BMJ Open

Prevalence of fluoroquinolone resistance-associated mutations in *Mycoplasma genitalium* among clients of two sexual health centres in the Netherlands: a cross-sectional study

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To cite: Braam JF, Zondag HCA, van Dam AP, *et al*. Prevalence of fluoroquinolone resistance-associated mutations in *Mycoplasma genitalium* among clients of two sexual health centres in the Netherlands: a cross-sectional study. *BMJ Open* 2022;**12**:e066368. doi:10.1136/bmjopen-2022-066368

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2022-066368).

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Received 13 July 2022
Accepted 14 November 2022

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ABSTRACT

Objective This study aimed to determine the prevalence of fluoroquinolone resistance-associated mutations (QRAMs) in *Mycoplasma genitalium* (MG) among clients of two sexual health centres (SHCs) in the Netherlands.

Design A cross-sectional study.

Setting and participants Between 2018 and 2019, 669 clients with MG were included from two previous studies: 375 male clients with urethritis from the SHC in Amsterdam; and 294 clients (male and female) from the SHC in Amsterdam and The Hague. Urogenital and anal samples (705 in total) that tested positive for MG by nucleic acid amplification tests were selected.

Outcome measures The presence of QRAM was detected by an MG-QRAM PCR targeting four mutations in the *parC* gene and investigated by sequence analysis of relevant regions of the *gyrA* and *parC* genes. Possible risk factors for the presence of QRAM were investigated.

Results We found QRAM in 58 of 669 (9%) clients with an MG infection: 36 of 375 (10%) in the study population of men with urethritis and 22 of 294 (7%) in the study population of other clients (including both men and women; p=0.334). Most prevalent mutations in the *parC* gene were S83I and D87N, occurring in 31 of 60 (52%) and 20 of 60 (33%) samples, respectively. Factors associated with the presence of QRAM were: men who have sex with men (adjusted OR (aOR) 3.4, 95% CI 1.7 to 6.9) and Asian origin (aOR 2.5, 95% CI 1.2 to 5.6). Multidrug resistance (QRAM plus macrolide resistance-associated mutations) was found in 46 of 669 (7%) clients.

Conclusions Nine per cent of MG-positive clients from two Dutch SHCs had QRAM. New treatment strategies and antibiotics are needed to treat symptomatic patients with multidrug-resistant MG.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ A large number of samples were included in this study.
⇒ We added fluoroquinolone resistance data for sexual health centre clients in the Netherlands that were obtained using a specific new PCR, and the mutations were confirmed by sequence analysis.
⇒ By using the *Mycoplasma genitalium*-fluoroquinolone resistance-associated mutation PCR from NYtor, which detects a set of mutations in the *parC* gene, there may be an underestimation of non-synonymous mutations in the *gyrA* gene as well as rare mutations in the *parC* gene.
⇒ Given the cross-sectional design, no follow-up information was available on the potential effectiveness of treatment.

INTRODUCTION

Mycoplasma genitalium (MG) is a sexually transmissible bacterium that is associated with urethritis in men[1] and pelvic inflammatory disease, cervicitis, preterm birth and spontaneous abortion in women.[2] In high-income countries, the summary prevalence in randomly selected samples from the general population was 1.3% (95% CI 1.0% to 1.8%) and in lower-income countries, this was 3.9% (95% CI 2.2% to 6.7%) according to a meta-analysis from 2018.[3] Compared with the general population, the prevalence of MG is much higher among men with urethritis, and was reported to be 23% in the Netherlands.[4]

Since the bacterium has no cell wall, β-lactam antibiotics cannot be used to treat MG infections. Therefore, treatment options include mainly antibiotics that disrupt protein synthesis, such as macrolides and tetracyclines, or DNA replication, such as fluoroquinolones. Although MG is susceptible to doxycycline in vitro, the clinical efficacy of doxycycline monotherapy against MG is only 22%–45%.[5] Most guidelines on treatment of MG now recommend resistance-guided therapy. As part of this approach, doxycycline is provided as initial empirical

therapy, reducing bacterial load and facilitating MG clearance.[6–8] Subsequently, macrolide-sensitive MG infections should be treated with azithromycin and macrolide-resistant infections with moxifloxacin. Other guidelines such as the European guidelines recommend azithromycin in case of macrolide-sensitive MG infections, moxifloxacin in case of macrolide-resistant MG infection and doxycycline or minocycline in case of a persistent MG infection after both azithromycin and moxifloxacin treatment.[9]

