevalence and High Rate of Antibiotic Resistance of *Mycoplasma genitalium* Infections in Men Who Have Sex With Men: A Substudy of the ANRS IPERGAY Pre-exposure Prophylaxis Trial

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Background. *Mycoplasma genitalium* (MG) is an emerging pathogen among men who have sex with men (MSM) with raising rates of antibiotic resistance. This study assessed the prevalence and incidence of MG infection in MSM enrolled in the open-label phase of the ANRS IPERGAY trial with on-demand tenofovir disoproxil fumarate/emtricitabine for human immunodeficiency virus prevention and the impact of doxycycline post-exposure prophylaxis (PEP).

Methods. 210 subjects were tested at baseline and at 6 months by real-time PCR assays for MG detection in urine samples and oropharyngeal and anal swabs. Resistance to azithromycin (AZM), to fluoroquinolones (FQs), and to doxycycline was investigated in the French National Reference Center of Bacterial Sexually Transmitted Infections (STIs).

Results. The all-site prevalence of MG at baseline was 10.5% (6.3% in urine samples, 4.3% in anal swabs, 0.5% in throat swabs) and remained unchanged at 6 months whether or not PEP was used: 9.9% overall, 10.2% with PEP, 9.6% without. The overall rate of MG resistance (prevalent and incident cases) to AZM and FQs was 67.6% and 9.1%, respectively, with no difference between arms. An in vivo mutation of the MG 16S rRNA, which could be associated with tetracycline resistance, was observed in 12.5% of specimens tested.

Conclusions. The prevalence of MG infection among MSM on pre-exposure prophylaxis was high and its incidence was not decreased by doxycycline prophylaxis with a similar high rate of AZM and FQ resistance, raising challenging issues for the treatment of this STI and supporting current recommendations to avoid testing or treatment of asymptomatic MG infection.

Keywords. *Mycoplasma genitalium*; HIV; PrEP; post-exposure prophylaxis; doxycycline.

Mycoplasma genitalium (MG) is an emerging sexually transmitted bacterium responsible for nongonococcal urethritis (NGU) in symptomatic men worldwide. It is the second leading cause of NGU after *Chlamydia trachomatis*, with a reported prevalence in NGU cases of 15–20% [1–4]. Its prevalence even exceeded that of *C. trachomatis* in the United Kingdom in 2014 (16.7% vs 14.7% in NGU, respectively) [2]. A recent study in China reported a prevalence of 19.7% for MG in men with symptomatic urethritis [3]. There is also a high prevalence of MG in asymptomatic men who have sex with men (MSM), ranging from 2.7% in urine to 7% in the rectum [5]. The emergence of antimicrobial resistance, mostly to azithromycin (AZM) and fluoroquinolones (FQs), has been increasingly reported in MG. The emergence of such resistance can be explained by mutations of the genes encoding the macrolide target (23S ribosomal RNA [rRNA]) or the FQ target (topoisomerase IV) [4]. Tetracyclines (TETs) provide a low rate of clinical response for MG infections [6], but no acquired resistance to this antibiotic class has yet been described. In other human *Mycoplasma* species, resistance or reduced susceptibility to tetracyclines is caused by the *tet* (M) determinant or mutations of the 16S rRNA gene, respectively [7].

In 2016, the recommended treatment for uncomplicated MG infections in Europe was a 5-day course of AZM (500 mg on the

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first day, then 250 mg on each of the next 4 days) or a 7- to 10-day course of moxifloxacin (MXF; 400 mg/day) [6]. However, the frequency of resistance to macrolides and fluoroquinolones is increasing worldwide and depends on the population tested [8]. In men with NGU, rates of resistance to AZM in the causal MG strain have been reported to range from 4.6% to 88.9% (4.6–6.7% in the former Soviet Union [9], 38–82.4% in Europe [1, 2, 10–12], 43–75.9% in Australia [13–16], 48–58% in the United States and Canada [17, 18], 70% in Japan [19], and 88.9% in China [3]). Conversely, resistance to FQs in MG worldwide has been estimated at 4.6% to 89.5%: 4.5–18% in Europe [1, 2, 10–12], 12–19% in Australia [13–16], 11.1–20% in the United States and Canada [17, 18], 31–70% in Japan [19], and 89.5% in China [3]. Macrolide resistance is highest in the population of MSM, with recent reported rates of 75% in Dublin [20], 79.9% in Berlin [21], 70.7–79.4% in Sydney [22, 23], and 58–75% in France [24, 25]. Resistance to FQs has been less studied and has been estimated at 13% in Berlin [21], 13.6% in the Asia-Pacific region [26], and 33.3% in Dublin [20]. In light of the high incidence of resistance to macrolides, resistance-guided sequential treatment after a course of doxycycline was recently proposed by the British Association for Sexual Health and HIV (BASHH) in the United Kingdom and Australia [27–29].

