Public Health England

Protecting and improving the nation's health

Mycoplasma genitalium Antimicrobial Resistance Surveillance (MARS)

Pilot report

Data to March 2019

Contents

Executive summary 3
Background 4
Aims and objectives 5
Methods 5
Results 8
Discussion 18
Conclusion 20
Appendices 21
References 25
Acknowledgements 27

Executive summary

Mycoplasma genitalium is a sexually transmitted pathogen, detectable in up to one-third of individuals attending sexual health clinics (SHCs) in the UK. With limited diagnostics available in some settings, syndromic management is occasionally used despite growing concerns regarding widespread antimicrobial resistance (AMR) and emerging untreatable infections.

The *M. genitalium* Antimicrobial Resistance Surveillance (MARS) pilot was established to assess the feasibility of conducting sentinel surveillance of *M. genitalium* AMR at sentinel SHCs in England. The pilot included data from all consecutive *M. genitalium* specimens collected from 17 clinics between January and March 2019. Clinics performed *M. genitalium* diagnostic testing for those presenting with non-gonococcal urethritis or pelvic inflammatory disease, and their current sex partners. Specimens sent to the Public Health England (PHE) Antimicrobial Resistance in STIs (AMRSTI) national reference laboratory were then tested for molecular markers predictive of macrolide and fluoroquinolone resistance in the *M. genitalium* 23S rRNA and *parC* gene, respectively.

Among 352 individuals included in the MARS pilot, 283 (80%) were symptomatic. Two hundred and fifty-four (72%) were men, 188 (74%) of whom identified as heterosexual. 144 (41%) were of White ethnicity, and 150 (43%) were aged 25- to 34-years-old. Of the corresponding 352 specimens submitted, 249 (71%) were successfully tested for macrolide resistance and, among these, 173 (69%) were predicted to be resistant. Most specimens from women (67%), heterosexual men (66%) and most notably, from gay, bisexual and other men who have sex with men (MSM) (85%) displayed macrolide resistance. Macrolide resistance mutations were frequent among specimens from people of White (66%) and Black or Black British (72%) ethnicity, and were more common among specimens from individuals who had a previous sexually transmitted infection (STI) in the past year (84%) than those who did not (66%). A total of 251 (71%) specimens were successfully tested for fluoroquinolone resistance and 21 (8%) were predicted to be resistant. Predicted resistance to both macrolides and fluoroquinolones was detected in 12 (5%) of 237 specimens.

Azithromycin was prescribed as (a component of) first treatment for 195 individuals. Among those, 21 (11%) failed treatment, as indicated by a positive test-of-cure, all of whom had specimens which had mutations associated with macrolide resistance. Moxifloxacin was prescribed as (a component of) first treatment for 139 individuals, of which 4 (3%) failed treatment. Among those, 3 (75%) had specimens which had a mutation associated with fluoroquinolone resistance.

MARS is a scalable means of continued *M. genitalium* surveillance and will provide a rich resource for informing future updates to management guidelines in the UK.

Background

Mycoplasma genitalium is a sexually transmitted pathogen causing non-gonococcal urethritis (NGU) in men, and cervicitis and pelvic inflammatory disease (PID) in women. The prevalence of *M. genitalium* infection is 1% in the general UK population (aged 16 to 44 years) [1], and 4% to 38% in individuals attending sexual health clinics (SHCs) [2]. Difficulties in culturing *M. genitalium*, compounded by limited molecular diagnostics in some settings, has led to widespread empirical treatment and the emergence of multi-drug resistant infection worldwide.

Azithromycin, a macrolide antibiotic, is recommended as the first-line treatment for *M. genitalium*, ideally when genotypic susceptibility has been confirmed [2]. Doxycycline, a second-generation tetracycline, is commonly given as pre-treatment to lower bacterial load and increase the effectiveness of azithromycin. Moxifloxacin, a broad-spectrum fluoroquinolone, is the second-line treatment.

Macrolide resistance is conferred by a single base mutation which inhibits antimicrobial binding, primarily at position A2058 or A2059 in region V of the 23S rRNA gene (*Escherichia coli* numbering) [3]. Fluoroquinolone resistance is associated with mutations in the quinolone resistance determining region (QRDR) of the *parC* gene, primarily substituting amino acids S83 and D87 (*M. genitalium* numbering) [4]. However, there is limited correlation between mutations in the *parC* gene and moxifloxacin resistance due to insufficient phenotypic minimum inhibitory concentration (MIC) data from clinical isolates and information on clinical outcomes. The relationship between other *M. genitalium* mutations with antimicrobial susceptibility and clinical outcomes is also currently unclear. As such, throughout this report the presence of resistance-associated mutations is conflated with resistance (macrolides only) and predicted resistance (fluoroquinolones).

There is a dearth of national data on *M. genitalium* antimicrobial resistance (AMR). Limited evidence suggests that the prevalence of macrolide resistance is 30% to 40% in the UK [5] and resistance to fluoroquinolones, although much lower, is likely to increase. As there are currently no reliable alternative treatment options, *M. genitalium* can be very challenging to treat. *M. genitalium* AMR data from the Public Health England (PHE) Antimicrobial Resistance in STIs (AMRSTI) national reference laboratory, obtained from 458 specimens received between 1 September 2017 and 28 November 2018, found 71% of referred *M. genitalium* specimens had a mutation associated with macrolide resistance, 8% had mutations predictive of fluoroquinolone resistance, and 7% had both [6]. The AMR data do, however, have some bias as they are from a charged-for-service, and lack important demographic, behavioural and clinical detail. Some samples are from individuals who have already failed treatment and therefore will over-represent resistant strains. As asymptomatic screening for *M. genitalium* is not recommended in the UK,

data held by the PHE AMRSTI national reference laboratory are also more likely to represent symptomatic SHC attendees, rather than be representative of all individuals diagnosed with infection with *M. genitalium* [2].

M. genitalium diagnoses in SHCs are reported through the national GUMCAD STI surveillance system, and *M. genitalium*-positive specimens in primary diagnostic laboratories are also reported through the Second-Generation Surveillance System (SGSS). However, these systems provide no information on treatment regimens and have low-quality AMR data for this species; SGSS reporting is also voluntary and some under-reporting is likely. As existing surveillance systems do not provide AMR and treatment data at a sufficient granularity to inform national management guidelines, the need for a bespoke *M. genitalium* surveillance system, which links enhanced surveillance and antimicrobial susceptibility data, is apparent.

Aims and objectives

Aim

The aim of the *M. genitalium* Antimicrobial Resistance Surveillance (MARS) pilot was to assess the feasibility of conducting sentinel surveillance of *M. genitalium* AMR to guide clinical treatment and inform updates to national management guidelines.

Objectives

The objectives were to:

- determine the demographic, behavioural and clinical characteristics of individuals diagnosed with *M. genitalium* infection at sentinel SHCs
- determine the prevalence of *M. genitalium* macrolide resistance and predicted fluoroquinolone resistance in individuals diagnosed with infection with *M. genitalium* at sentinel SHCs
- investigate risk factors associated with infection with an AMR strain
- describe treatment outcomes for those with infection with an AMR strain

Methods

Participating sites

A convenience sample of 17 SHCs and their 7 associated laboratories agreed to participate in the MARS pilot. These SHCs performed *M. genitalium* testing according to current management guidelines [2], testing people presenting with NGU or PID, and their current sex partners. Pilot sites were geographically dispersed throughout England

and were selected as they were able to:

- perform *M. genitalium* testing and submit specimens
- report enhanced surveillance data on people diagnosed with *M. genitalium* to PHE

Five laboratories serving 14 study sites already performed *M. genitalium* testing locally and routinely sent sequential *M. genitalium* specimens to the PHE AMRSTI national reference laboratory for resistance analysis. Two sites did not have access to local *M. genitalium* testing. For these, all specimens (where testing was indicated) were sent to PHE as usual, and only positives were included in the dataset. The study period was January 2019 to March 2019, however the last date for specimen submission varied by participating site, depending on the number of specimens received.

M. genitalium identification and AMR testing

PHE used an in-house multiplex real-time PCR that incorporates 2 *M. genitalium* targets, *MgPa* and *gap*. The *MgPa* component targets a 78 base pair (bp) region of the *M. genitalium* adhesion protein [7]. The *gap* component targets a 187 bp fragment of the *M. genitalium* glyceraldehyde-3-phosphate dehydrogenase enzyme [8].