Antibiotic-resistant MG has been reported for both azithromycin and moxifloxacin. Macrolide resistance is caused by mutations at positions 2058 and 2059 (*Escherichia coli* numbering) in region V of the *23S rRNA* gene.[10] The main cause of fluoroquinolone resistance or reduced susceptibility in MG are mutations in the topoisomerase IV *parC* gene. Amino acid changes S83R, S83I, D87N and D87Y in the DNA topoisomerase 4 subunit A coding region, as a result of mutations in the *parC* gene, are most frequently reported to be associated with fluoroquinolone resistance.[11 12] Mutations in the *gyrA* subunit of DNA gyrase are usually combined with mutations in the *parC* gene and are reported less frequently.[11] Amino acid changes D99N, G93C and M95I in DNA gyrase as a result of mutations in the *gyrA* gene have been associated with increased minimum inhibitory concentration in MG isolates, but these isolates also had an S83I amino acid change in the *parC* subunit.[11] Murray *et al* found that treatment was more likely to fail in patients who had, besides a mutation in the *parC* gene, a concurrent mutation in the *gyrA* gene (M95I or D99N), suggesting an additive effect of mutations in the *gyrA* gene.[12]

A systematic review and meta-analysis by Machalek *et al* included data of macrolide resistance-associated mutations (MRAMs) and fluoroquinolone resistance-associated mutations (QRAMs) in MG from 59 studies including 21 countries to investigate the prevalence and trends of resistance. Prevalence of MRAM is increasing worldwide, from 10% before 2010 to 51% in 2017.[13] Prevalence of QRAM did not change significantly over time and has been reported to be worldwide on average 8%,[13] except for Japan where a significant increase was observed from 5% before 2010 to 29% in 2016–2017.[11] Multidrug-resistant MG, carrying both MRAM and QRAM, occurred in 3% of the MG samples.[13] In the Netherlands, MG-MRAM is also frequently reported and varies between 40% and 74%, depending on the population included in the study.[4 14 15] Nijhuis *et al*,[16] the only report on QRAM in MG in the Netherlands, found a prevalence of 8% QRAM among primary care and hospital patients, but not including patients visiting sexual health centres (SHCs).[16]

Because prevalence of macrolide resistance in MG is increasing, the use of moxifloxacin for the treatment of MG is also likely to increase. It is important to monitor the prevalence of QRAM, since alternative treatment options are limited. In this study, we aimed to determine the prevalence of, and risk factors for, MG-QRAM among participants from two previous studies, consisting of male patients with urethritis and clients visiting two SHCs in the Netherlands.

METHODS

Study population and sample selection

In this study, we used a subset of samples of two large studies performed at two SHCs in the Netherlands. The subset included in the current study consisted of all transcription-mediated amplification (TMA) MG-positive samples that were also positive either with the MG-MRAM PCR[14] or the MgPa PCR[17] from the two studies, which are briefly described below. Only samples from the first clinical visit during the study period were included.

The first study (MG prevalence study) was a cross-sectional MG prevalence study in which all clients—symptomatic and asymptomatic—at the SHCs in Amsterdam in February and March 2018 and in The Hague in May and June 2018 were eligible to be included.[18] Symptomatic clients had either urethral, vaginal and/or anal symptoms. Included clients from this study represent the total population attending the SHC. First-void urine was collected from all men and anal swabs were collected from men who have sex with men (MSM). Cervical-vaginal swabs were taken from all female clients. Anal samples were taken according to the policy of the local SHC: in The Hague from all women attending the SHC and in Amsterdam anal samples were taken only from women if they reported anal sex or anal symptoms, were notified for a sexually transmitted infection (STI) or if they reported to perform sex work. All samples were tested for *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT) (Aptima Combo 2, Hologic, San Diego, California, USA) and MG (Aptima, Hologic). From all samples that tested positive for MG in the MG-TMA assay, DNA was extracted with isopropanol precipitation.[14] They were subsequently tested with the MgPa PCR to detect MG and for macrolide resistance using the MG-MRAM PCR to detect wildtype (MG-WT) or any mutations (MRAM) in the *23S rRNA* gene at nucleotide positions 2058 and 2059 (*E. coli* numbering).[14] If two samples were available from different anatomical locations from a client, of which at least one contained MG-MRAM, the infection was considered to be caused by a resistant strain.

We refer to the clients from this MG prevalence study as ‘SHC clients’.

The second study included all men with urethritis at the clinic in Amsterdam between May 2018 and November 2019.[4] Urethritis was defined as presence of >10 leucocytes per high-power field in Gram stains of urethral discharge. Symptoms were dysuria, discharge or urethral discomfort. First-void urine was collected from all men. Similar procedures as in the MG prevalence study were performed in this study to test for NG, CT, MG and MRAM. We refer to these patients as ‘men with urethritis’.

