Public Health England

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Mycoplasma genitalium Antimicrobial Resistance Surveillance (MARS)

Second pilot report

Data to March 2020

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Executive summary

Mycoplasma genitalium is a sexually transmitted pathogen, detectable in up to one in 3 individuals attending sexual health clinics (SHCs) in the UK. There is growing concern regarding widespread antimicrobial resistance (AMR) and untreatable infections.

Following the first pilot in 2019, a second pilot of *M. genitalium* Antimicrobial Resistance Surveillance (MARS) was conducted to improve data quality and to inform the development of routine surveillance of *M. genitalium* AMR at sentinel SHCs in England. It included data from all consecutive *M. genitalium* specimens collected from 15 SHCs between January and March 2020. Clinics performed *M. genitalium* diagnostic testing for those presenting with non-gonococcal urethritis or pelvic inflammatory disease, and for the current sex partners of those who tested positive for *M. genitalium*. Positive specimens sent to the Public Health England (PHE) Antimicrobial Resistance in STIs (AMRSTI) national reference laboratory were tested for molecular markers predictive of macrolide and fluoroquinolone resistance in the *M. genitalium* 23S rRNA and *parC* genes, respectively.

Among 251 individuals included in the second MARS pilot, 190 (76%) were symptomatic. The sample included 131 (52%) heterosexual men, 54 (22%) gay, bisexual or other men who have sex with men (MSM) and 61 (24%) women. One-hundred and twenty-four (49%) individuals were of White ethnicity, and 110 (44%) were aged 25 to 34 years old. Of the corresponding 251 specimens submitted, 230 (92%) were successfully tested for macrolide resistance and, among these, 159 (69%) were predicted to be resistant. Most specimens from women (55%), heterosexual men (69%) and notably, from MSM (88%) displayed macrolide resistance. Macrolide resistance mutations were more commonly detected among specimens from people of Black or Black British (80%) ethnicity compared to those who were White (62%), as well as among specimens from individuals who had a previous sexually transmitted infection (STI) in the past year (79%) compared to those who did not (68%). Of the 251 specimens, 233 (93%) were successfully tested for fluoroquinolone resistance and 26 (11%) were predicted to be resistant. Predicted resistance to both macrolides and fluoroquinolones was detected in 23 (10%) of 223 specimens.

As recommended in the national management guidelines, most individuals received either azithromycin or moxifloxacin (with or without doxycycline pre-treatment) for treating infection with *M. genitalium*. Azithromycin was prescribed either alone or in combination with another antibiotic for 105 individuals; of these, 16 (15%) failed initial treatment, as indicated by a positive test-of-cure, 14 of whom had specimens with mutations associated with macrolide resistance. Moxifloxacin was prescribed either alone or in combination with another antibiotic for 99 individuals, of which one (1%) failed initial treatment.

Background

Mycoplasma genitalium is a sexually transmitted pathogen which is associated with 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 up 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 infections 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 recommended second-line treatment (2).

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 substitutions in ParC and moxifloxacin resistance due to insufficient phenotypic minimum inhibitory concentration (MIC) data from isolates and associated clinical outcomes. The relationship between other *M. genitalium* mutations (including mutations within the *gyrA* gene) with antimicrobial susceptibility and clinical outcomes is also currently unclear, however there have been reports of clinical treatment failure where these mutations are present (5, 6, 7). As such, throughout this report the presence of resistance-associated mutations or amino acid substitutions is assumed to confer either resistance to macrolides or predicted resistance to fluoroquinolones.

Retrospective analysis of *M. genitalium* antimicrobial resistance (AMR) data from the Public Health England (PHE) Antimicrobial Resistance in STIs (AMRSTI) national reference laboratory found that 71% of 458 *M. genitalium* specimens referred between 1 September 2017 and 28 November 2018 had a mutation associated with macrolide resistance, 8% had substitutions predictive of fluoroquinolone resistance, and 7% had both (8). Similarly, data from the first pilot of *M. genitalium* Antimicrobial Resistance Surveillance (MARS) in 2019 (9), which included 352 specimens, found 69% of specimens had macrolide resistance and 8% had fluoroquinolone resistance. The first pilot highlighted that additional data were required to identify population groups at greater risk of resistant infection and treatment failure. The need for more granular information regarding clinical outcomes was also identified from the first pilot, including timings of sequential therapies and reason(s) for repeat testing or failure to return for a test-of-cure, to ensure accuracy of clinical cure rates.

Existing data on *M. genitalium* are limited in scale and lack sufficient detail regarding AMR and clinical outcomes to inform national management guidelines. Therefore, there is need for a strengthened *M. genitalium* surveillance system, which links epidemiological antimicrobial susceptibility and clinical outcome data.

Aims and objectives

Aim

The aim of the second *M. genitalium* Antimicrobial Resistance Surveillance (MARS) pilot was to refine, improve and gather more recent data to assess the distribution and trends of *M. genitalium* AMR and guide national management guidelines.

Objectives

The objectives of the second pilot are shown below. These were to:

- gather data on 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 *M. genitalium* at sentinel SHCs
- implement data collection improvements based on the lessons learnt from the first MARS pilot in 2019
- improve the monitoring of treatment outcomes for those infected with an AMR strain