Specimens that were positive on the PHE *M. genitalium* assay were tested for mutations associated with macrolide and predicted fluoroquinolone resistance [4,9]. Region V of the 23S rRNA gene (macrolide) and the quinolone resistance determining region (QRDR) of the *parC* gene (fluoroquinolone) were amplified, followed by Sanger sequencing. Predicted antibiotic resistance was inferred from the detection of known *M. genitalium* mutations in these genes. The PHE AMRSTI national reference laboratory reported the results to the referring laboratories within the published turn-around time (8 days).

Enhanced data collection

Enhanced demographic, behavioural and clinical information was collected for each individual with a submitted *M. genitalium*-positive specimen. A list of positive specimens from each SHC and the clinic attendee's date of attendance was securely shared with the clinics at the end of each month during the pilot to request the enhanced surveillance data. Clinic patient identification code, gender and age were used to identify specimens. Clinicians from the participating sites were asked to complete a secure web-based questionnaire to collect the enhanced data for each individual.

Data management

M. genitalium AMR test results from the PHE AMRSTI national reference laboratory and enhanced data from participating sites were linked for each individual on clinic ID, patient ID, age and gender. Linked data were used to create the dataset for analysis.

Specimens were removed from the dataset if they were a duplicated entry (n=4) or were not from a participating SHC (n=6). Where individuals had more than one sample taken at first attendance (n=5), urine samples were prioritised for men (n=3) and vaginal samples for women (n=2) [2]. Due to small sample size in some cells, specimen site, number of sexual partners in the UK and abroad in the 3 months prior to diagnosis of infection with *M. genitalium*, concurrent and previous sexually transmitted infections (STIs) diagnosis variables were re-categorised. Specimen site was grouped: urethral, urine, vaginal and other (cervical and rectal). Number of sexual partners in the UK was grouped: none or one, 2 to 5, 6 or more. Number of partners abroad was grouped: none, one or more. Concurrent STI and previous STI were grouped as 'Yes', 'No' and 'Unknown' and 'Yes', 'No', respectively.

Analysis

The frequencies of demographic, behavioural and clinical characteristics for individuals included in the MARS pilot, as well as (predicted) resistance to macrolides and fluoroquinolones, were determined. The relationships between *M. genitalium* resistance-associated mutations and antimicrobial treatment outcomes were descriptively analysed. Subsequent positive test results (within the pilot period) were used as a proxy for treatment failure.

Data analysis was carried out using STATA v15.1 (StataCorp LP, College Station, TX, USA).

Ethical considerations

The proposal to collect *M. genitalium* surveillance data was approved by the Caldicott Panel of the National Infection Service at PHE (ref #46). PHE's Research Governance, Research Translation & Innovation Division agreed that ethical approval was not required for this pilot. Data are stored according to PHE's Data Protection and Information Governance policies.

Results

Sample characteristics

Between January and March 2019, 362 *M. genitalium*-positive specimens were sent to the PHE AMRSTI national reference laboratory for molecular antimicrobial resistance testing. Enhanced surveillance data were obtained for 352 (97%) individuals, from which 352 specimens were taken, and all of these were included in descriptive analyses.

Among 352 individuals, irrespective of those who had specimens which were successfully screened for mutations associated with resistance, 188 (53%) were heterosexual men, 95 (27%) were women, and 66 (19%) were MSM (Table 1). Most (n=150, 43%) were aged 25- to 34-years-old and were of White (n=144, 41%) or Black or Black British (n=104, 30%) ethnicity.

The majority (n=310, 88%) of individuals were HIV negative. Individuals commonly reported none or one sexual partner in the UK (n=191, 54%), and no sexual partners abroad in the 3 months prior to their diagnosis of infection with *M. genitalium* (n=179, 51%). However, data on whether individuals had had sex abroad were poorly completed (n=188, 53%).

Individuals were predominantly symptomatic (n=283, 80%), although notably fewer women displayed symptoms. Few had more than one *M. genitalium* test per episode (n=72, 21%); only 14 (4%) had more than 3 tests.

Where information on concurrent and previous STI diagnosis was available, 81 (23%) individuals were reported to have one or more concurrent STIs and 61 (17%) had a history of STIs in the past year, most commonly with gonorrhoea or chlamydia.

Table 1. Number of all individuals diagnosed with *M. genitalium* in the MARS pilot, by individuals' characteristics (N=352)†

	Women n (% of N)	Men		Unknown n (% of N)	Total n (% of N)
		Het. Men n (% of N)	MSM n (% of N)		
Individuals (N)	**95**	**188**	**66**	**3**	**352**
Age group (years)					
15-19	9 (9%)	15 (8%)	2 (3%)	1 (33%)	27 (8%)
20-24	33 (35%)	56 (30%)	7 (11%)	1 (33%)	97 (28%)
25-34	40 (42%)	84 (45%)	25 (38%)	1 (33%)	150 (43%)
35-44	13 (14%)	30 (16%)	18 (27%)	0 (0%)	61 (17%)
45-64	0 (0%)	3 (2%)	14 (21%)	0 (0%)	17 (5%)

Ethnicity					
White	43 (45%)	52 (28%)	47 (71%)	2 (67%)	144 (41%)
Mixed	6 (6%)	12 (6%)	2 (3%)	0 (0%)	20 (6%)
Asian or Asian British	7 (7%)	9 (5%)	2 (3%)	0 (0%)	18 (5%)
Black or Black British	14 (15%)	80 (43%)	9 (14%)	1 (33%)	104 (30%)
Other Ethnic Groups	3 (3%)	8 (4%)	1 (2%)	0 (0%)	12 (3%)
Unclassified	22 (23%)	27 (14%)	5 (8%)	0 (0%)	54 (15%)
HIV status					
Positive	2 (2%)	1 (1%)	8 (12%)	0 (0%)	11 (3%)
Negative	86 (91%)	167 (89%)	54 (82%)	3 (100%)	310 (88%)
Unknown	7 (7%)	20 (11%)	4 (6%)	0 (0%)	31 (9%)
Number of UK sexual partners (past three months)					
0-1	73 (77%)	102 (54%)	14 (21%)	2 (67%)	191 (54%)
2-5	16 (17%)	72 (38%)	36 (55%)	1 (33%)	125 (36%)
6+	0 (0%)	4 (2%)	9 (14%)	0 (0%)	13 (4%)
Not reported	6 (6%)	10 (5%)	7 (11%)	0 (0%)	23 (7%)
Number of sexual partners whilst abroad (past three months)					
0	53 (56%)	96 (51%)	28 (42%)	2 (67%)	179 (51%)
1+	6 (6%)	1 (1%)	2 (3%)	0 (0%)	9 (3%)
Not reported	36 (38%)	91 (48%)	36 (55%)	1 (33%)	164 (47%)
Symptoms (at first test)					
Yes	54 (57%)	167 (89%)	60 (91%)	2 (67%)	283 (80%)
No	39 (41%)	17 (9%)	3 (5%)	1 (33%)	60 (17%)
Not reported	2 (2%)	4 (2%)	3 (5%)	0 (0%)	9 (3%)
Specimen					
Urethral	0 (0%)	45 (24%)	9 (14%)	2 (67%)	56 (16%)
Urine	22 (23%)	139 (74%)	55 (83%)	1 (33%)	217 (62%)
Vaginal	65 (68%)	1 (1%)	0 (0%)	0 (0%)	66 (19%)
Other	7 (7%)	0 (0%)	1 (2%)	0 (0%)	8 (2%)
Unknown	1 (1%)	3 (2%)	1 (2%)	0 (0%)	5 (1%)
Tests per *M. genitalium* episode					
One	76 (80%)	149 (79%)	53 (80%)	2 (67%)	280 (79%)
Two or more	19 (20%)	39 (21%)	13 (20%)	1 (33%)	72 (21%)
Grouped concurrent STI					
Yes	17 (18%)	43 (23%)	21 (32%)	0 (0%)	81 (23%)
No	73 (77%)	140 (75%)	43 (65%)	3 (100%)	259 (74%)
Unknown	5 (5%)	5 (3%)	2 (3%)	0 (0%)	12 (3%)

Concurrent STI*					
Chlamydia	8 (8%)	27 (14%)	2 (3%)	0 (0%)	37 (10%)
Gonorrhoea	6 (6%)	16 (8%)	18 (26%)	0 (0%)	40 (11%)
Other Concurrent STI	6 (6%)	3 (2%)	5 (7%)	0 (0%)	14 (4%)
No STI	73 (74%)	141 (73%)	43 (61%)	3 (1%)	260 (72%)
Unknown	5 (5%)	5 (3%)	2 (3%)	0 (0%)	12 (3%)
Grouped previous STI diagnosis (past year)					
Yes	10 (11%)	27 (14%)	24 (36%)	0 (0%)	61 (17%)
No	85 (89%)	161 (86%)	42 (64%)	3 (100%)	291 (83%)
Previous STI diagnosis (past year)*					
Chlamydia	5 (5%)	17 (9%)	8 (11%)	0 (0%)	30 (8%)
Gonorrhoea	1 (1%)	7 (4%)	14 (19%)	0 (0%)	22 (6%)
M. genitalium	2 (2%)	4 (2%)	1 (1%)	0 (0%)	7 (2%)
Other STI	2 (2%)	1 (1%)	10 (13%)	0 (0%)	13 (4%)
No STI	85 (89%)	161 (85%)	42 (56%)	3 (100%)	291 (80%)

† 'Not reported' and 'Unknown' refer to instances where information was unknown or not stated.
* For concurrent or previous STI variables, individuals may have more than one infection.