Fluoroquinolone resistance-associated mutation PCR

All included samples from both studies were tested using the MG fluoroquinolone RES real-time PCR kit (in short MG-QRAM PCR, NYtor, Nijmegen, the Netherlands) for the detection of QRAM in the *parC* gene according to the specifications of the manufacturer. This multiplex PCR targets the following *parC* single-nucleotide polymorphisms: G248T, A247C, G259A and G259T, causing amino acid changes S83R, S83I, D87N and D87Y in the ParC subunit.[16]

Sequencing

Sanger sequencing was performed on relevant genetic regions of the *parC* (nucleotides 164–483) and *gyrA* (nucleotides 172–402) genes in order to identify mutations present in the samples containing mutations as detected by MG-QRAM PCR. Primers described by Deguchi *et al* were used with the addition of M13 sequences at the forward tail (5' TGTAAAACGACGGCCAGT) and reverse tail (5' CAGGAAACAGCTATGACC) primers. These were used to amplify the *gyrA* and *parC* regions of interest using PCR with the following cycling conditions: 40 cycles of 95°C (60 s), 52°C (50 s) and 72°C (50 s).[19] All PCR products were checked by gel electrophoresis using Qiaxcel (Qiagen) and sent for subsequent product purification and high-throughput Sanger sequencing at the Core Facility Genomics of the Amsterdam University Medical Center. The sequence analysis was done using Bionumerics V.7.6.3 (Applied Maths, BioMérieux). Whether a mutation was synonymous or not was analysed using MEGA X V.10.1.8.[20]

Statistical analysis

Sociodemographic data, sexual behaviour, the presence of clinical symptoms and recent antibiotic treatment (antibiotic use in the previous 3 months) were extracted from electronic patient files. The variable 'educational level' was categorised into low (no education, primary school, lower secondary vocational education and intermediate secondary general education), mid (higher secondary general education, senior secondary vocational education and pre-university secondary education) and high (higher professional or university education). Multidrug resistance (simultaneous resistance against macrolides and fluoroquinolones) was determined on a sample level. X^2 test was used to compare the participants included in the two different studies. Factors associated with MG-QRAM were examined using univariable and multivariable logistic regression analyses on client level. Variables with $p<0.20$ in univariable analysis were considered in multivariable analysis, but only retained if $p<0.05$. Significance was assessed two sided for all variables, applying a cut-off value of $p<0.05$. Data were analysed using Stata Intercooled V.15 (StataCorp, Texas, USA).

Patient and public involvement

None.

RESULTS

Demographics

We included 705 MG-positive samples derived from 669 clients. This included 375 men with urethritis and 330 samples from 294 SHC clients. The majority of the 669 clients were men (550, 82%) and almost half were MSM (292, 44%). Clients were mostly from the Netherlands (285, 43%) followed by Latin America (166, 25%). The majority were highly educated (356, 58%), had not used antibiotics in the previous 3 months (581, 87%) and 60 (9%) were living with HIV. Coinfections with NG and CT were present among 86 (13%) and 111 (17%) clients, respectively (table 1).

There were some demographic differences between clients from the two substudies. Among the men with urethritis, age was significantly higher compared with SHC clients (table 1). The majority of SHC clients were from the Netherlands (54%), and the country of origin was more diverse among clients with urethritis and consisted of a two times higher proportion of people from Latin America (table 1). Also, more men with urethritis were diagnosed with NG (62 of 375, 17%) as compared with the SHC clients (24 of 294, 8%). Furthermore, HIV status was more often unknown among SHC clients compared with clients with urethritis.

Mutations

Successful identification of presence or absence of MRAM and QRAM was possible in 689 of 705 (98%) samples. For 16 of 705 samples, the MRAM status was unknown of which one sample contained QRAM. Only 206 of 705 (29%) of samples were found to be MG-WT. Almost two-thirds (426 of 705, 60%) contained MRAM only, 11 of 705 (2%) contained QRAM only, and 46 of 705 (7%) contained both MRAM and QRAM (table 2). QRAM was found in 58 of 669 (9%) MG-positive clients: 36 of 375 (10%) from men with urethritis and 22 of 294 (7%) from SHC clients.

Four different non-synonymous mutations that have previously been associated with fluoroquinolone resistance were found in the *parC* genes of 54 of 58 samples: S83R, S83I, D87N and D87Y. In three samples, the sequence could not be determined and in one sample, QRAM could not be confirmed since a WT sequence was detected by sequencing. The most prevalent mutations were S83I and D87N, occurring in 28 of 54 (52%) and 18 of 54 (33%) samples, respectively (table 3). Double non-synonymous mutations in *parC* were not detected. Besides a *parC* mutation, one mutation was detected in the *gyrA* gene that resulted in the amino acid change D99Y in 1 of 54 (2%) samples (table 3).