Methods

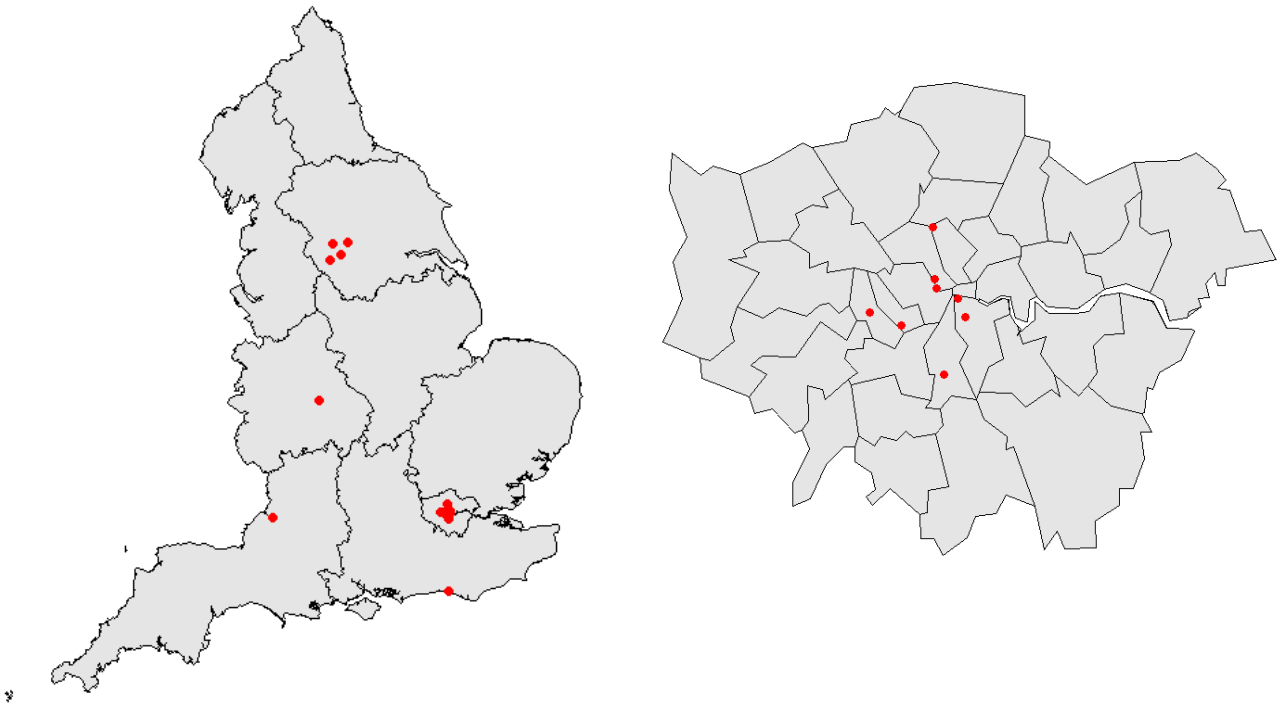
Participating sites

A convenience sample of 15 SHCs and their 7 associated laboratories agreed to be part of the second pilot. These SHCs performed *M. genitalium* testing according to current management guidelines (2), testing people presenting with NGU or PID, and current sex partners of those who tested positive for *M. genitalium*. Figure 1 shows the distribution of SHCs participating in the second pilot within England, 8 of which were in London. Sites were selected that were able to:

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

The data collection period was January to March 2020.

Figure 1. Map showing 15 sentinel sexual health clinics across England, with London shown at larger scale



M. genitalium identification and AMR testing

PHE used an in-house multiplex real-time PCR that incorporates 2 *M. genitalium*-specific targets, *MgPa* and *gap* to confirm specimen positivity. The *MgPa* component targets a 78-base pair (bp) region of the *M. genitalium* adhesion protein (10). The *gap* component targets a 187 bp fragment of the *M. genitalium* glyceraldehyde-3-phosphate dehydrogenase enzyme (11).

Specimens that were positive on the PHE *M. genitalium* assay were tested for mutations associated with macrolide and fluoroquinolone resistance (4, 12). 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. The QRDR of the *gyrA* gene was also amplified and sequenced in isolates where the *parC* sequence was non-wild-type (4). 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 standard 8-day turn-around time (not including *gyrA*, as this was done for surveillance purposes only).

Enhanced data collection

A list of positive specimen IDs from each SHC with the date of attendance was securely shared with the clinics at the end of each month during the pilot to request enhanced surveillance data. Clinicians from the participating sites were asked to complete a secure web-based

questionnaire to collect enhanced data for each individual. Clinic patient identification code, gender and age were used to link enhanced clinical records from SHCs to a *M. genitalium* specimen.

Data management

Specimens were removed from the dataset if they were duplicates (n=19) or if enhanced data were not available (n=16).

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 result(s) within the pilot period were used to indicate 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. PHE Research Governance, Research Translation and Innovation representatives confirmed 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 2020, 286 *M. genitalium*-positive specimens were sent to the PHE AMRSTI national reference laboratory for molecular AMR testing as part of the second MARS pilot. Enhanced surveillance data were obtained for 254 individuals, of which 251 could be matched to a specimen and were thus included in descriptive analyses.

Among 251 individuals whose specimens were successfully screened for genetic markers associated with resistance, 131 (52%) were heterosexual men, 61 (24%) were women and 54 (22%) were MSM (Table 1). Forty-four percent (n=110) of individuals were 25 to 34 years of age, and the most commonly reported ethnicities were White or White British (n=124, 49%) or Black or Black British (n=58, 23%). More than half of individuals lived outside of London (n=139, 55%), although a notably large proportion of the 54 MSM were London residents (70%).

The majority (n=215, 86%) of individuals were HIV negative. Individuals commonly reported one or no sexual partners in the UK (n=138, 55%), and no sexual partners abroad in the 3 months prior to their diagnosis of infection with *M. genitalium* (n=128, 51%).

Individuals were predominantly symptomatic (n=190, 76%), although fewer women (51%) displayed symptoms compared to heterosexual men (76%) and MSM (88%). Few had more than one *M. genitalium* test per episode (n=57, 23%). The majority (79%) of individuals were tested according to national management guidelines as they had PID or NGU or were a sexual contact of someone diagnosed with *M. genitalium*.

Where information on previous STI diagnosis was available, 32 (13%) individuals had a history of an STI in the past year, most commonly gonorrhoea or chlamydia. Ten individuals had more than one previous STI diagnosis and only one individual had 3 previous STI diagnoses in the past year.

Table 1. Number of all individuals diagnosed with *M. genitalium* among sentinel sexual health clinics in England, by individuals' characteristics, January to March 2020 (N=251)†

		Men			
	Women	**Het Men**	**MSM**	**Unknown**	**Total**
	n (% of N)	**n (% of N)**	**n (% of N)**	**n (% of N)**	**n (% of N)**
Individuals	61	131	54	5	251
Age group (years)					
15 to 19	9 (15%)	4 (3%)	0 (0%)	0 (0%)	13 (5%)
20 to 24	25 (41%)	41 (31%)	8 (15%)	2 (40%)	76 (30%)
25 to 34	22 (36%)	64 (49%)	23 (43%)	1 (20%)	110 (44%)
35 to 44	3 (5%)	19 (15%)	16 (30%)	1 (20%)	39 (16%)
45 to 64	0 (0%)	1 (1%)	7 (13%)	1 (20%)	9 (4%)
Unknown	2 (3%)	2 (2%)	0 (0%)	0 (0%)	4 (2%)
Ethnicity					
White	29 (48%)	65 (50%)	30 (56%)	0 (0%)	124 (49%)
Mixed	6 (10%)	7 (5%)	5 (9%)	3 (60%)	21 (8%)
Asian or Asian British	4 (7%)	5 (4%)	1 (2%)	1 (20%)	11 (4%)
Black or Black British	13 (21%)	35 (27%)	10 (19%)	0 (0%)	58 (23%)
Other Ethnic Groups	2 (3%)	6 (5%)	2 (4%)	1 (20%)	11 (4%)
Unclassified	7 (11%)	13 (10%)	6 (11%)	0 (0%)	26 (10%)
Region					
London	19 (31%)	50 (38%)	38 (70%)	2 (40%)	109 (43%)
Outside London	41 (67%)	81 (62%)	15 (28%)	2 (40%)	139 (55%)