Little is known about the prevalence and resistance of MG in MSM participants on pre-exposure prophylaxis (PrEP) for human immunodeficiency virus (HIV) prophylaxis, although rates of bacterial sexually transmitted infections (STIs) are high in these populations [30, 31]. Our team recently studied among MSM on PrEP the benefit of post-exposure prophylaxis (PEP) with doxycycline to reduce the incidence of bacterial STIs [32]. Significantly lower rates of *Chlamydia* infections and syphilis were reported in participants using doxycycline PEP, with relative risk reductions of 70% for *Chlamydia* and 73% for syphilis. No change was observed for gonococcal infections, possibly due to the high levels of resistance to tetracycline of these bacteria [32].

In this study, we wished to investigate the prevalence of MG infection among asymptomatic MSM enrolled in this doxycycline PEP study. The objectives of this study were (1) to assess the prevalence of MG infection at baseline and after 6 months and (2) to screen MG for mutations associated with resistance to macrolides, FQs, and TETs.

METHODS

During the open-label phase of the ANRS IPERGAY trial with on-demand PrEP for HIV with the combined pill of tenofovir disoproxil fumarate and emtricitabine (TDF/FTC), participants from 6 French hospitals (Tenon, Saint Louis, Nantes, Nice, Tourcoing, Lyon) were also enrolled in a prospective, randomized (1:1), open-label substudy of PEP with doxycycline [32]. Participants were instructed to take 200 mg of doxycycline within 24 hours after each sexual intercourse (with a limit of 600 mg/week) or no prophylaxis. During the study, all participants were tested every 2 months for the detection of *C. trachomatis* and *Neisseria gonorrhoeae* by polymerase chain reaction (PCR) in urine and oral and anal swabs. In addition, in 5 of the 6 hospitals, all patients were tested at baseline and at 6 months by real-time PCR assays for the detection of MG in urine samples, with the results given to clinicians to guide treatment. Almost all patients, except for one, were asymptomatic at baseline. By contrast, the 2 other sites of testing (oropharyngeal and anal sites) were retrospectively screened by batch testing of frozen samples, so the results were not available for the clinicians. The detection of MG was performed with the *C. trachomatis/Ureaplasma/M. genitalium* Real-TM kit (Sacace Biotechnologies) on urine and the Cobas TV/MG kit for use on the Cobas 6800 System (Roche Diagnostics) for anal and throat swab samples.

All samples testing positive for MG by real-time PCR were frozen at −80°C. Resistance to AZM, FQs, and TET was then investigated retrospectively at the National Reference Center for Bacterial STIs, Bordeaux University Hospital, in Bordeaux, France. We checked for 23S rRNA mutations associated with AZM resistance by using the ResistancePlus MG test (SpeeDx, Australia) directly on specimens and amplifying and sequencing the 23S rRNA [33]. We screened for MXF-associated mutations by amplifying and sequencing the quinolone resistance-determining region (QRDR) of the *ParC* gene [33]. We detected doxycycline-associated mutations by amplifying and sequencing the 16S rRNA gene of MG as previously described, with the MG16–439F and MG16–1301R primers [34].

We also assessed whether participant baseline characteristics (demographics and sexual behavior) could be associated with the detection of MG at baseline.

The significance of differences in percentages was assessed using chi-square or Fisher's exact tests, as appropriate. All tests and confidence intervals (CIs) were 2-tailed. Analyses were performed with SAS software (version 9.3; SAS Institute).