There were 30 SHC clients who had two MG-positive samples from two anatomical locations, of which the MRAM and QRAM profiles were available. Twenty-nine had a combination of anal and vaginal samples and one had an anal and a urine sample. The majority (27 of 30, 90%) had identical resistance genes profiles. Three sample sets (10%) were discordant: one client with an

Table 1 Characteristics of clients from the SHC of Amsterdam and The Hague in 2018 and male clients with urethritis from the SHC in Amsterdam between 2018 and 2019 with a positive MG test result

		Total (N=669)	SHC clients (N=294)	Male clients with urethritis (N=375)	P value
Age in years, n (%)	<25	231 (35)	120 (41)	111 (30)	0.001
	25–34	266 (40)	92 (31)	174 (46)	
	35–44	95 (14)	43 (15)	52 (14)	
	≥45	77 (12)	39 (13)	38 (10)	
Sex	Men	550 (82%)	175 (60%)	375 (100%)	n.a.
	Women	115 (17%)	115 (39%)	n.a.	
	Transgender	4 (1%)	4 (1%)	n.a.	
Sexual risk group, n (%)	MSW	256 (38)	43 (15)	213 (57)	n.a.
	MSM	292 (44)	132 (45)	160 (43)	
	Women	115 (17)	115 (39)	n.a.	
	Unknown/other	6 (1)	4 (1)	2 (1)	
Country/region of origin, n (%)	The Netherlands	285 (43)	159 (54)	126 (34)	<0.001
	Africa	57 (9)	20 (7)	37 (10)	
	Central and South America	166 (25)	47 (16)	119 (32)	
	Asia	55 (8)	24 (8)	31 (8)	
	Europe outside the Netherlands	88 (13)	36 (12)	52 (14)	
	Other	18 (3)	8 (3)	10 (3)	
Educational level, n (%)	Low	71 (11)	33 (12)	38 (11)	0.582
	Medium	192 (31)	91 (33)	101 (30)	
	High	356 (58)	153 (55)	203 (59)	
Antibiotic use in previous 3 months, n (%)	None	581 (87)	261 (89)	320 (85)	0.219
	Previous 14 days	24 (4)	6 (2)	18 (5)	
	Previous 15–30 days	15 (2)	5 (2)	10 (3)	
	Previous 31–90 days	49 (7)	22 (7)	27 (7)	
HIV status, n (%)	Negative	448 (67)	170 (58)	278 (74)	<0.001
	Positive	60 (9)	28 (10)	32 (9)	
	Unknown	161 (24)	96 (33)	65 (17)	
No of sex partners in last 6 months, n (%)	0 or 1	69 (10)	37 (13)	32 (9)	0.022
	2–4	272 (41)	108 (37)	164 (44)	
	5–9	148 (22)	58 (20)	90 (24)	
	≥10	176 (26)	90 (31)	86 (23)	
Any symptoms	No	213 (32%)	213 (72%)	n.a.	n.a.
	Yes	456 (68%)	81 (28%)	375 (100%)	
NG, n (%)	Negative	583 (87)	270 (92)	313 (83)	0.001
	Positive	86 (13)	24 (8)	62 (17)	
CT, n (%)	Negative	558 (83)	252 (86)	306 (82)	0.156
	Positive	111 (17)	42 (14)	69 (18)	

X^2 test was performed to determine differences between participants included in both studies.
*Information was missing for 50 participants.
CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; MSM, men who have sex with men; MSW, men who have sex with women only; n.a., not applicable; NG, *Neisseria gonorrhoeae*; SHC, sexual health centre.

anal MG-WT and a vaginal MG-MRAM; one client with an anal multidrug-resistant MG and a vaginal MG-MRAM; and one client with an anal MG-MRAM and a vaginal MG-WT.

Risk factors for MG-QRAM

QRAM was present among 7% of clients from the SHCs and 10% of men with urethritis (p=0.334) (table 4). MSM were more frequently infected with MG-QRAM

Table 2 Resistance profile of MRAM* and QRAM by anatomical location from clients of the SHC of Amsterdam and The Hague in 2018 and male clients with urethritis from the SHC in Amsterdam between 2018 and 2019 with a positive MG test result