		Men			
	Women	**Het Men**	**MSM**	**Unknown**	**Total**
	n (% of N)	**n (% of N)**	**n (% of N)**	**n (% of N)**	**n (% of N)**
Not reported	1 (2%)	0 (0%)	1 (2%)	1 (20%)	3 (1%)
HIV status					
HIV negative	48 (79%)	120 (92%)	43 (80%)	4 (80%)	215 (86%)
HIV positive	0 (0%)	1 (1%)	9 (17%)	1 (20%)	11 (4%)
Unknown	13 (21%)	10 (8%)	2 (4%)	0 (0%)	25 (10%)
Number of UK sexual partners (past 3 months)					
0 to 1*	46 (75%)	76 (58%)	14 (26%)	2 (40%)	138 (55%)
2 to 5	9 (15%)	42 (32%)	32 (59%)	2 (40%)	85 (34%)
6 or more	1 (2%)	3 (2%)	3 (6%)	0 (0%)	7 (3%)
Not reported	5 (8%)	10 (8%)	5 (9%)	1 (20%)	21 (8%)
Number of sexual partners while abroad (past 3 months)					
0	41 (67%)	63 (48%)	21 (39%)	3 (60%)	128 (51%)
1 or more	4 (7%)	10 (8%)	1 (2%)	0 (0%)	15 (6%)
Not reported	16 (26%)	58 (44%)	32 (59%)	2 (40%)	108 (43%)
Symptoms (at first test)					
Yes	31 (51%)	115 (88%)	41 (76%)	3 (60%)	190 (76%)
No	30 (49%)	16 (12%)	13 (24%)	2 (40%)	61 (24%)
Specimen					
Urethral	1 (2%)	18 (14%)	5 (9%)	0 (0%)	24 (10%)
Urine	3 (5%)	108 (82%)	47 (87%)	4 (80%)	162 (65%)
Cervical, rectal, other	2 (3%)	0 (0%)	2 (4%)	0 (0%)	4 (2%)
Vaginal	52 (85%)	0 (0%)	0 (0%)	0 (0%)	52 (21%)
Unknown	3 (5%)	5 (4%)	0 (0%)	1 (20%)	9 (4%)
Tests per *M. genitalium* episode					
1	37 (61%)	78 (60%)	26 (48%)	2 (40%)	143 (57%)
2 or more	24 (39%)	52 (40%)	28 (52%)	3 (60%)	107 (43%)
Unknown	0 (0%)	1 (1%)	0 (0%)	0 (0%)	1 (0%)
Testing indication					
NGU	0 (0%)	107 (82%)	35 (65%)	3 (60%)	145 (58%)
PID	20 (33%)	0 (0%)	0 (0%)	0 (0%)	20 (8%)
Contact	23 (38%)	8 (6%)	2 (4%)	0 (0%)	33 (13%)
Other	3 (5%)	0 (0%)	0 (0%)	1 (20%)	4 (2%)
Unknown	15 (25%)	16 (12%)	17 (31%)	1 (20%)	49 (20%)

		Men			
	Women	Het Men	MSM	Unknown	Total
	n (% of N)	n (% of N)	n (% of N)	n (% of N)	n (% of N)
Grouped previous STI diagnosis (past year)*					
No	59 (97%)	115 (88%)	41 (76%)	4 (80%)	219 (87%)
Yes	2 (3%)	16 (12%)	13 (24%)	1 (20%)	32 (13%)
Previous STI diagnosis (past year)					
Chlamydia	2 (3%)	5 (4%)	2 (4%)	0 (0%)	9 (5%)
Gonorrhoea	0 (0%)	3 (2%)	4 (7%)	0 (0%)	7 (3%)
M. genitalium	0 (0%)	2 (2%)	1 (2%)	1 (20%)	4 (2%)
Other STI	0 (0%)	6 (5%)	6 (11%)	0 (0%)	12 (5%)
None	59 (97%)	115 (88%)	41 (76%)	4 (80%)	219 (87%)

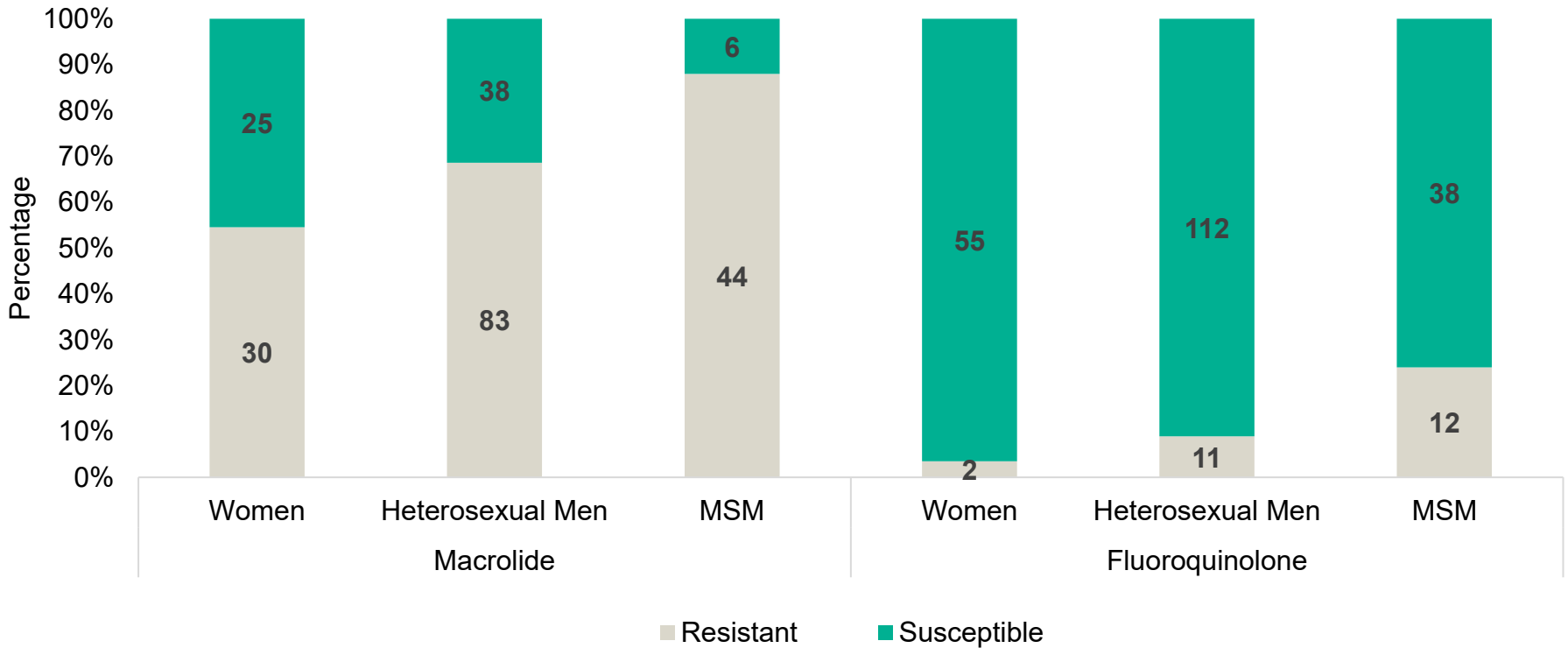
† 'Not reported' and 'Unknown' refer to instances where information was unknown or not stated'.
ⁿ Fewer than 5 individuals reported less than one recent sexual partner in the UK in the past 3 months.
* For grouped previous STI, STI episodes are counted only once for individuals.