RESULTS

Mycoplasma genitalium Prevalence at Baseline and New Infections at 6 Months

From July 2015 to June 2016, 210 of the 232 (90.5%) participants randomized in the PEP study were tested for MG. Participants characteristics are described in Table 1 and were well balanced across arms. All participants underwent urinary tests or tests on swabs from oral or anal sites for MG at baseline, with 179 (85%) being tested at all 3 sites. At 6 months, 192 participants underwent urinary tests or tests on swabs from oral or anal sites for MG, with 145 (76%) being tested at all 3 sites. In total, 1102 samples from 210 participants (378 urine samples, 353 anal swabs, and 371 throat swabs) were screened for MG (Figure 1).

In total, 32 participants were found to have MG infections (22 at baseline and 19 at the 6-month visit). The prevalence of MG at baseline was 10.5% (95% CI, 6.7–15.4%] for all sites combined and was close to that of *Chlamydia* infection and

Table 1. Clinical Features of the Participants Included in the *Mycoplasma genitalium* Substudy

Characteristics at Baseline	PEP (n = 107)	No PEP (n = 103)	Total (N = 210)	*P*
Age, median [IQR], years	37.4 [32.5, 47.1]	38.8 [32.0, 44.5]	38.5 [32.0, 46.0]	.74
Postsecondary education, n/N (%)	99/105 (94)	90/101 (89)	189/206 (92)	.17
Male circumcision, n/N (%)	27/107 (25)	19/103 (18)	46/210 (22)	.24
Number of sexual partners in last 2 months, median [IQR]	10 [5, 15]	10 [3, 20]	10 [4, 20]	.87
Number of times had sexual intercourse in last 4 weeks, median [IQR]	10 [5, 15]	10 [5, 20]	10 [5, 20]	.37
Type of last sexual intercourse, n/N (%)				
Oral only	10/98 (10)	10/98 (10)	20/196 (10)	
Anal insertive only	43/98 (44)	32/98 (33)	75/196 (38)	.25
Anal receptive	45/98 (46)	56/98 (57)	101/196 (52)	
Last receptive anal intercourse without condom, n/N (%)	35/45 (78)	43/56 (77)	78/101 (77)	.91
STI baseline	21/107 (20)	15/103 (15)	36/210 (17)	.33
Chlamydia	17[a]/107 (16)	6/103 (6)	23[a]/210 (11)	
Gonorrhea	9[a]/107 (8)	8/103 (8)	17[a]/210 (8)	
Syphilis	0/107 (0)	1/103 (1)	1/210 (1)	
Recreational drug use in last 12 months, n/N (%)	35/104 (34)	36/100 (36)	71/204 (35)	.73

Abbreviations: IQR, interquartile range; PEP, post-exposure prophylaxis; STI, sexually transmitted infection.

[a]Five participants in the PEP arm (and total) had both a *Chlamydia* infection and gonorrhea.

gonorrhea. The highest prevalence of MG infection was reported in urine (6.3%), followed by anal (4.3%) and throat (0.5%) samples (Table 2). At this stage, each participant had only 1 site positive for MG and only 1 participant was mildly symptomatic (burning on urination). At the 6-month visit, 19 participants (10 in the PEP arm and 9 in the non-PEP arm) tested positive for MG, with an overall prevalence of 9.9% (95% CI, 6.1–15.0%), 10.2% with PEP and 9.6% without PEP ($P = .89$) (Table 2). The median use of doxycycline in the PEP arm over 6 months in this study was 670 mg/month (interquartile range, 270–1200 mg). Only 11 participants acquired a new MG infection during this 6-month period: 7 participants in the PEP arm and 4 in the non-PEP arm. These infections were detected in urine (n = 5) and anal (n = 6) or throat (n = 1, also positive for an anal sample) samples (Figure 1). All participants were asymptomatic. The percentage of participants with a new MG infection did not significantly differ between the 2 study arms.