	Total	SHC clients: anal	SHC clients: vaginal	SHC clients: urine	SHC clients: total	Male patients with urethritis: urine
WT	206 (30%)	44 (31%)	39 (41%)	22 (29%)	105 (33%)	101 (27%)
MRAM only	426 (62%)	86 (60%)	50 (53%)	52 (68%)	188 (60%)	238 (63%)
QRAM only	11 (2%)	2 (1%)	3 (3%)	1 (1%)	6 (2%)	5 (1%)
Multidrug resistant (MRAM+QRAM)	46 (7%)	11 (8%)	2 (2%)	2 (3%)	15 (5%)	31 (8%)
Subtotal	689 (100%)	143 (100%)	94 (100%)	77 (100%)	314 (100%)	375 (100%)
Not identifiable*	16	10	6	0	16	0
Total	705	153	100	77	330	375

Results are given on sample level.
*Information on MRAM PCR was missing for 6 vaginal samples and 10 anal samples, of which 1 vaginal sample contained QRAM.
MG, *Mycoplasma genitalium*; MRAM, macrolide resistance-associated mutation; QRAM, fluoroquinolone resistance-associated mutation; SHC, sexual health centre; WT, wildtype.

(37 of 292, 13%) compared with men who have sex with women only (12 of 256, 5%) and women (9 of 115, 8%) (p=0.008). In addition, clients with MG-QRAM were more often of Asian origin (11 of 55, 20%) (p=0.017) and had also MRAM (46 of 456, 10%) (p=0.049). In multivariable logistic regression, being MSM (adjusted OR (aOR) 3.4, 95% CI 1.7 to 6.9) and being of Asian origin (aOR 2.5, 95% CI 1.2 to 5.6) remained associated with the presence of QRAM (table 4).

The combination of QRAM and MRAM was found in 5% of the clients from the SHCs and 8% from the men with urethritis (online supplemental table 1). Being MSM (aOR 4.7, 95% CI 2.1 to 10.9) was again found to be significantly associated with the presence of multidrug-resistant MG.

Table 3 Identified QRAMs determined by sequencing in samples that tested positive with MG-QRAM PCR (NYtor) from clients of the SHC of Amsterdam and The Hague in 2018 and male clients with urethritis from the SHC in Amsterdam between 2018 and 2019 with a positive MG test result

Gene	SNP	Amino acid change	Total	SHC clients	Male patients with urethritis
gyrA	G70T	D99Y	1	1	0
parC	A247C	S83R	1	0	1
	G248T	S83I	28	11	17
	G259A	D87N	18	3	15
	G259T	D87Y	7	4	3
Total *parC**			54	18	36

*None of the strains contained two or more SNPs in the sequenced DNA at positions 247, 248 and 259. All samples derived from individual clients.
MG, *Mycoplasma genitalium*; QRAMs, fluoroquinolone resistance-associated mutations; SHC, sexual health centre; SNP, single-nucleotide polymorphism.

DISCUSSION

QRAMs were detected among 9% (58 of 669) of MG-positive clients in this study. Seventy-nine per cent (46 of 58) also harboured A2058 or A2059 mutations on the *23S* rRNA locus resulting in 7% (46 of 669) multidrug-resistant MG among MG-positive clients of two SHCs in the Netherlands. Only 29% (206 of 705) of samples were found to contain MG-WT.

Recent reviews estimated the prevalence of MG-QRAM in Europe at 3%–5%.[13 21] These reviews did not present MG-QRAM prevalence data from the Netherlands or Belgium. However, reports from the Netherlands and Belgium found an MG-QRAM prevalence of 8%[16] and 26%,[22] respectively. In the Belgian study, 18% of the MG infections were found to be multidrug resistant and the difference between women (3%) and MSM (24%) was remarkable.[22] In the Netherlands, the prevalence of multidrug-resistant MG was previously reported to be 7% among samples that originated from both primary care and hospital care patients—no samples from SHCs were included[16]—and we found the same prevalence in our study among clients of the SHCs. However, the prevalence of MG-MRAM was lower among primary care and hospital care patients (41%)[16] compared with our study (69%). The difference found between MRAM prevalences from both studies and not between QRAM prevalences might be explained by the frequent use of macrolides and the so far conservative use of fluoroquinolones in the Netherlands. In addition, a study in the USA found QRAM in 11% of included patients with urethritis,[23] which is

Table 4 Prevalence of MG-QRAM and results of univariable and multivariable logistic regression analyses for association of characteristics with presence of MG-QRAM in clients of the SHC of Amsterdam and The Hague in 2018 and male clients with urethritis from the SHC in Amsterdam between 2018 and 2019 with a positive MG test result