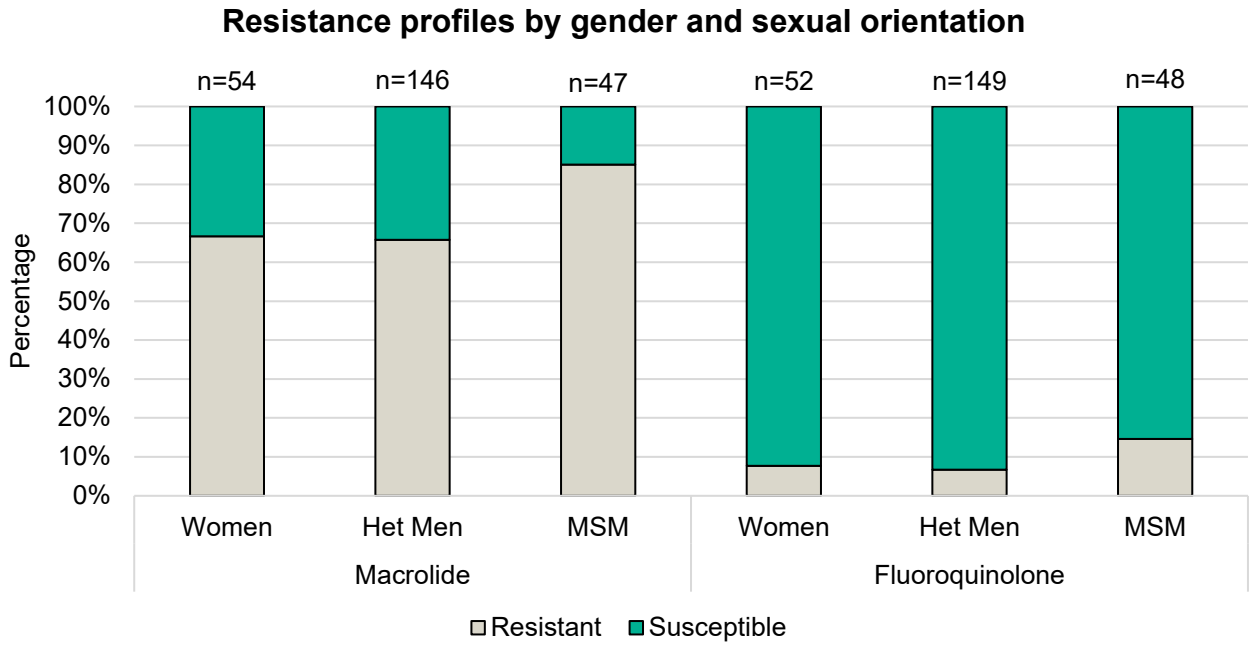
Macrolide and predicted fluoroquinolone resistance

Among 352 specimens, most of which were from urine (62%) or vaginal (19%) samples, macrolide sequence data were available for 249 (71%), fluoroquinolone sequence data were available for 251 (71%) and data for both were available for 237 (67%) specimens. Specimens that could not be tested for macrolide resistance were largely (87%) those that could not be tested for fluoroquinolone resistance, due to low levels or degradation of DNA in the specimen. Specimens which could not be sequenced over-represented women ($p<0.01$).

One hundred and seventy-three (69%) sequenced specimens had a macrolide resistance mutation, consisting of 84 (49%) A2059G, 81 (47%) A2058G, 4 (2%) A2058T and 4 (2%) A2058C mutations. Twenty-one (8%) specimens had a mutation predictive of fluoroquinolone resistance, consisting of 10 (48%) S83I, 9 (43%) D87N, one (5%) D87Y and one (5%) S83R mutations. Predicted dual-drug resistance (i.e. macrolides and fluoroquinolones) was detected in 12 (5%) of 237 specimens which were successfully sequenced for macrolide and fluoroquinolone resistance.

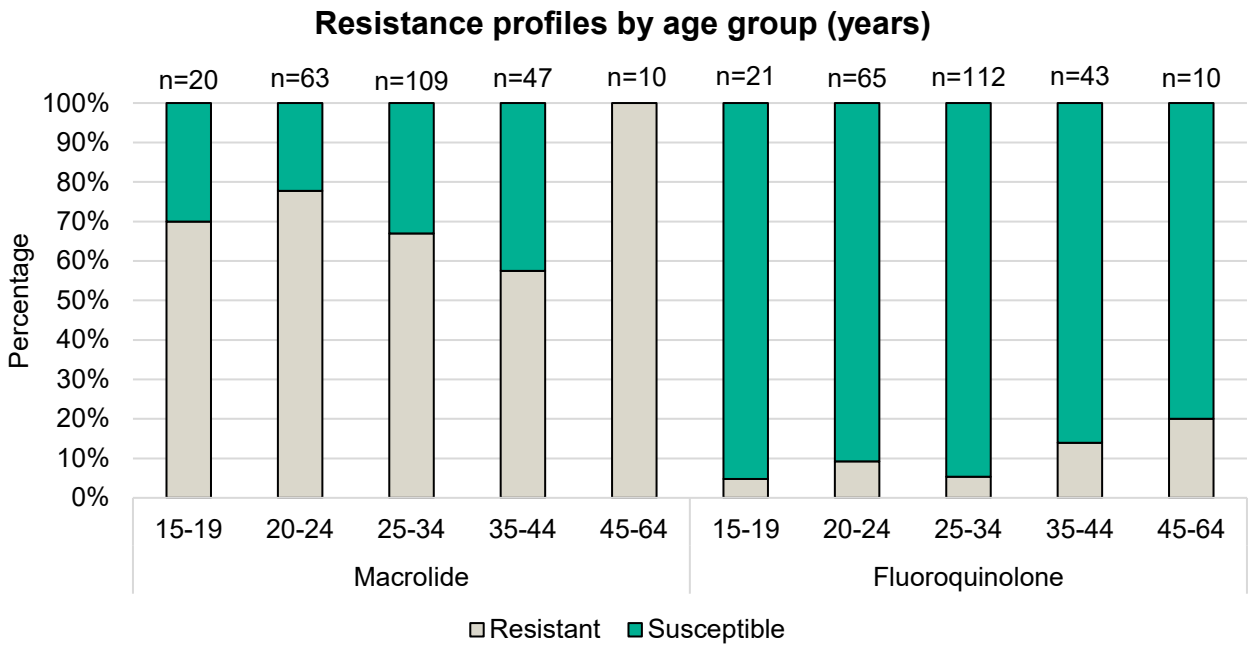
Figures 1 to 6 show the percentage of *M. genitalium* specimens with genotypic macrolide or fluoroquinolone resistance by selected characteristics.