Antimicrobial Drug Resistance of all *Mycoplasma genitalium*–Positive Specimens

Thirty-nine specimens from 30 participants were available for the detection of mutations associated with resistance to AZM, FQs, and TET. Nine (23.1%) and 6 (15.4%) of these 39 specimens gave no amplicons for the detection of resistance to AZM and FQs, respectively. The overall frequencies of specimens harboring mutations associated with resistance to AZM and FQs in MG were 66.7% (20/30) and 9.1% (3/33), respectively. Overall, 69.6% (16/23; 95% CI, 47–87%) and 11.1% (3/27; 95% CI, 2–29%) of patients harbored AZM and FQ resistance-associated mutations, respectively. At day 0, 60% and 5.6% of samples harbored AZM and FQ resistance-associated mutations, respectively; the corresponding proportions were 73.3% and 13.3% at 6 months (Table 3). For TET, we found in vivo mutations of the MG 16S rRNA shown to be involved or associated with TET resistance in other bacteria in 2 of 16 (12.5%) specimens tested, corresponding to an overall rate of MG 16S rRNA mutations of 14.3% (2/14; 95% CI, 1.8–42.8%).

The 23S rRNA mutations associated with AZM resistance were A2058G, A2058T, or A2059G (*Escherichia coli* numbering) (Supplementary Table 1, Table 3) [35]. The S83I and D87Y (*E. coli* numbering) substitutions possibly associated with FQ resistance in MG affect residues in the *ParC* QRDR. The A88T mutation in *ParC* has never been associated with FQ resistance to date. However, it may be involved in resistance as it is located next to the position D87 shown to be associated with MXF resistance. For TET, several mutations of the 16S rRNA gene of MG-positive specimens were identified, including mutations at positions C1192G, G966T, and C967T within or very close to the TET target site, in 2 samples (Supplementary Table 1, Table 3) from each arm of the study. In this study, C1192G was detected in MG DNA from an anal swab from 1 participant in the PEP doxycycline arm, whereas G966T and C967T were detected in the MG DNA from an anal swab from 1 participant from the control group previously treated 3 times with doxycycline for *Lymphogranuloma venereum* (LGV) before inclusion in the no-PEP arm. For this last patient, the first doxycycline regimen took place in July 2014, followed by a second one in December 2014 and a third one in April 2015. The patient was enrolled in the study in September 2015. However, no minimum inhibitory concentration (MIC) data were available to confirm any doxycycline resistance.

One participant had multiple MG-positive anatomical sites (throat and anus) in the doxycycline PEP arm at 6 months (patient 6 in Supplementary Table 1), and the 2 isolates differed by their resistance profile, suggesting that the 2 strains were not related.