		Number of clients with MG-QRAM as a fraction of total clients with MG*	P value†	Univariable analysis OR	95% CI	Multivariable analysis aOR	95% CI
Total		58/669 (9%)					
Study group	SHC clients	22/294 (7%)	0.334	1			
	Men with urethritis	36/375 (10%)		1.31	0.75 to 2.28		
Age in years, n (%)	<25	18/231 (8)	0.662	1			
	25–34	26/266 (10)		1.28	0.68 to 2.40		
	35–44	6/95 (6)		0.80	0.31 to 2.08		
	≥45	8/77 (10)		1.37	0.57 to 3.29		
Sex	Men	49/550 (9%)	0.770	1			
	Women	9/115 (8%)		0.87	0.41 to 1.82		
	Transgender	0/4 (0%)		n.a.			
Sexual risk group, n (%)	MSW	12/256 (5)	0.008	1		1	
	MSM	37/292 (13)		2.95	1.50 to 5.79	3.40	1.66 to 6.94
	Women	9/115 (8)		1.73	0.71 to 4.22	1.81	0.71 to 4.61
	Unknown/other	0/6 (0)		n.a.		n.a.	
Country/region of origin, n (%)	The Netherlands	25/285 (9)	0.017	1		1	
	Africa	4/57 (7)		0.78	0.26 to 2.35	0.98	0.32 to 2.99
	Central and South America	14/166 (8)		0.96	0.48 to 1.90	1.36	0.66 to 2.81
	Asia	11/55 (20)		2.6	1.19 to 5.66	2.54	1.15 to 5.62
	Europe outside the Netherlands	2/88 (2)		0.24	0.06 to 1.04	0.20	0.05 to 0.88
	Other	4/23 (17)		1.3	0.28 to 5.98	1.25	0.27 to 5.87
Educational level‡, n (%)	Low	3/71 (4)	0.357	1			
	Medium	15/192 (8)		1.92	0.54 to 6.85		
	High	33/356 (9)		2.32	0.69 to 7.77		
Antibiotic use in previous 3 months, n (%)	None	46/581 (8)	0.068	1			
	Previous 14 days	2/24 (13)		1.66	0.48 to 5.78		
	Previous 30 days	4/15 (27)		4.23	1.30 to 13.81		
	Previous 90 days	5/49 (10)		1.32	0.50 to 3.50		
HIV status, n (%)	Negative	40/448 (9)	0.233	1			
	Positive	8/60 (13)		1.57	0.70 to 3.53		
	Unknown	10/161 (6)		0.68	0.33 to 1.38		
No of sex partners in last 6 months, n (%)	0 or 1	4/69 (6)	0.069	1			
	2–4	17/272 (6)		1.08	0.35 to 3.33		
	5–9	14/148 (9)		1.70	0.54 to 5.36		
	≥10	23/176 (13)		2.44	0.81 to 7.34		
Any symptoms§	No	17/213 (8%)	0.599	1			
	Yes	5/81 (6%)		0.76	0.27 to 2.13		

Continued

Table 4 Continued

		Number of clients with MG-QRAM as a fraction of total clients with MG*	P value†	Univariable analysis OR	95% CI	Multivariable analysis aOR	95% CI
MRAM¶	WT	11/203 (5%)	0.049	1			
	Mutant	46/456 (10%)		1.96	0.99 to 3.86		
NG, n (%)	Negative	50/583 (9)	0.823	1			
	Positive	8/86 (9)		1.09	0.50 to 2.39		
CT, n (%)	Negative	50/558 (9)	0.549	1			
	Positive	8/111 (7)		0.79	0.36 to 1.71		

*If at least one of the samples on the same visit had MG-QRAM, the client was defined as having QRAM.
†X^2 test was used to determine significance.
‡Information was missing for 50 clients.
§Only given for SHC clients, and not from the group of men who had urethritis.
¶Information was missing for 10 clients.
aOR, adjusted OR; CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; MRAM, macrolide resistance-associated mutation; MSM, men who have sex with men; MSW, men who have sex with women only; n.a., not applicable; NG, *Neisseria gonorrhoeae*; QRAM, fluoroquinolone resistance-associated mutation; SHC, sexual health centre; WT, wildtype.

similar to the 10% found in this study among patients with urethritis of the SHC in Amsterdam.

The two risk factors associated with the presence of QRAM in MG-positive clients in our study were being MSM and being from an Asian origin. Previous studies also reported that MSM were disproportionally burdened with QRAM.[22] Yet, Asian origin has not been identified as a risk factor before. Nonetheless, the prevalence of QRAM in Japan,[11] Singapore[24] and China[25] is much higher than in Europe. Travelling to a country with a high prevalence of QRAM or having sex with a person from such a country might be a risk factor for QRAM. MG resistance figures are limited for Asian countries and we do not have travel information, nor period of residence in the Netherlands or information about foreign sex partners from our clients. Future studies should investigate this further.