Figure 1. Proportion of *M. genitalium* specimens by macrolide (n=247) or fluoroquinolone (n=249) resistance profile by gender and sexual orientation*



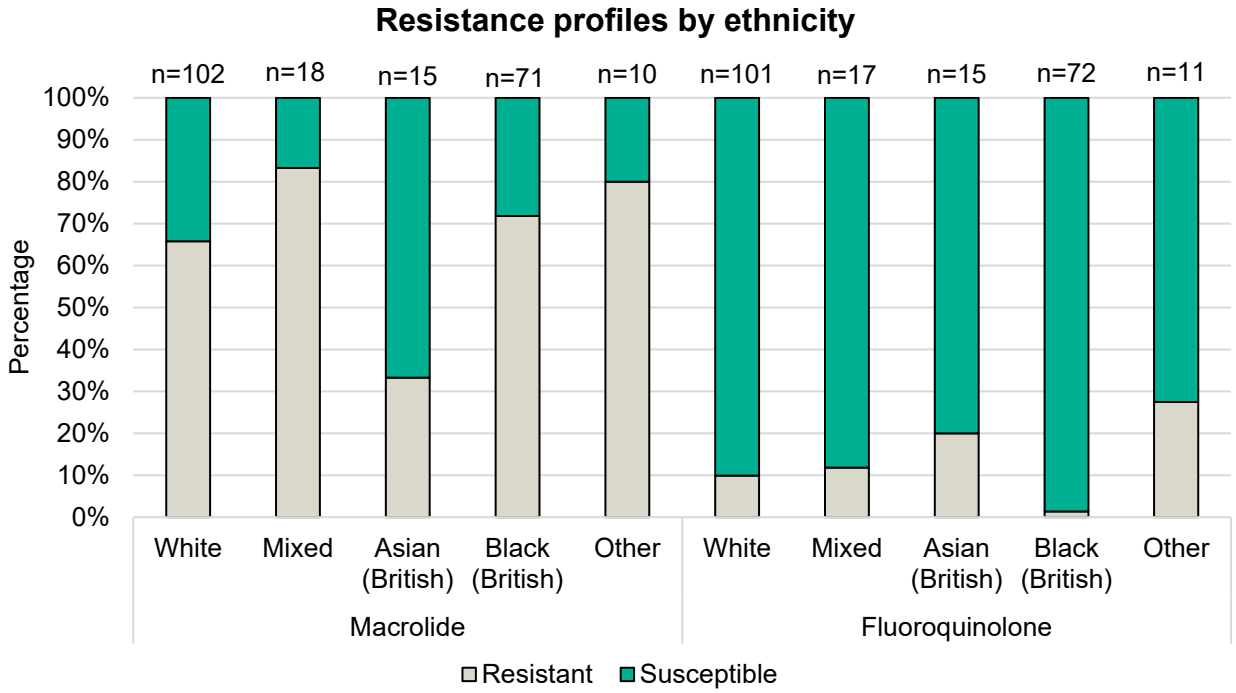
* Graph excludes individuals with unknown gender and sexual orientation for macrolide (n=2) and fluoroquinolone (n=2) analyses, in addition to specimens which could not be tested for mutations associated with macrolide (n=103) or fluoroquinolone resistance (n=101).

Figure 2. Proportion of *M. genitalium* specimens by macrolide (n=249) or fluoroquinolone (n=251) resistance profile by age group*



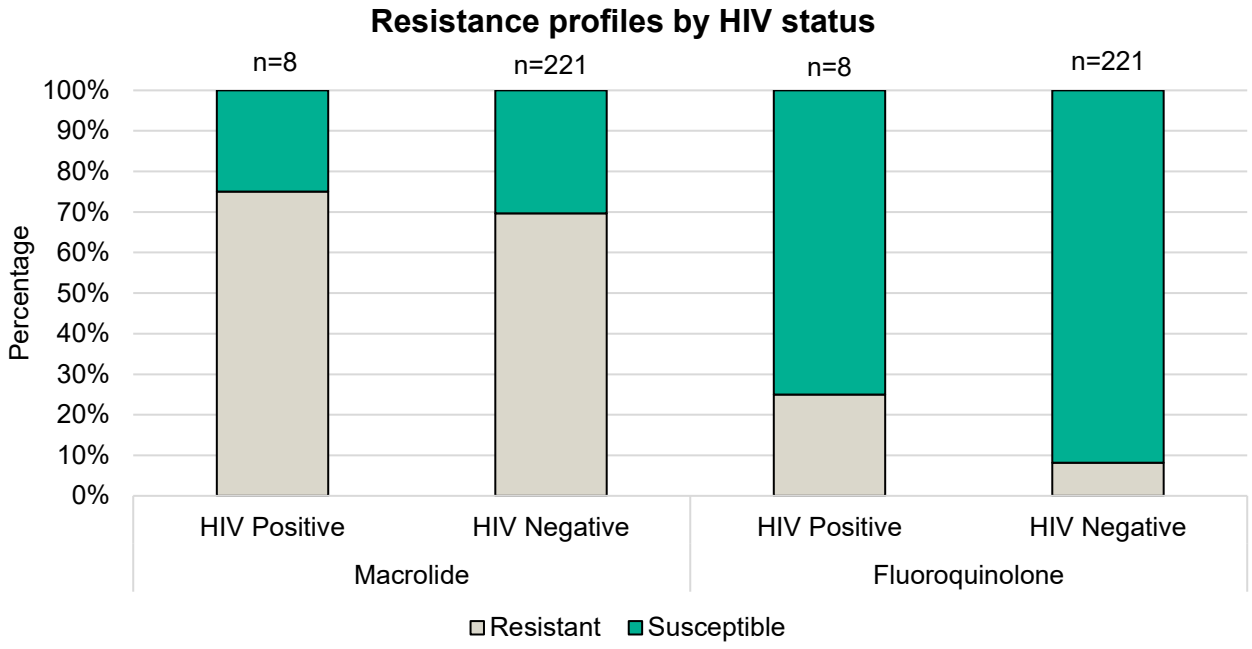
* Graph excludes specimens which could not be tested for mutations associated with macrolide (n=103) or fluoroquinolone resistance (n=101).

Figure 3. Proportion of *M. genitalium* specimens by macrolide (n=216) or fluoroquinolone (n=216) resistance profile by ethnicity*



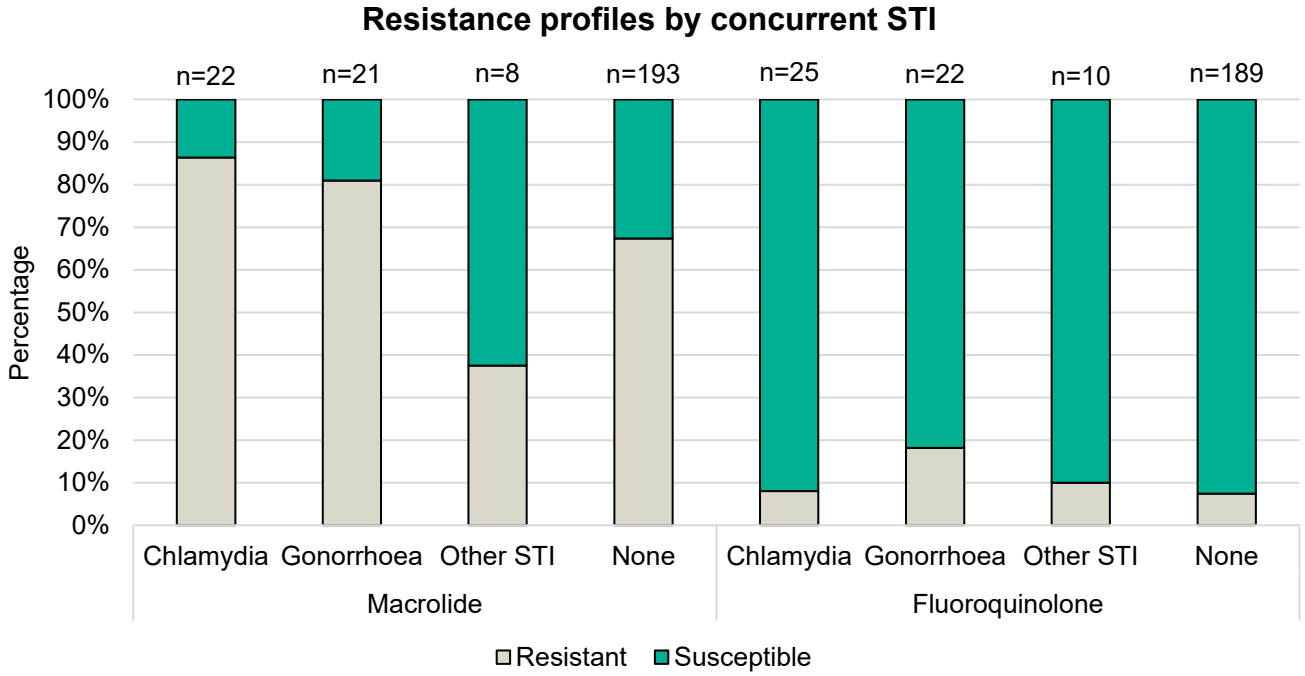
* Graph excludes individuals with unclassified ethnicity for macrolide (n=33) and fluoroquinolone (n=35) analyses, in addition to specimens which could not be tested for mutations associated with macrolide (n=103) or fluoroquinolone resistance (n=101).