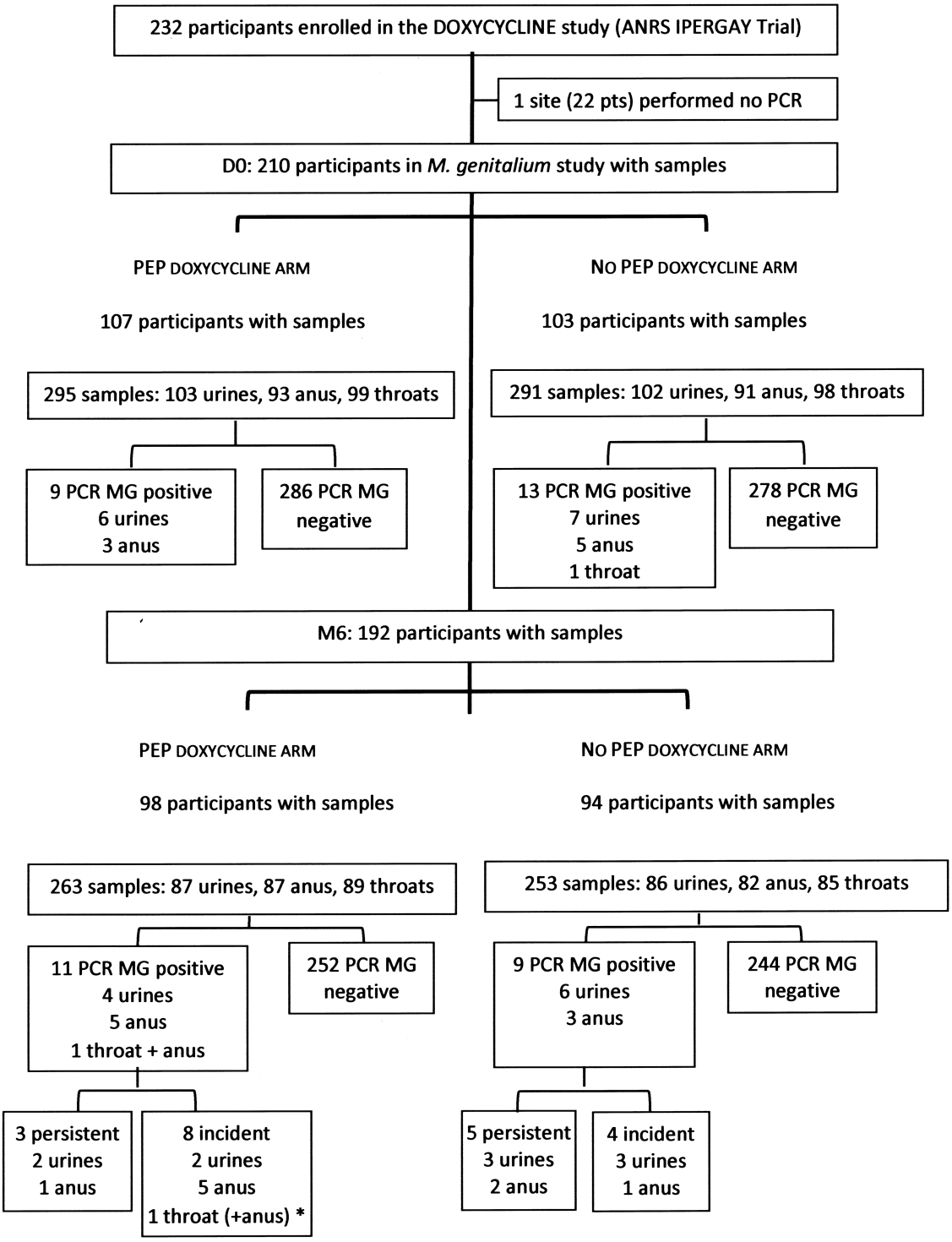

Figure 1. Flowchart of the doxycycline PEP and MG study. *This participant had MG detected both in throat and anal swabs. Abbreviations: D0, day 0; M6, month 6; MG, *Mycoplasma genitalium*; PCR, polymerase chain reaction; PEP, post-exposure prophylaxis.

Outcome of Urinary *Mycoplasma genitalium* Infections

The response of baseline urinary MG infections to treatment was investigated according to the detection of MG at 6 months and antibiotic resistance. At baseline, 13 urinary infections were diagnosed and treated: 8 of 13 by a 5-day course of AZM (500 mg [day 1], then 250 mg [days 2–5]), as recommended by the European guidelines [6], 4 of 13 with AZM 1 g, and 1 of 13 with a 14-day course of doxycycline (200 mg/day). Finally, 8 of 13 (62%) were cured, with a negative PCR at 6 months, whereas 5 of 13 (38%) remained PCR positive at the 6-month visit, including 2 of 5 infections not treated with the recommended regimen treatment (one receiving doxycycline and

Table 2. Prevalence of *Mycoplasma genitalium* Infection at Baseline and at 6 Months in Men Who Have Sex With Men on Pre-exposure Prophylaxis With or Without Post-prophylaxis With Doxycycline

	Prevalence of MG at Baseline			Prevalence of MG at 6 Months			
Participants with Positive PCR	Global Prevalence, % [95% CI]	PEP, n/N (%)	No PEP, n/N (%)	Global Prevalence, % [95% CI]	PEP, n/N (%)	No PEP, n/N (%)	*P*[a]
Urine	6.3 [3.4–10.6]	6/103 (6)	7/102 (7)	5.8 [2.8–10.4]	4/87 (4.6)	6/86 (7.0)	.51
Anus	4.3 [1.9–8.6]	3/93 (3)	5/91 (5)	5.3 [2.5–9.9]	6/87 (6.9)	3/82 (3.7)	.50
Throat	.5 [.01–2.8]	0/99 (0)	1/98 (1)	.6 [.01–3.2]	1/89 (1.1)	0/85 (0)	1.00
Total (patients)	10.5 [6.7–15.4]	9/107 (8)	13/103 (13)	9.9 [6.1–15.0]	10/98 (10.2)	9/94 (9.6)	.89

Abbreviations: CI, confidence interval; MG, *Mycoplasma genitalium*; PCR, polymerase chain reaction; PEP, post-exposure prophylaxis.