Treatment options for patients with multidrug-resistant MG are limited. Doxycycline has been frequently used to treat other STIs such as CT, but this drug has a poor clinical efficacy in treatment of urogenital MG infections with cure rates of 22%–45%.[5] Australian studies showed promising results for the treatment of MG-MRAM and multidrug-resistant MG with pristinamycin.[26–28] However, the use of this antibiotic has several disadvantages. Since the drug is registered in only a few countries, patient compliance is low and the antibiotic is relatively expensive.[29] Another option that is more widely available is minocycline, which was also reported to be active against MG-MRAM and multidrug-resistant MG.[28] Up to 46% of the patients reported mild side effects, such as dizziness and headache.[28] More research needs to be done into the effectiveness of both antibiotics.

The difference in resistance profiles of 3 of 30 SHC clients, with two samples from different anatomical locations, can be explained by the clients being infected with different strains at each anatomical location, or by de novo development of mutations associated with resistance. Previously, Cadosch *et al* developed a model to determine the acquired resistance mutations and they found a probability of 12% de novo resistance for MRAM.[30] This is comparable with the percentage that we found.

Strengths of this study include the large number of samples included and the inclusion of samples from patients with urethritis—the main group of patients with an MG infection that will be given antibiotic treatment. As a limitation, we may have underestimated non-synonymous mutations in the *gyrA* gene in the study population as well as rare mutations in the *parC* gene as the QRAM PCR only focuses on the four main mutations of the *parC* gene of MG after which sequence analysis was performed based on QRAM PCR-positive samples for both genetic regions. However, mutations in the *gyrA* gene are less frequently reported and, if detected, usually in combination with mutations in the *parC* gene.[11] The concurrence of non-synonymous mutations in both genes in MG has been described to have an additive effect on fluoroquinolone resistance,[12] and therefore it remains important to monitor for mutations in *gyrA* besides the *parC* gene. Unfortunately, no follow-up information was available on the potential effectiveness of treatment because of the cross-sectional study design.

In this study, we found a 9% prevalence of fluoroquinolone-resistant mutations in clients at the SHCs of Amsterdam and The Hague in the Netherlands. The emergence of antibiotic resistance against the second-line treatment (fluoroquinolones) of MG underlines the necessity of resistance testing for QRAM and the importance of investigating new treatment options to treat symptomatic patients with multidrug-resistant MG.

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Acknowledgements The authors would like to thank Vanessa van Schaik and Nassima Allabbass of the Public Health Service of Amsterdam for assistance with performing the molecular tests.

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Funding Funding was provided by the Public Health Laboratory of GGD Amsterdam. Hologic, San Diego, USA, provided part of the diagnostic tests to detect MG.

Disclaimer Hologic had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval Patients with urethritis and clients of the SHCs in Amsterdam and The Hague were informed of the 'opt-out' system regarding research on remnants of patient material. All data were pseudonymised before analysis. The study protocol was evaluated by the Medical Ethics Committee of the Academic Medical Center in Amsterdam (W18.013#18.024) and deemed not to require a full review of the board, and signed informed consent was not deemed to be required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available.