Figure 4. Proportion of *M. genitalium* specimens by macrolide (n=229) or fluoroquinolone (n=229) resistance profile by HIV status*



* Graph excludes individuals with unknown HIV status for macrolide (n=20) and fluoroquinolone (n=22) analyses, in addition to specimens which could not be tested for mutations associated with macrolide (n=103) or fluoroquinolone resistance (n=101).

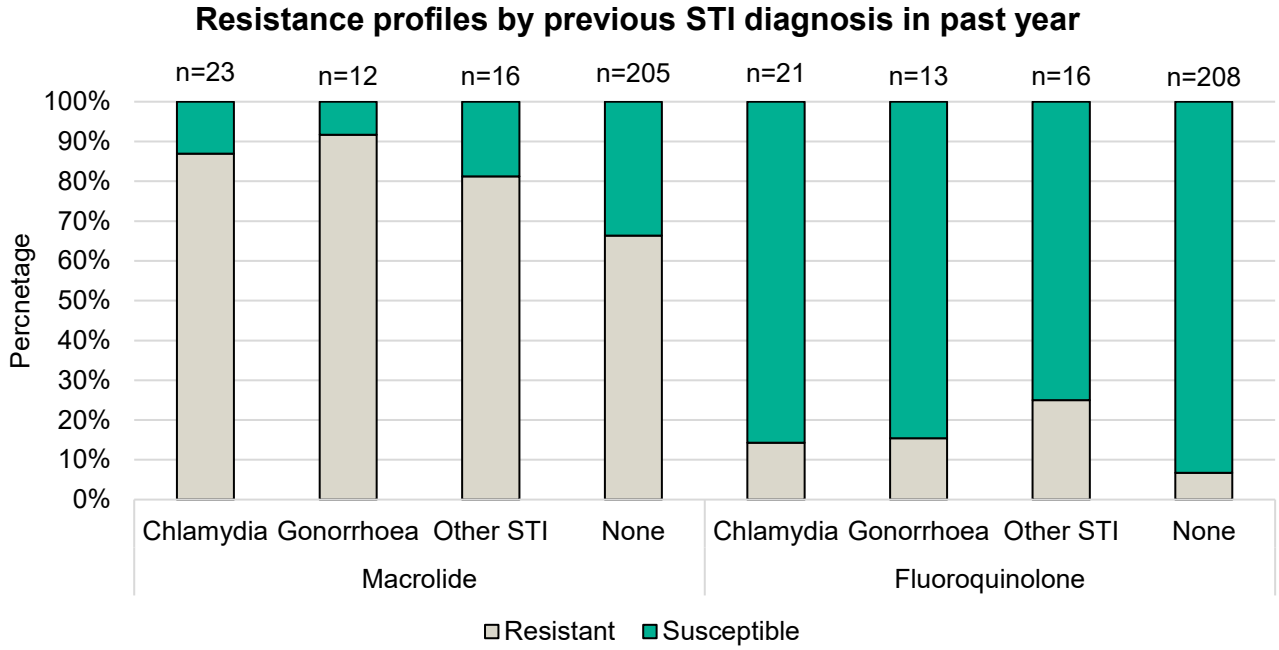
Figure 5. Proportion of *M. genitalium* specimens by macrolide (N=244) or fluoroquinolone (n=246) resistance profile by concurrent STI status*



Note: STI episodes are counted separately.

* Graph excludes individuals with unknown concurrent STI status for macrolide (n=9) and fluoroquinolone (n=10) analyses.

Figure 6. Proportion of *M. genitalium* specimens by macrolide (n=256) or fluoroquinolone (n=258) resistance profile by previous STI diagnosis status



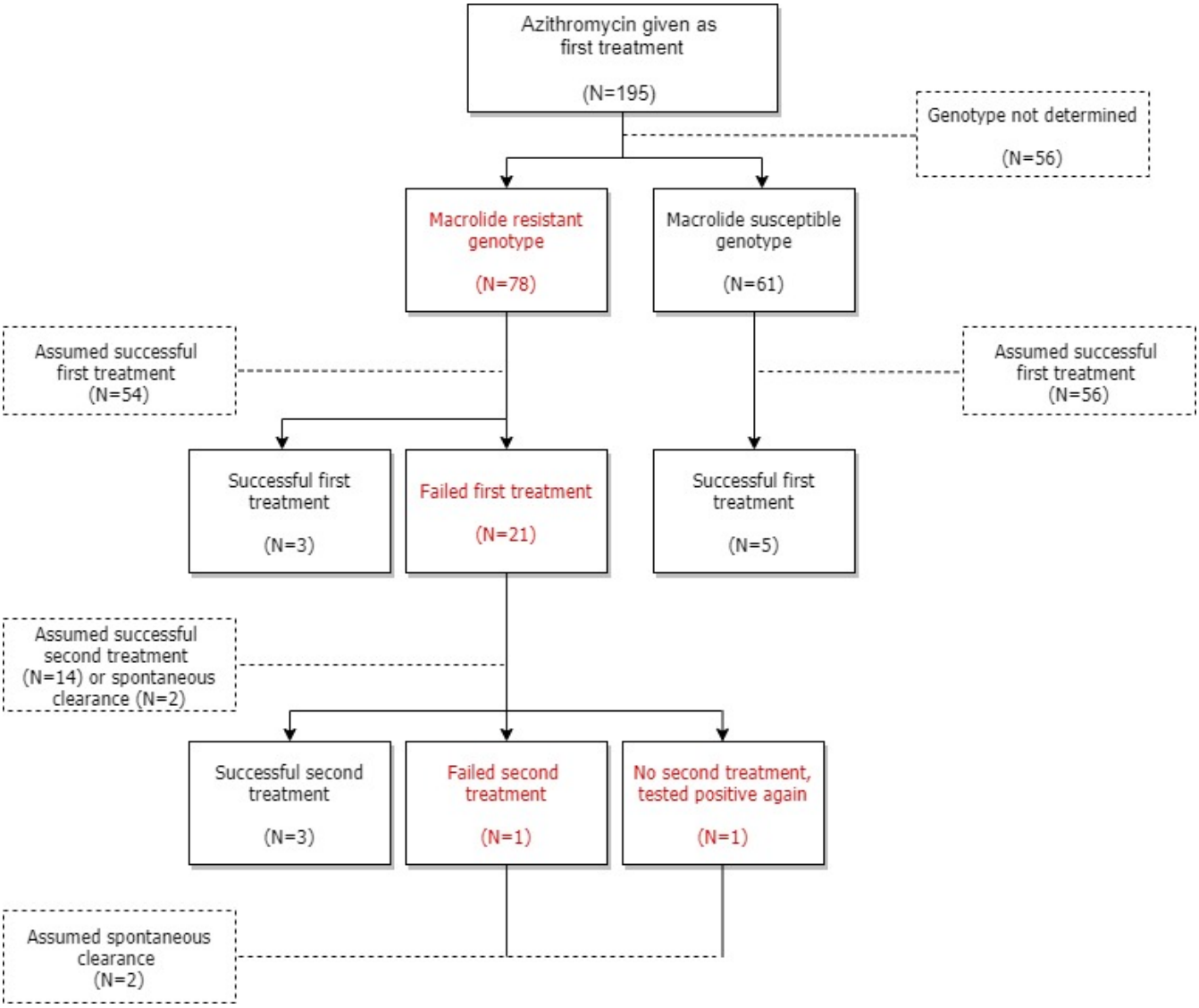
Note: STI episodes are counted separately.

Resistance-associated mutations and treatment outcomes

Management with azithromycin

One hundred and ninety-five individuals were prescribed azithromycin, either alone or as a component of their first treatment for *M. genitalium* (Figure 7). Among those, 169 (87%) individuals also received doxycycline; date of treatment(s) were available for 161/169 (95%) of these. The average time interval between treatment with doxycycline and azithromycin was 15 days. Among the 195 individuals, 16 (8%) received azithromycin only and 10 (5%) received azithromycin in combination with another antibiotic.

Figure 7. Outcome flowchart for those given azithromycin as their first treatment for *M. genitalium* infection (n=195)*



* Individuals who did not have a subsequent test result were assumed to have been successfully treated.

Of 195 individuals who were prescribed azithromycin as their first treatment, 61 (31%) were infected with a macrolide susceptible genotype; all were assumed to be successfully treated as follow-up test-of-cure data were only provided for 5 individuals, all of whom retested negative.