Macrolide and predicted fluoroquinolone resistance

Among 251 specimens, most were from urine (65%) or vaginal swabs (21%). Macrolide resistance data were available for 230 (92%); fluoroquinolone (*parC*) resistance data were available for 233 (93%) and data for both were available for 223 (89%) specimens. Sequencing failed in 28 (11%) specimens due to low DNA load. This was a marked improvement on the first pilot, where 33% of specimens could not be successfully sequenced. One hundred and fifty-nine (159 out of 230, 69%) sequenced specimens had a macrolide resistance-associated mutation, consisting of 74 (47%) A2059G, 74 (47%) A2058G, 5 (3%) A2058T, 5 (3%) A2059C and one (1%) A2058C mutation. Twenty-six (26/233, 11%) specimens had a substitution predictive of fluoroquinolone resistance in ParC, consisting of 19 (73%) serine to isoleucine83 (S83I), 4 (15%) aspartic acid to asparagine87 (D87N) and 3 (11%) aspartic acid to tyrosine87 (D87Y). A further specimen had a ParC substitution (serine to asparagine83 [S83N]) whose significance with regards to resistance is, at present, unknown; this individual was treated using doxycycline and azithromycin. Predicted dual-drug resistance (such as, macrolides and fluoroquinolones) was detected in 23 (10%) of 223 specimens which were successfully sequenced for macrolide and fluoroquinolone resistance. Among the 27 *parC* non wild-type specimens, sequencing of the *gyrA* QRDR was successful for 25 (93%). Analysis revealed mutations resulting in amino acid substitutions in 4 out of 25 (16%), specifically 2 (50%) methionine to isoleucine95 (M95I), one (25%) aspartic acid to asparagine99 (D99N) and one (25%) alanine to threonine105 (A105T). Of these 4 specimens, 3 (75%) were harbouring a S83I ParC substitution, one (25%) was harbouring a D87N ParC substitution and all were resistant to macrolides. All *gyrA* substitutions detected have been reported previously (5, 7).

Figures 2 to 6 show the percentage of *M. genitalium* specimens with genotypic macrolide or fluoroquinolone resistance by selected characteristics. The majority (88%) of specimens from MSM were macrolide resistant, with high levels of resistance also observed among specimens from heterosexual men (69%) and women (55%) (Figure 2). While rarer, fluroquinolone resistance was also higher among specimens from MSM (24%) compared to heterosexual men (10%) and women (4%).

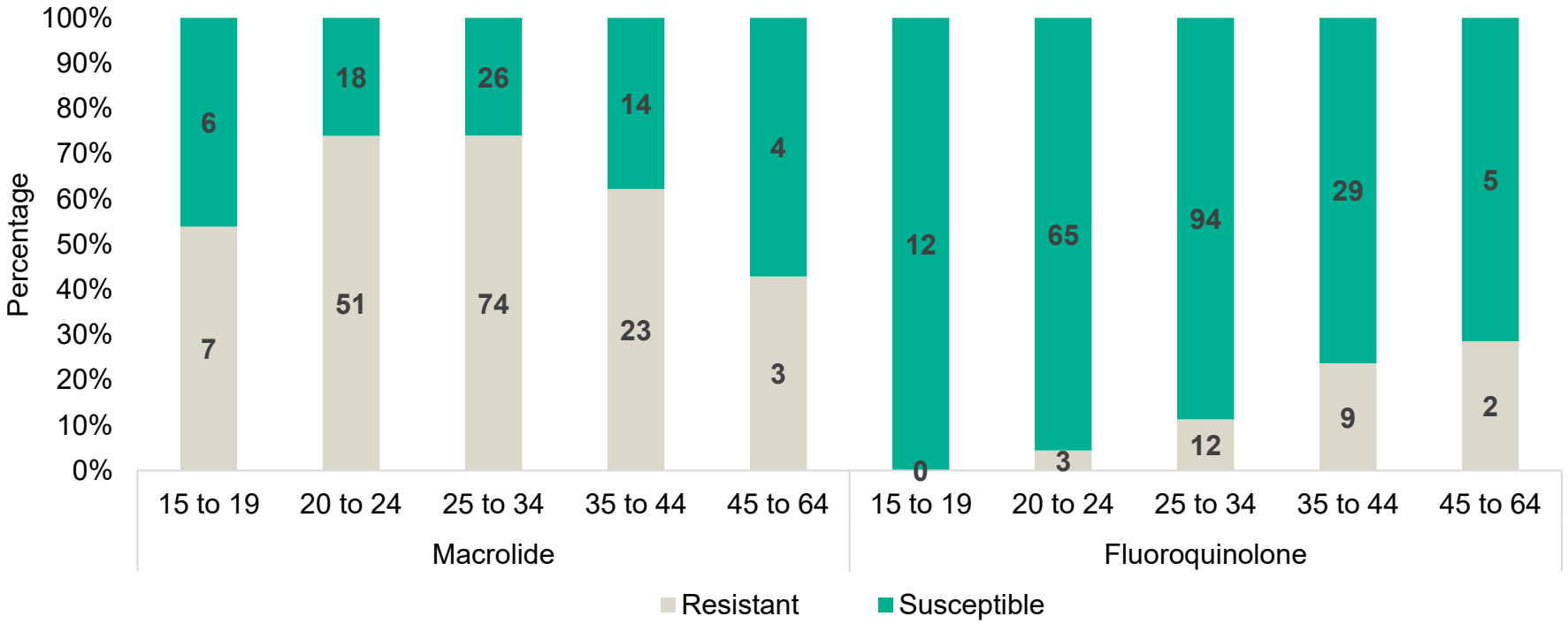
Figure 2. Proportion of M. genitalium specimens by macrolide (n=226) or fluoroquinolone (n=229) resistance profile among sentinel sexual health clinics in England, by gender and sexual orientation, January to March 2020*



* Numbers shown on the bars indicate the number of individuals in each group. Graph excludes individuals with unknown gender and sexual orientation for macrolide (n=4) and fluoroquinolone (n=4) analyses, in addition to specimens which could not be tested for mutations associated with macrolide (n=21) or fluoroquinolone resistance (n=18). While MSM includes both homosexual and bisexual men, women include heterosexual and homosexual women due to small sample size.

Macrolide resistance was most common (74%) among specimens from those aged 20 to 24 and 25 to 34 years old (Figure 3). Conversely, fluoroquinolone resistance increased with age, although few individuals were aged over 34 years old.