[a]Fisher's exact or chi-square test comparing prevalence between PEP arm and no-PEP arm at 6 months.

one receiving 1 g of AZM). Among the 12 participants who received AZM, 9 had specimens retrospectively tested for resistance and 5 of 9 had macrolide resistance. Four participants with no macrolide resistance were cured, as compared with only 2 of 5 (40%) of those with resistance mutations in the 23s rRNA (P = .17). Unfortunately, for all patients who had a positive PCR test at baseline and at 6 months, no difference could be made between infection, reinfection, and/or colonization since the patients were asymptomatic and there was no test of cure performed at 3 weeks to control that the PCR had become negative.

Risk Factors Associated with *Mycoplasma genitalium* Infection at Baseline

We investigated whether participants baseline demographics, sexual behavior, or STI coinfections were associated with MG infection at baseline, and found no significant association (Supplementary Table 2).

DISCUSSION

The use of PrEP with oral TDF/FTC during condomless sex constitutes a major advance in the prevention of HIV [30, 31]. The impact of this prophylaxis was reported in 2018 in Paris, with a decrease in observed rates of new HIV diagnoses by 28% among MSM born in France [36]. Unfortunately, a resurgence of bacterial STIs, such as *Chlamydia* infection, gonorrhea, and syphilis, has also been reported among MSM over the last 10 years, due to an increase in sexual risk behaviors and a decrease in condom use. Increases in the incidence of bacterial STIs have also been reported in MSM and bisexual individuals using PrEP [37]. We previously showed that PEP with

Table 3. Resistance-associated Mutations in *Mycoplasma genitalium* Strains Detected at Baseline and at 6 Months, by Arm (Doxycycline PEP or no-PEP Arm)

	23S rRNA Mutations (AZM Resistance)		*ParC* Mutations (FQ Resistance)		16S rRNA Mutations (Suspicion of TET Resistance)	
	PEP	No PEP	PEP	No PEP	PEP	No PEP
At baseline						
No. with RAMs/no. tested	2/4	7[a]/11	0/6	1/12	0/3	1[a]/8
Mutations observed (no. of isolates)	A2059G (2)	A2058G (2), A2058T (1), A2059G (4)	…	S83I (1)	…	G966T + C967T (1)
Rate of RAMs (number of MG infections n = 22), n/N (%)	9/15 (60.0)		1/18 (5.6)		1/11 (9.1)	
At 6 months						
No. with RAMs/no. tested	5[b,c]/8	6/7	2[b,c]/6	0/9	1[c]/2	0/3
Mutations observed (no. of isolates)	A2058G (3), A2058T (1), A2059G (1)	A2058G (1), A2058T (1), A2059G (4)	D87Y (1), A88T (1)[d]	…	C1192G (1)	…
Rate of RAMs (no. of MG infections n = 19), n/N (%)	11/15 (73.3)		2/15 (13.3)		1/5 (20.0)	
Overall RAMs, n/N (%)	20/30 (66.7)		3/33 (9.1)		2/16 (12.5)	
No. of participants with RAMs at baseline and/or at 6 months (no. of participants with MG infections n = 32)	16/23 (69.6)		3/27 (11.1)		2/14 (14.3)	

Abbreviations: AZM, azithromycin; FQ, fluoroquinolone; MG, *Mycoplasma genitalium*; PEP, post-exposure prophylaxis; RAM, resistance-associated mutation; rRNA, ribosomal RNA; TET, tetracycline.

[a]One of the isolates harbored mutations associated with resistance to AZM + TET.