Seventy-eight (40%) of the 195 individuals who were prescribed azithromycin were infected with a macrolide resistant genotype, consisting of 42/78 (54%) A2059G, 33/78 (42%) A2058G and 3/78 (4%) A2058T mutations. Among these, 57/78 (73%) were successfully treated; 3/57 had a negative test-of-cure while the remaining 54/57 were assumed to be successfully treated as follow-up test-of-cure data were not provided. The majority, 45/57 (79%), of individuals were symptomatic at the time of their first positive test. The 3 who had a negative test-of-cure also received a 7-day course of doxycycline, and 2 of these also received moxifloxacin. Specimens from these 3 individuals had the A2059G mutation, and one also had a D87Y mutation. Of the remaining individuals who were assumed to have been successfully treated, 40/54 (74%) also received either a 7 or 14-day course of doxycycline. Thirty-five individuals had date of treatment(s) provided and among these, the average time between treatment with doxycycline and azithromycin was 16 days.

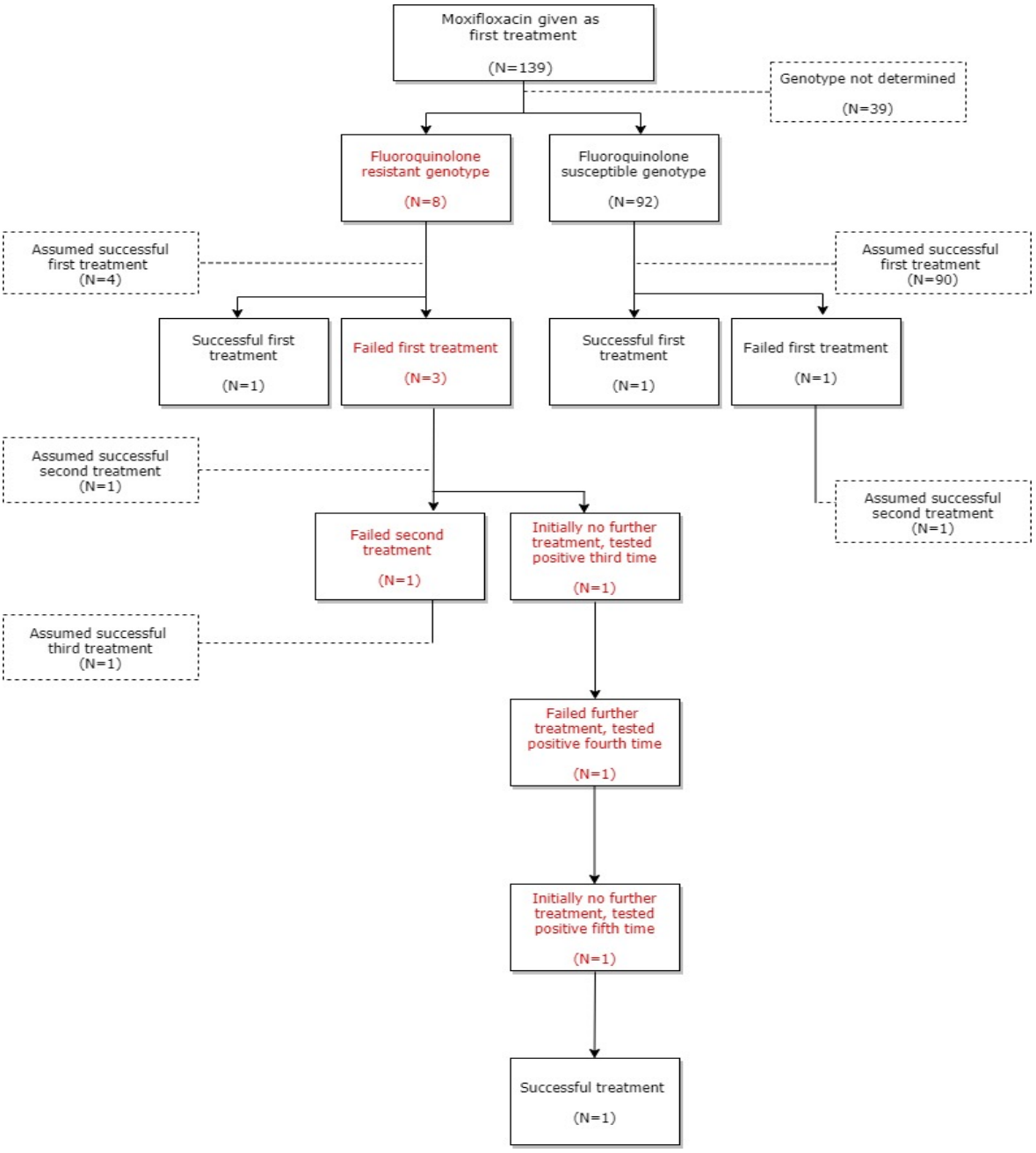
Among the 78 individuals who were prescribed azithromycin and were infected with a macrolide resistant genotype, 21/78 (27%) failed their first treatment, as indicated by a second positive test: 20/21 (95%) had the A2058G mutation and one the A2058T mutation. 18/21 (86%) were symptomatic at the time of their first positive test, and 13/21 (62%) were symptomatic at their second test. 18/21 (86%) who failed treatment with azithromycin had also received a 7-day course of doxycycline. Sixteen of these individuals had date of treatment(s) provided and among these, the average time between treatment with doxycycline and azithromycin was 19 days.

Among the 21 individuals who failed azithromycin treatment, 12 were further treated with moxifloxacin, 4 with moxifloxacin and doxycycline, one with azithromycin and doxycycline and one with pristinamycin. Three individuals received no further treatment, although 2 of these remained symptomatic. Infection resolved in 19/21; 3/19 were negative when tested for a third time and 16/19 were assumed to be successfully treated or to have cleared the infection as further testing data were not available. One individual failed their second treatment (doxycycline and azithromycin, given 8 days apart), as indicated by a third positive test. Another individual also tested positive for a third time, but had not received a second treatment. Both individuals remained symptomatic at the time of their third test, but as neither received any further treatment, nor had a fourth test result reported, it is assumed their infection spontaneously cleared.

Management with moxifloxacin

One hundred and thirty-nine individuals were prescribed moxifloxacin, either alone or as a component of their first treatment for *M. genitalium* (Figure 8). Among those, 84 (60%) also received doxycycline; date of treatment(s) were available for 83 (99%) of these. The average time interval between treatment with moxifloxacin and doxycycline was 19 days. 46 (33%) individuals received moxifloxacin only and 9 (6%) received moxifloxacin in combination with another antibiotic.

Figure 8. Outcome flowchart for those treated with moxifloxacin as their first treatment for *M. genitalium* infection (n=195)*



* Individuals who did not have a subsequent test result were assumed to have been successfully treated.

Of 139 individuals who were prescribed moxifloxacin as their first treatment, 92 (66%) were infected with a fluoroquinolone susceptible genotype. Nearly all (99%) of the 92 individuals were successfully treated, except one who received moxifloxacin in combination with doxycycline and azithromycin and whose specimen had the A2059G mutation associated with macrolide resistance. Upon testing positive for a second time 6 months later, the individual was still symptomatic and was prescribed pristinamycin, which was assumed to have resolved the infection as the individual did not return for test-of-cure.

A further 8/139 (6%) individuals were infected with a fluoroquinolone resistant genotype: 5/8 (63%) had the S83I mutation, 2/8 (25%) had the D87N mutation, and one had the D87Y mutation; 7/8 (88%) also had the A2059G mutation, indicative of dual-drug resistance. Among these, 5/8 (63%) were successfully treated; 1/5 had a negative test-of-cure while the remaining 4/5 were assumed to be successfully treated as follow-up test-of-cure data were not provided. All 5 were symptomatic at the time of their first positive test. The individual who had a negative test-of-cure had a specimen with D87Y and A2059G mutations and also received a 7-day course of doxycycline and azithromycin, 4 days apart. Of the remaining individuals who were assumed to be successfully treated, 4/4 also received either a 7 or 14-day course of doxycycline and 2/4 were given additional treatment with azithromycin. Two specimens from these 4 individuals had the D87N and A2059G mutations, one had S83I and A2058G mutations, and one had the S83I mutation.