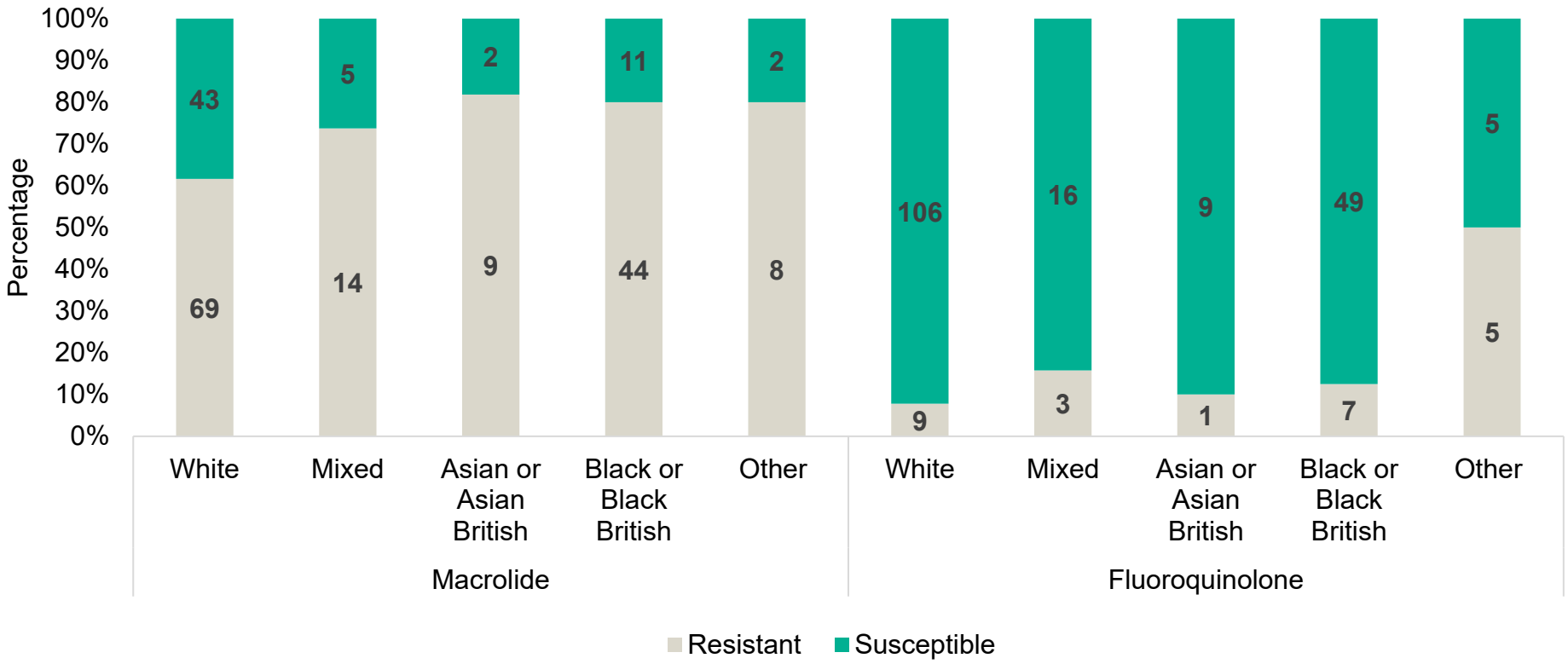
Figure 3. Proportion of *M. genitalium* specimens by macrolide (n=226) or fluoroquinolone (n=229) resistance profile among sentinel sexual health clinics in England by age group, January to March 2020*



* Numbers shown on the bars indicate the number of individuals in each group. Age group shown in years. Graph excludes specimens which could not be tested for mutations associated with macrolide (n=21) or fluoroquinolone resistance (n=18).

Specimens from people of Black British ethnicity had higher rates of macrolide resistance (80%) compared to those who were White (62%) (Figure 4). Few discernible differences were observed across ethnic groups for fluoroquinolone resistance due to small sample size.

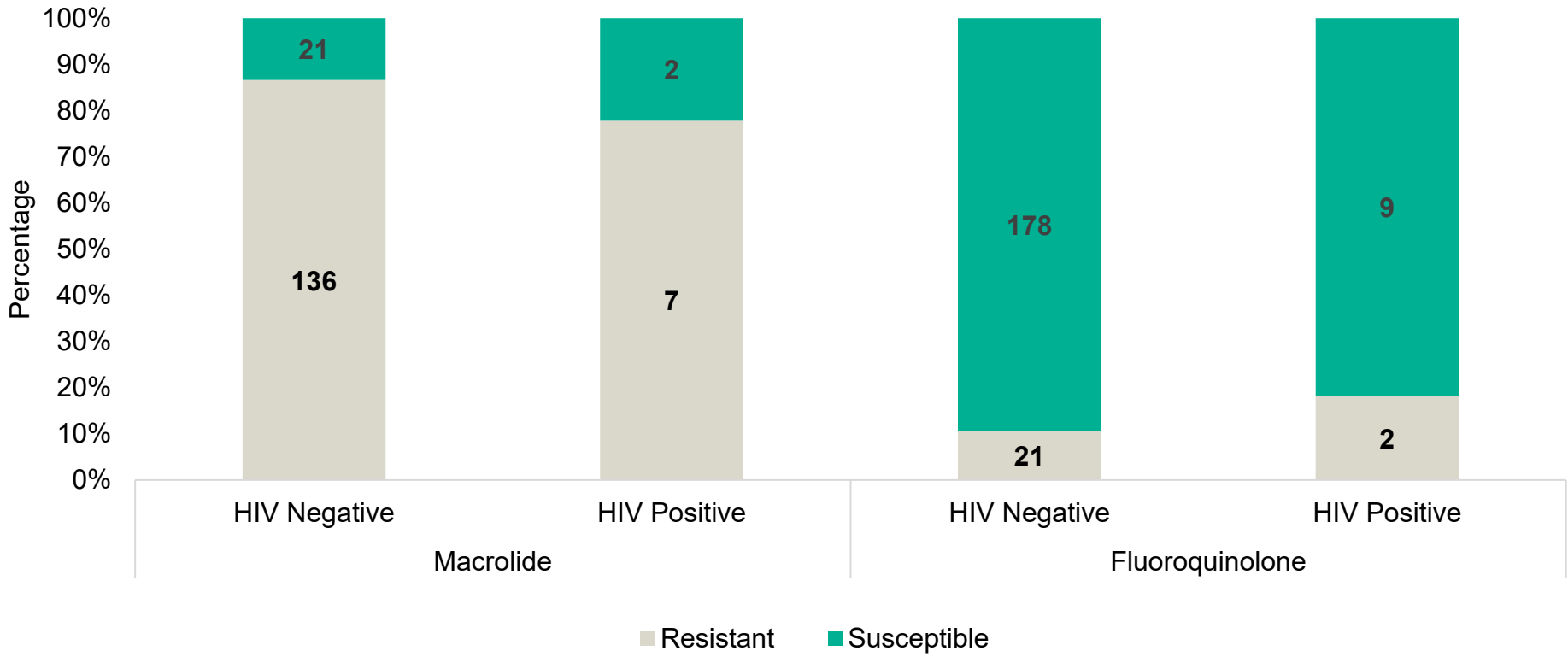
Figure 4. Proportion of *M. genitalium* specimens by macrolide (n=207) or fluoroquinolone (n=209) resistance profile among sentinel sexual health clinics in England by ethnic group, January to March 2020*



* Numbers shown on the bars indicate the number of individuals in each group. Graph excludes individuals with unclassified ethnicity for macrolide (n=23) and fluoroquinolone (n=24) analyses, in addition to specimens which could not be tested for mutations associated with macrolide (n=21) or fluoroquinolone resistance (n=18).