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REFERENCES

1 Horner PJ, Martin DH. Mycoplasma genitalium infection in men. *J Infect Dis* 2017;216:S396–405.
2 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.
3 Baumann L, Cina M, Egli-Gany D, *et al*. Prevalence of *Mycoplasma genitalium* in different population groups: systematic review andmeta-analysis. *Sex Transm Infect* 2018;94:255–62.
4 Braam JF, van Dam AP, Bruisten SM, *et al*. Macrolide-Resistant Mycoplasma genitalium impairs clinical improvement of male urethritis after empirical treatment. *Sex Transm Dis* 2022;49:360–7.
5 Jensen JS, Bradshaw C. Management of Mycoplasma genitalium infections - can we hit a moving target? *BMC Infect Dis* 2015;15:343.
6 Centers for Disease Control and Prevention. Mycoplasma genitalium, 2021. Available: https://www.cdc.gov/std/treatment-guidelines/mycoplasmagenitalium.htm
7 British Association for Sexual Health and HIV. British association for sexual health and HIV national guideline for the management of infection with Mycoplasma genitalium, 2018. Available: https://www.guidelines.co.uk/sexual-health/bashh-mycoplasma-genitalium-guideline/454722.article
8 Australasian Sexual Health Alliance. STI management guidelines for use in primary care: Mycoplasma genitalium 2021, 2021. Available: https://sti.guidelines.org.au/sexually-transmissible-infections/mycoplasma-genitalium/
9 Jensen JS, Cusini M, Gomberg M, *et al*. *2021 European guideline on the management of Mycoplasma genitalium infections*, 2021.
10 Jensen JS, Bradshaw CS, Tabrizi SN, *et al*. Azithromycin treatment failure in Mycoplasma genitalium-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. *Clin Infect Dis* 2008;47:1546–53.
11 Hamasuna R, Le PT, Kutsuna S, *et al*. Mutations in parC and gyrA of moxifloxacin-resistant and susceptible Mycoplasma genitalium strains. *PLoS One* 2018;13:e0198355.
12 Murray GL, Bodiyabadu K, Danielewski J, *et al*. Moxifloxacin and Sitafloxacin treatment failure in Mycoplasma genitalium infection: association with parC mutation G248T (S83I) and concurrent gyrA mutations. *J Infect Dis* 2020;221:1017–24.
13 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.
14 Braam JF, Hetem DJ, Vergunst CE, *et al*. Evaluating the prevalence and risk factors for macrolide resistance in Mycoplasma genitalium using a newly developed qPCR assay. *PLoS One* 2020;15:e0240836.
15 Martens L, Kuster S, de Vos W, *et al*. Macrolide-Resistant Mycoplasma genitalium in southeastern region of the Netherlands, 2014-2017. *Emerg Infect Dis* 2019;25:1297–303.
16 Nijhuis RHT, Duinsbergen RG, Pol A, *et al*. Prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium and Trichomonas vaginalis including relevant resistance-associated mutations in a single center in the Netherlands. *Eur J Clin Microbiol Infect Dis* 2021;40:591-595.
17 Jensen JS, Björnelius E, Dohn B, *et al*. Use of TaqMan 5' nuclease real-time PCR for quantitative detection of Mycoplasma genitalium DNA in males with and without urethritis who were attendees at a sexually transmitted disease clinic. *J Clin Microbiol* 2004;42:683–92.
18 Hetem DJ, Kuizenga Wessel S, Bruisten SM, *et al*. High prevalence and resistance rates of *Mycoplasma genitalium* among patients visiting two sexually transmitted infection clinics in the Netherlands. *Int J STD AIDS* 2021;32:837–44.
19 Deguchi T, Maeda S, Tamaki M, *et al*. Analysis of the gyrA and parC genes of Mycoplasma genitalium detected in first-pass urine of men with non-gonococcal urethritis before and after fluoroquinolone treatment. *J Antimicrob Chemother* 2001;48:742–4.
20 Kumar S, Stecher G, Li M, *et al*. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–9.
21 Fernández-Huerta M, Barberá MJ, Serra-Pladevall J, *et al*. *Mycoplasma genitalium* and antimicrobial resistance in Europe: a comprehensive review. *Int J STD AIDS* 2020;31:190–7.
22 De Baetselier I, Kenyon C, Vanden Berghe W, *et al*. An alarming high prevalence of resistance-associated mutations to macrolides and fluoroquinolones in *Mycoplasma genitalium* in Belgium: results from samples collected between 2015 and 2018. *Sex Transm Infect* 2021;97:297–303.
23 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.

24 Hart T, Tang WY, Mansoor SAB, *et al*. Mycoplasma genitalium in Singapore is associated with Chlamydia trachomatis infection and displays high macrolide and fluoroquinolone resistance rates. *BMC Infect Dis* 2020;20:314.
25 Ke W, Li D, Tso LS, *et al*. Macrolide and fluoroquinolone associated mutations in Mycoplasma genitalium in a retrospective study of male and female patients seeking care at a STI clinic in Guangzhou, China, 2016-2018. *BMC Infect Dis* 2020;20:950.
26 Bissessor M, Tabrizi SN, Twin J, *et al*. Macrolide resistance and azithromycin failure in a Mycoplasma genitalium-infected cohort and response of azithromycin failures to alternative antibiotic regimens. *Clin Infect Dis* 2015;60:1228–36.
27 Read TRH, Jensen JS, Fairley CK, *et al*. Use of pristinamycin for macrolide-resistant Mycoplasma genitalium infection. *Emerg Infect Dis* 2018;24:328–35.
28 Doyle M, Vodstrcil LA, Plummer EL, *et al*. Nonquinolone Options for the Treatment of *Mycoplasma genitalium* in the Era of Increased Resistance. *Open Forum Infect Dis* 2020;7:ofaa291.
29 Unemo M, Jensen JS. Antimicrobial-Resistant sexually transmitted infections: gonorrhoea and Mycoplasma genitalium. *Nat Rev Urol* 2017;14:139–52.
30 Cadosch D, Garcia V, Althaus CL. De novo mutations drive the spread of macrolide resistant Mycoplasma genitalium: a mathematical modelling study. *BioRxiv* 2018;1:321216.