Among the 8 individuals who were prescribed moxifloxacin and were infected with a fluoroquinolone resistant genotype, 3/8 (38%) individuals failed their first treatment (10-day course of moxifloxacin-only), as indicated by a second positive test; all 3 specimens had both S83I and A2059G mutations. Of the 3 who failed treatment, one individual, who was symptomatic at the time of their first test but asymptomatic at the second, was treated with a second (14-day) course of moxifloxacin-only. It is assumed the individual cleared the infection as a third test result was not reported. The second individual, who had remained symptomatic following the first treatment, received doxycycline and, 5 days later, pristinamycin as a secondary treatment, which failed, and was subsequently given 100 mg doxycycline twice-daily for 4 weeks. This treatment was assumed successful as no further test results were reported. Initially, the third individual did not receive further treatment, despite remaining symptomatic at the time of their second test. They did, however, subsequently receive azithromycin and doxycycline, which failed, after testing positive for a third time, at which point they were still symptomatic. After testing positive for a fourth and fifth time, the latter occurring after the MARS study period, the individual was prescribed pristinamycin. Their symptoms (dyspareunia) then resolved, and they tested negative when tested for the sixth time.

Discussion

In this pilot of enhanced surveillance for *M. genitalium* AMR, we found very high levels of macrolide resistance (69%), while predicted fluoroquinolone (8%) and dual-drug (macrolide and fluoroquinolone) resistance were less prevalent (5%). Although macrolide resistance was universally high, specimens from MSM (85%), people of Black or Black British ethnicity (72%), and those who had a previous STI diagnosis in the past year (84%) had notably high rates of resistance. Greater rates of macrolide resistance were also observed among specimens from people of Mixed ethnicity, those from "Other" ethnicities and those aged 45- to 64-years-old, but there were few individuals in these groups (Appendix 1). Where fluoroquinolone resistance was detected, specimens taken from people with a previous STI diagnosis in the past year were twice as likely to have a mutation associated with fluoroquinolone resistance compared to specimens from individuals who did not have a previous STI diagnosis, although the sample size was small (Appendix 2).

Where azithromycin was prescribed, either alone or as a component of first treatment for *M. genitalium*, treatment failure was recorded in one in 10 instances (21/195). Macrolide resistance mutations, mostly A2058G, were detected in all instances of treatment failure with azithromycin. All individuals with infection with macrolide susceptible *M. genitalium* were successfully treated with azithromycin, although few returned for a test-of-cure. Treatment success was therefore inferred from the absence of a positive follow-up test, likely over-estimating the number of individuals who cleared infection. Interestingly, 57/78 (73%) *M. genitalium* infections with mutations associated with macrolide resistance, predominantly A2059G, were clinically cured using azithromycin as a component of the first treatment; note, 54/57 (95%) were assumed to be successfully treated, as they did not return for a test-of-cure.

Given that the majority of macrolide resistant infections were also treated with doxycycline, with an average time interval of 16 days between different treatments, it is possible that doxycycline contributed towards the successful treatment of macrolide resistant infections. However, of the 21 individuals who were infected with a macrolide resistant strain and who had a positive test-of-cure, 18 (86%) were given doxycycline in addition to azithromycin, yet failed treatment. Among these, the average time interval between doxycycline and azithromycin treatments was 19 days. Although it was possible to discern the time interval between different treatments where date of treatment(s) data were provided, it was assumed that doxycycline was given prior to azithromycin if multiple antibiotics were prescribed at one attendance, as national guidelines currently advise giving doxycycline prior to treatment with azithromycin [2]. However, as it was not possible to definitively determine which antibiotics were given first where multiple antibiotics were recorded at one appointment, further data are needed to provide insight into the effectiveness of using doxycycline pre-treatment. Nevertheless, while it cannot

be excluded that some infections may have spontaneously cleared or that doxycycline pre-treatment improved treatment effectiveness, these data suggest that genotypic macrolide resistance is not unequivocally predictive of treatment failure.

The number of infections with mutations associated with fluoroquinolone resistance was too low to allow firm conclusions about the significance of different *parC* mutations. However, the D87N and S83I mutations have the strongest published evidence for being predictive of treatment failure [4,10]. Among the 8 specimens that displayed a fluoroquinolone resistant genotype, 5 had the S83I mutation, 3 of which failed treatment; the 2 additional infections which had the S83I mutation were assumed to be clinically cured with moxifloxacin as no test-of-cure result was reported. The 2 infections with the D87N mutation were also assumed to be clinically cured for this reason. The remaining individual with a specimen that displayed the fluoroquinolone resistant genotype, the only D87Y mutation, had a negative test-of-cure after receiving treatment with doxycycline, azithromycin and moxifloxacin, staggered at 4 and 12 days between different treatments. Despite a small sample size, data from the MARS pilot are therefore suggestive of an association between the S83I mutation and clinical fluoroquinolone resistance. Of note, all individuals with infections with predicted fluoroquinolone resistance who were given doxycycline as well as moxifloxacin (5/8) were assumed to be clinically cured. The remaining 3 who were given moxifloxacin alone did not clear their infection. This implies that doxycycline may have a role in combination therapy with moxifloxacin, but more data are needed.

In addition to the need for more data, there were several limitations of the MARS pilot. National guidelines stipulate *M. genitalium* testing is indicated if an individual is symptomatic or is a current sexual partner of persons infected with *M. genitalium* [2]. As the clinics included in this pilot follow the national testing criteria, results reported here are not representative of all individuals with infection with *M. genitalium*. Indeed, the vast majority of individuals included in this pilot were symptomatic, so particular caution is needed before extrapolating findings to asymptomatic *M. genitalium* infections. Moreover, in addition to a relatively small sample size, the absence of a question on reason for repeat testing hampered interpretation. Positive (second, third, fourth) test was used as a proxy for treatment failure, however, repeat tests may have been due to reinfection or persistent infection due to poor compliance with treatment, rather than true treatment failure. As the absence of a repeat positive test was used as a proxy for successful treatment, clinical cure rates also may have been over-estimated by classifying those who failed to return for a test-of-cure as successfully treated.

Conclusion

The MARS pilot showed that a sentinel surveillance programme for monitoring *M. genitalium* AMR is feasible. Data presented here corroborate earlier reports of extensive macrolide resistance in *M. genitalium* with accompanying demographic, behavioural and clinical detail, in addition to evidence of dual-drug resistance [6]. As with PHE's Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP), the 2-month collection period for the MARS pilot provided sufficient data for preliminary analyses and, based on enthusiasm for further rounds, was achievable for collaborating sites and the PHE AMRSTI national reference laboratory. Subsequent MARS surveys will seek to address the limitations of this pilot by increasing the sample size to improve statistical power for determining AMR risk factors and by including additional questions about treatment, such as the timings of sequential therapies, follow-up, and reason(s) for repeat testing or failure to return for test-of-cure. Continued surveillance through MARS will provide evidence to inform clinical management guidelines.

Appendices

1. Macrolide resistance by individuals' characteristics

Table 2. *M. genitalium* macrolide resistance by individuals' characteristics (n=249)*

Characteristics	Resistant (n)	Susceptible	Total (N)	Resistant (n of N, %)
Specimens	**173**	**76**	**249**	**69%**
Gender and sexual orientation				
Women	36	18	54	**67%**
Het. Men	96	50	146	**66%**
MSM	40	7	47	**85%**
Unknown	1	1	2	**50%**
Age group (years)				
15-19	14	6	20	**70%**
20-24	49	14	63	**78%**
25-34	73	36	109	**67%**
35-44	27	20	47	**57%**
45-64	10	0	10	**100%**
Ethnicity				
White	67	35	102	**66%**
Mixed	15	3	18	**83%**
Asian or Asian British	5	10	15	**33%**
Black or Black British	51	20	71	**72%**
Other Ethnic Groups	8	2	10	**80%**
Unclassified	27	6	33	**82%**
HIV status				
Negative	154	67	221	**70%**
Positive	6	2	8	**75%**
Unknown	13	7	20	**65%**
Total UK partners (past 3 months)				
0-1	91	41	132	**69%**
2-5	62	28	90	**69%**
6+	7	2	9	**78%**
Not reported	13	5	18	**72%**
Number of partners whilst abroad (past 3 months)				
0	88	42	130	**68%**
1+	5	1	6	**83%**
Not reported	80	33	113	**71%**
Symptoms (at first test)				
No	24	14	38	**63%**
Yes	143	61	204	**70%**
Unknown	6	1	7	**86%**
Specimen				
Urethral	33	16	49	**67%**
Urine	111	44	155	**72%**
Vaginal	24	11	35	**69%**
Other	4	2	6	**67%**
Unknown	1	3	4	**25%**

Concurrent STI				
No	129	63	192	**67%**
Yes	38	10	48	**79%**
Unknown	6	3	9	**67%**
Concurrent chlamydia				
No	148	70	218	**68%**
Yes	19	3	22	**86%**
Unknown	6	3	9	**67%**
Concurrent gonorrhoea				
No	150	69	219	**69%**
Yes	17	4	21	**81%**
Unknown	6	3	9	**67%**
Previous STI diagnosis (in the past year)				
No	136	69	205	**66%**
Yes	37	7	34	**84%**
Previous chlamydia diagnosis (in the past year)				
No	153	73	226	**68%**
Yes	20	3	23	**87%**
Previous gonorrhoea diagnosis (in the past year)				
No	162	75	237	**68%**
Yes	11	1	12	**92%**
Previous *M. genitalium* diagnosis (in the past year)				
No	169	75	244	**69%**
Yes	4	1	5	**80%**

* Excludes specimens which could not be tested for mutations associated with macrolide resistance (n=103).