There were no notable differences between individuals living with HIV and those who were not with regards to macrolide or fluoroquinolone resistance (Figure 5).

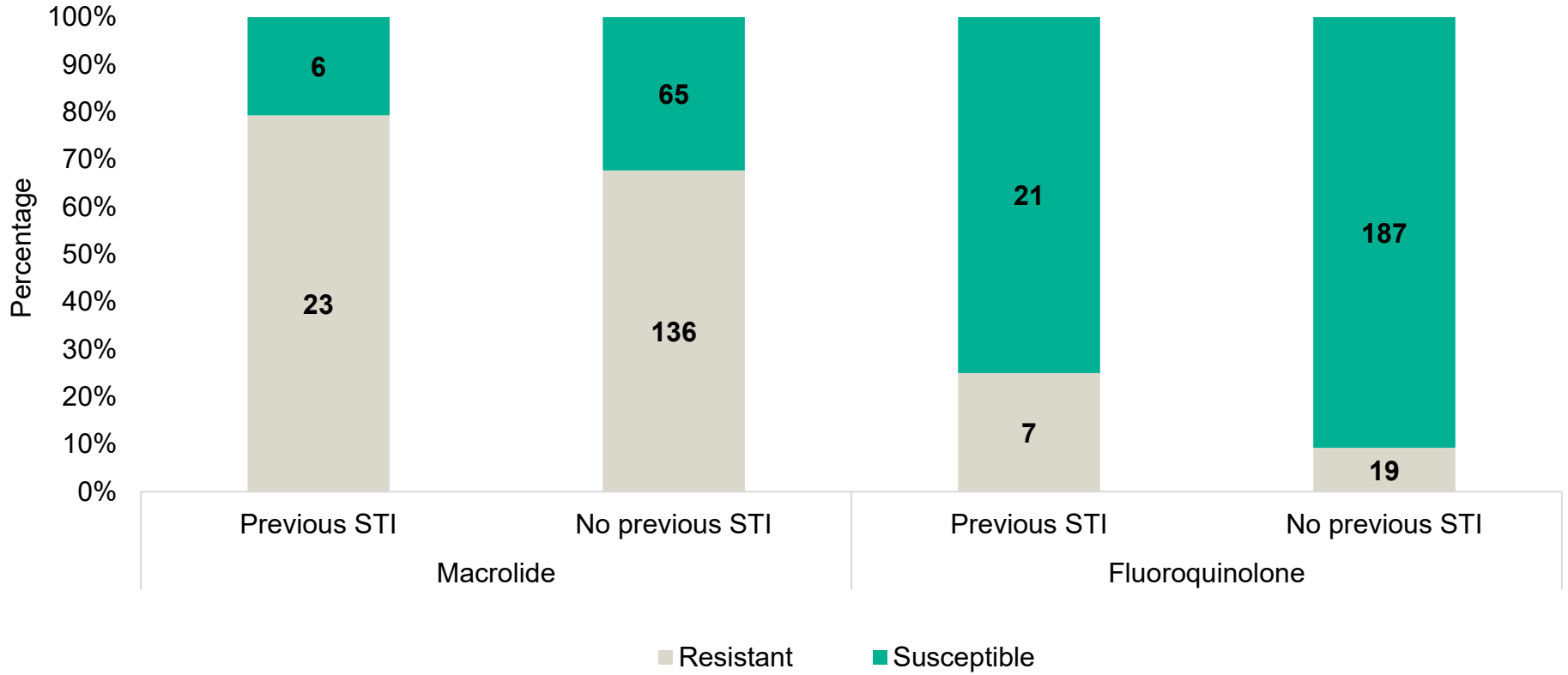
Figure 5. Proportion of *M. genitalium* specimens by macrolide (n=206) or fluoroquinolone (n=209) resistance profile among sentinel sexual health clinics in England by HIV status, January to March 2020*



* Numbers shown on the bars indicate the number of individuals in each group. Graph excludes individuals with unknown HIV status for macrolide (n=24) and fluoroquinolone (n=24) analyses, in addition to specimens which could not be tested for mutations associated with macrolide (n=21) or fluoroquinolone resistance (n=18).

Macrolide (79%) and fluoroquinolone (25%) resistance was more common in specimens from those diagnosed with a previous STI in the past year compared to those who were not (68% and 9%, respectively) (Figure 6).

Figure 6. Proportion of *M. genitalium* specimens by macrolide (n=230) or fluoroquinolone (n=233) resistance profile among sentinel sexual health clinics in England by previous STI diagnosis status, January to March 2020*

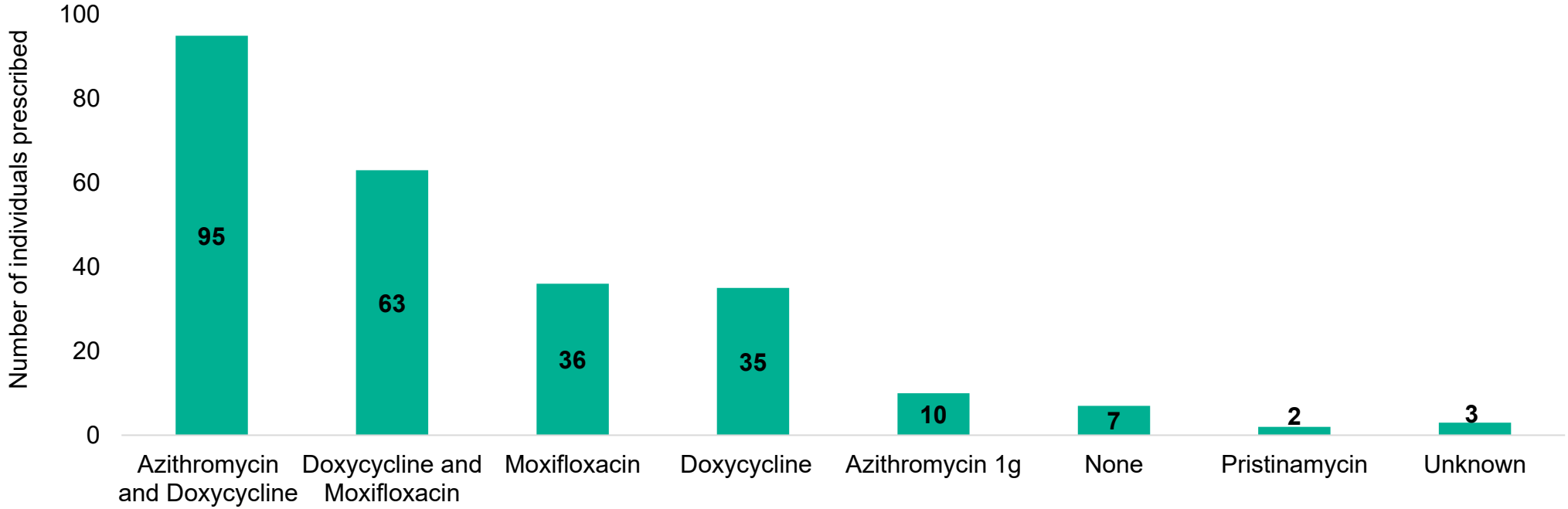


Resistance-associated mutations and treatment outcomes

Prescribing practices

Antimicrobial prescribing data were available for 248 out of 251 (99%) individuals with a *M. genitalium* infection (Figure 7). Among these, most individuals were prescribed the recommended treatments of azithromycin (n=105, 42%) or moxifloxacin (n=99, 40%). Only 2 out of 248 individuals received pristinamycin, both of which had macrolide and fluoroquinolone resistant infections. The majority of those who received doxycycline only (n=35) or no treatment (n=7) failed to return for (further) treatment, were asymptomatic at their second attendance or had a subsequent negative test-of-cure.