[b]One of the isolates harbored mutations associated with resistance to AZM + FQ.

[c]One isolate harbored mutations associated with resistance to AZM + FQ + TET.

[d]The A88T mutation in *ParC* has never been associated with FQ resistance to date. However, it may be involved in resistance as it is located next to the position D87 shown to be associated with moxifloxacin resistance.

doxycycline could reduce the incidence of *Chlamydia* infection and syphilis in this population [32].

This ancillary study was performed to assess the prevalence of MG infection in these participants and to assess their resistance to antibiotics. In our study, a high rate of MG infection (10.5%, corresponding to 22 participants) was observed at baseline, mostly in the urinary tract (6.3%) and the anus (4.3%), with levels similar to those of recent reports among MSM on PrEP [22, 38, 39]. The prevalence of MG in throat swabs was very low (0.5%), consistent with previous reports [40]. Our findings also indicate that MG prevalence in asymptomatic MSM is quite similar to that of *C. trachomatis* and *N. gonorrhoeae* [32]. We could not find any association between MG infection and participants' baseline characteristics, and the rate of STI coinfection was low (9%) in participants with MG infection. The prevalence of MG infection remained stable at 6 months in our study, without significant differences between the doxycycline PEP arm or the no-PEP arm, suggesting that doxycycline PEP has no impact on the MG incidence contrary to syphilis or *Chlamydia* infections [32].

We then screened the MG isolates for mutations associated with resistance to macrolides, FQs, and TETs. In our study, MG isolates were frequently resistant to AZM (66.7%) and MXF (9.1%). Our results are similar to those reported for MSM with and without HIV, in whom the resistance of MG to AZM and FQs ranged from 70.7% to 79.9% [20–25] and from 13% to 33.3% [20, 21, 26], respectively. This high level of AZM resistance highlights the need for routine resistance testing on any MG isolate obtained, particularly from MSM, before treatment. Our results also highlight the clinical benefit of not testing and therefore not treating asymptomatic patients in order to avoid a therapeutic impasse with an untreatable MG. Indeed, we showed that the response rate to AZM in our participants was lower when macrolide-resistance mutations were detected. In line with this, given the high rate of multidrug-resistant MG isolates, BASHH and Australian STI guidelines no longer recommend the screening and treatment of MG infections in asymptomatic participants, including MSM, to limit the increase in untreatable MG strains. Furthermore, these guidelines recommend resistance-guided therapy, which relies on the use of doxycycline for the management of NGU, followed by the administration of either AZM or MXF, depending on the MG resistance status provided by a molecular diagnostic assay [27, 28]. In France, and in light of our study, new recommendations proposed by the French Dermatologic Society and the French National Reference Center for Bacterial STIs have, since 2019, also recommended against screening of asymptomatic participants for MG.

Interestingly, in our study, several mutations within or very close to the TET target site in the 16S rRNA gene were identified in MG-positive specimens from 2 participants; these mutations have already been involved or associated with TET resistance in other bacterial species [41], including animal mycoplasmas [42] and the human urogenital species *Mycoplasma hominis* [43], but have never been reported in MG. These mutations were observed in participants who had received doxycycline (one for prophylaxis and the other for LGV treatment). These data require experimental confirmation in cultured MG isolates containing 16S rRNA mutations, for studies of the profile of MG sensitivity to TETs, and to determine the impact of these mutations on TET MICs. These resistant strains were observed in vivo in participants who had taken doxycycline for treatment or prophylaxis, suggesting that the acquisition of these mutations may have been enhanced by the use of doxycycline. These data deserve confirmation with a larger number of isolates and could be a limitation in the use of doxycycline PEP.

In conclusion, the prevalence of MG infection was high in this cohort of asymptomatic MSM on PrEP with TDF/FTC and randomized in a study assessing the benefit of doxycycline PEP. We found no evidence of a change in MG prevalence at 6 months with the use of PEP. However, we found a high rate of resistance to AZM and MXF and potential TET resistance-associated mutations, raising important issues for the screening and treatment of this STI in asymptomatic individuals. Our results support current recommendations to avoid testing or treatment of asymptomatic MG infection.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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