2. Fluoroquinolone resistance by individuals' characteristics

Table 3. *M. genitalium* fluoroquinolone resistance by individuals' characteristics (n=251)*

Characteristics	Resistant (n)	Susceptible	Total (N)	Resistant (n of N, %)
Specimens	**21**	**230**	**251**	**8%**
Gender and sexual orientation				
Women	4	48	52	**8%**
Het. Men	10	139	149	**7%**
MSM	7	41	48	**15%**
Unknown	0	2	2	**0%**
Age group (years)				
15-19	1	20	21	**5%**
20-24	6	59	65	**9%**
25-34	6	106	112	**5%**
35-44	6	37	43	**14%**
45-64	2	8	10	**20%**
Ethnicity				
White	10	91	101	**10%**
Mixed	2	15	17	**12%**
Asian or Asian British	3	12	15	**20%**
Black or Black British	1	71	72	**1%**
Other Ethnic Groups	3	8	9	**27%**
Unclassified	2	33	35	**6%**
HIV status				
Negative	18	203	221	**8%**
Positive	2	6	8	**25%**
Unknown	1	21	22	**5%**
Total UK partners (past 3 months)				
0-1	9	124	133	**7%**
2-5	11	81	92	**12%**
6+	1	9	10	**10%**
Not reported	0	16	16	**0%**
Number of partners whilst abroad (past 3 months)				
0	10	123	133	**8%**
1+	1	4	5	**20%**
Not reported	10	103	113	**9%**
Symptoms (at first test)				
No	1	37	38	**3%**
Yes	20	186	206	**10%**
Unknown	0	7	7	**0%**
Specimen				
Urethral	2	47	49	**4%**
Urine	17	142	159	**11%**
Vaginal	1	22	23	**3%**
Other	1	4	5	**20%**
Unknown	0	4	4	**0%**

Concurrent STI				
No	14	174	188	**7%**
Yes	6	47	53	**11%**
Unknown	1	9	10	**10%**
Concurrent chlamydia				
No	18	198	216	**8%**
Yes	2	23	25	**8%**
Unknown	1	9	10	**10%**
Concurrent gonorrhoea				
No	16	203	219	**7%**
Yes	4	18	22	**18%**
Unknown	1	9	10	**10%**
Previous STI diagnosis (in the past year)				
No	14	194	208	**7%**
Yes	7	36	43	**16%**
Previous chlamydia diagnosis (in the past year)				
No	18	212	230	**8%**
Yes	3	18	21	**14%**
Previous gonorrhoea diagnosis (in the past year)				
No	19	219	238	**8%**
Yes	2	11	13	**15%**
Previous *M. genitalium* diagnosis (in the past year)				
No	20	226	246	**8%**
Yes	1	4	5	**20%**

* Excludes specimens which could not be tested for mutations associated with fluoroquinolone resistance (n=101).

References

[1] Sonnenberg P, Ison CA, Clifton S, Field N, Tanton C, Soldan K, 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 [cited 2020 June 05]; 44(6): 1982-94. Available from DOI: 10.1093/ije/dyv194

[2] Soni S, Horner P, Rayment M, Pinto-Sander N, Naous N, Parkhouse A, et al. British Association for Sexual Health and HIV national guideline for the management of infection with Mycoplasma genitalium (2018) [Online]. 2018 [cited 2020 June 03]. Available from: https://www.bashh.org/guidelines/

[3] Bradshaw C, Jensen J, Tabrizi S, Read T, Garland S, Hopkins C, et al. Azithromycin failure in Mycoplasma genitalium urethritis. Emerg Infect Dis. 2006 [cited 2020 July 07];12(7):1149–52. Available from DOI:10.3201/eid1207.051558

[4] Deguchi T, Maeda S, Tamaki M, Yoshida T, Ishiko H, Ito 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 [cited 2020 June 05]; 48(5): 742-4. Available from DOI: 10.1093/jac/48.5.742

[5] Conway R, Cook S, Soni S. Antibiotic treatment of Mycoplasma genitalium infection [Online]. Pharm J. 2019 [cited 2020 July 30]. Available from: https://www.pharmaceutical-journal.com/cpd-and-learning/learning-article/antibiotic-treatment-of-mycoplasma-genitalium-infection/20206592.article?firstPass=false

[6] Day M, Cole M, Patel H, Fifer H, Woodford N, Pitt R. P616 Predictive macrolide and fluoroquinolone resistance markers in mycoplasma genitalium from the UK and Ireland. Sexually Transmitted Infections. 2019 [cited 2020 July 30]; 95: A272.2-A272. Available from DOI: 10.1136/sextrans-2019-sti.684

[7] Jensen JS, Bjornelius E, Dohn B, Lidrink P. 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 [cited 2020 June 05]; 42(2): 683-92. Available from DOI: 10.1128/jcm.42.2.683-692.2004

[8] Svenstrup HF, Jensen JS, Bjornelius E, Lidbrink P, Birkelund S, Christiansen G. Development of a quantitative real-time PCR assay for detection of Mycoplasma genitalium. J Clin Microbiol. 2005 [cited 2020 June 05]; 43(7): 3121-8. Available from DOI: 10.1128/JCM.43.7.3121-3128.2005

[9] Li X, Atkinson TP, Hagood J, Makris C, Duffy L, Waites K. Emerging macrolide resistance in Mycoplasma pneumoniae in children: detection and characterization of resistant isolates. Pediatr Infect Dis J. 2009 [cited 2020 June 05]; 28(8): 693-6. Available from DOI: 10.1097/INF.0b013e31819e3f7a

[10] Pitt R, Fifer H, Woodford N, Alexander S. Detection of Markers Predictive of Macrolide and Fluoroquinolone Resistance in Mycoplasma genitalium From Patients Attending Sexual Health Services in England. Sex Transm Infect. 2018 [cited 2020 June 26]; 94(1):9-13. Available from DOI: 10.1136/sextrans-2017-053164

Acknowledgements

The MARS team would like to thank the collaborating centres for their support, SHCs for the prompt submission of clinical data and laboratories for sending isolates to the PHE AMRSTI national reference laboratory in Colindale.

Collaborating centres:
Birmingham (J Phattey, J Ross, N Wickramasinghe, K Dedicoat), Bristol (P Horner, M Clarke, P Muir), Carlisle (M Phillips, C Graham, D Padgett), Leeds (A Evans, J Bulman, E Page, N Fearnley), London Charing Cross, Chelsea and Westminster (M Rayment, M Rebec, N Nwokolo, V Tittle, L Garvey, A Sullivan, R Brum), CNWL Sexual Health (E Jungmann, R Browne, B Macrae, A Richardson, S Candfield, D Nugent), London St Thomas' (A Nori, P Cliff).

About Public Health England

Public Health England exists to protect and improve the nation's health and wellbeing, and reduce health inequalities. We do this through world-leading science, research, knowledge and intelligence, advocacy, partnerships and the delivery of specialist public health services. We are an executive agency of the Department of Health and Social Care, and a distinct delivery organisation with operational autonomy. We provide government, local government, the NHS, Parliament, industry and the public with evidence-based professional, scientific and delivery expertise and support.

Public Health England
Wellington House
133-155 Waterloo Road
London SE1 8UG
Tel: 020 7654 8000

www.gov.uk/phe
Twitter: @PHE_uk
www.facebook.com/PublicHealthEngland

Prepared by: Helen Fifer, Rachel Merrick, Mandy Yung, Rachel Pitt, Hester Allen, Michaela Day, Katy Sinka, Gwenda Hughes, Neil Woodford, Hamish Mohammed, Michelle Cole

For queries relating to this document, please contact: helen.fifer@phe.gov.uk

Published: November 2020
PHE gateway number: GW-1722

PHE supports the UN Sustainable Development Goals

SUSTAINABLE DEVELOPMENT GOALS