Figure 7. Number of individuals prescribed each antibiotic (combination) as their first treatment for M. genitalium infection, among sentinel sexual health clinics in England, January to March 2020



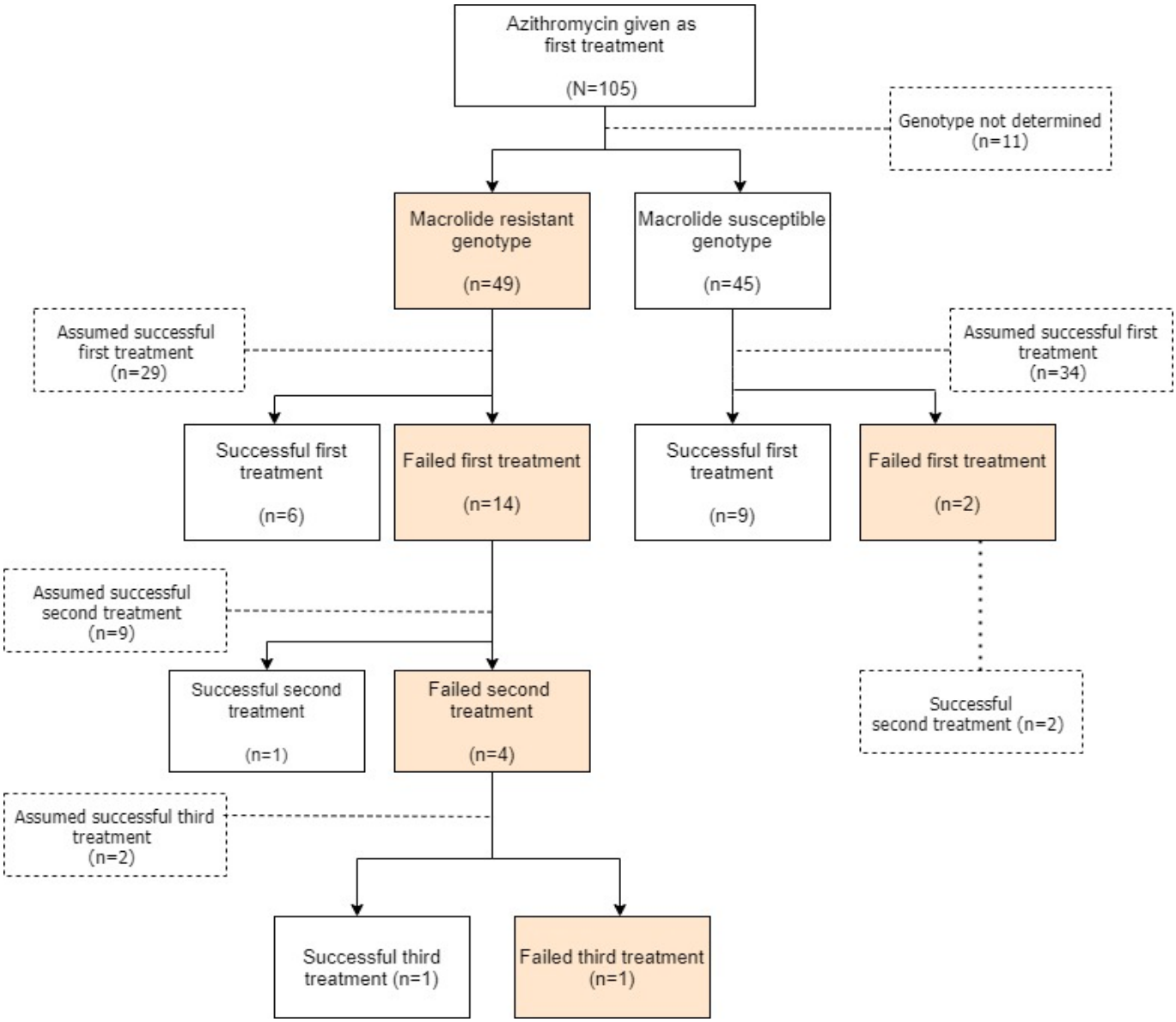
* Numbers shown on the bars indicate the number of specimens in each group.

Management with azithromycin

All individuals given azithromycin

One-hundred and five individuals were prescribed azithromycin, either alone or with doxycycline. Treatment outcomes for each of these individuals are described in Figure 8 and in detail in the text below. Among the 105 individuals, 95 (90%) also received doxycycline; dates of treatment(s) were available for 92 individuals. Treatments were delivered a median of 7 days apart, ranging from a minimum and maximum time interval of 0 and 28 days, respectively, between doxycycline and azithromycin treatments. Among the 105 individuals, 10 (10%) received azithromycin only.

Figure 8. Outcome flowchart for individuals given azithromycin as their first treatment for *M. genitalium* among sentinel sexual health clinics in England (N=105), January to March 2020*



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

Macrolide susceptible

Among 105 individuals who were prescribed azithromycin (either alone or in combination with doxycycline) as their first treatment, 45 (43%) were infected with a macrolide susceptible genotype; 34 were assumed to be successfully treated as, among these, 12 failed to respond to recall, symptoms resolved in 9 and follow-up information was unavailable for the remainder. Follow-up test-of-cure data were provided for 11 individuals, 9 of whom retested negative. The 2 individuals with a macrolide susceptible genotype who retested positive had received the recommended therapy of doxycycline followed by azithromycin, of which one was asymptomatic, and one was symptomatic at the time of their second test. After receiving further treatments with doxycycline and moxifloxacin, the 2 individuals did not return for a third test and were therefore assumed to have been clinically cured.

Macrolide resistant

Forty-nine (47%) of the 105 individuals who were prescribed azithromycin were infected with a macrolide resistant genotype, consisting of 26 (53%) A2059G, 19 (39%) A2058G, one (2%) A2058C, one (2%) A2059C and 2 (4%) A2058T mutations in the 23S rRNA gene. The majority, 42 out of 49 (86%), of individuals were symptomatic at the time of their first positive test. Thirty-five out of 49 (71%) were known or assumed to be successfully treated with their first treatment regimen; 6 had a negative test-of-cure while the remaining 29 did not have a follow-up test-of-cure (3 failed to respond to recall, 26 unknown). Five of the 6 (83%) who had a negative test-of-cure also received a 7-day course of doxycycline, a median time of 7 days prior to azithromycin treatment (ranging from 7 to 10 days), while the other received azithromycin only. Among the 6 specimens from these individuals, 4 had A2059G, one had A2058G and one had A2058T mutations, and one also had a D87Y ParC substitution. Of the remaining 29 individuals who were assumed to have been successfully treated, 25 out of 29 (86%) also received a 7 days course of doxycycline, a median of 7 days prior to azithromycin treatment (ranging from 3 to 11 days).

Among the 49 individuals who were prescribed azithromycin and were infected with a macrolide resistant genotype, 14 out of 49 (29%) failed their first treatment, as indicated by a second positive test; 8 out of 14 (57%) had a A2059G mutation, 6 (43%) had a A2058G mutation, and 2 had additional S83I ParC substitutions. Twelve out 14 (86%) were symptomatic at the time of their first positive test, and 9 (64%) were symptomatic at their second test. Thirteen out of 14 (93%) who failed treatment with azithromycin had also received a 7-day course of doxycycline. Twelve out of 14 of these individuals had date of treatment(s) provided, with a median time of 7 days between treatment with doxycycline and azithromycin (ranging from 0 to 20 days).

Infection subsequently resolved in 10 out of 14 individuals who had a macrolide-resistant infection and who failed initial treatment with azithromycin; one re-tested negative following further treatment with doxycycline and moxifloxacin and the remaining 9 were assumed to be successfully treated as re-testing data were not available. Among these 9, 3 received further treatment with doxycycline, one with moxifloxacin, one with doxycycline and moxifloxacin, 3 did not have further treatment and one did not have additional treatment information provided.

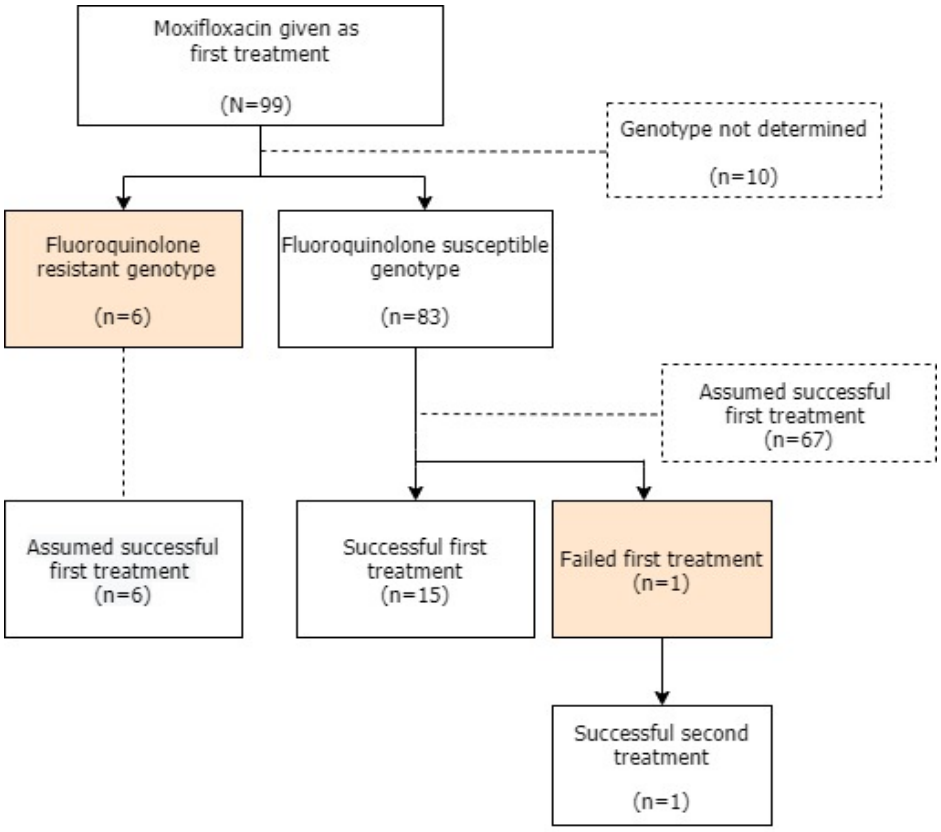
Of the 4 individuals who tested positive for a third time, 2 individuals were assumed to have cleared their infection as further testing data were not provided; one of these received second-line treatment with moxifloxacin and one did not receive further treatment. Another of these 4 individuals had a negative test after treatment with moxifloxacin. The final individual re-tested positive after failing further treatment with doxycycline and azithromycin. After the pilot ended, this individual received further treatment with moxifloxacin, but did not return for a test of cure.

Management with moxifloxacin

All individuals given moxifloxacin

Ninety-nine individuals were prescribed moxifloxacin, either alone or with doxycycline. Treatment outcomes for each of these individuals are described in Figure 9 and in detail in the text below. Among the 99 individuals, 63 (64%) also received doxycycline; dates of treatment(s) were available for all 63 individuals. Treatments were delivered a median of 15 days apart, ranging from a minimum and maximum time interval of 0 and 205 days, respectively, between doxycycline and moxifloxacin treatments. Among the 99 individuals, 36 (36%) received moxifloxacin only.

Figure 9. Outcome flowchart for individuals given moxifloxacin as their first treatment for *M. genitalium* among sentinel sexual health clinics in England (N=99) *, January to March 2020*



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

Fluoroquinolone susceptible

Of 83 (84%) infected with a fluoroquinolone susceptible genotype, 82 out of 83 (99%) were successfully treated; 15 had a negative test-of-cure while the remaining 67 were assumed to be

successfully treated as follow-up test-of-cure data were not provided. Among the 67 who did not have a test-of-cure, symptoms were reported to have resolved in 40, 15 failed to respond and 12 had no further information. The individual with a susceptible infection who retested positive had received doxycycline in addition to moxifloxacin. After receiving further treatment with moxifloxacin, the individual re-tested negative.

Fluoroquinolone resistant

A further 6 out of 99 (6%) individuals were infected with a fluoroquinolone resistant genotype: all 6 had a S83I ParC substitution, as well as a mutation associated with macrolide resistance (4 A2059G, 2 A2058T). One of which also had a M95I GyrA substitution. Four out of 6 (67%) were symptomatic at the time of their first test. Four out of 6 (67%) received doxycycline in combination with moxifloxacin, with a median time of 11 days between treatments (ranging from 7 to 19 days). All individuals failed to return for a test of cure, so all were assumed to not require further treatment; 3 failed to respond to recall, symptoms resolved in one individual (given 2-days doxycycline, 2-days moxifloxacin and 7-days doxycycline) and no follow-up data were available for the remaining 2.

Discussion

In this second pilot of enhanced surveillance for *M. genitalium* AMR, macrolide resistance was detected in more than two thirds of specimens (69%, 159 out of 230); this is consistent with the findings from the first pilot (69%, 173 out of 249). Meanwhile, predicted fluoroquinolone and dual-drug (macrolide and fluoroquinolone) resistance was identified in one in 10 specimens. Although macrolide resistance was universally high, specimens from MSM (88%), people of Black or Black British ethnicity (80%) and those who had a previous STI diagnosis in the past year (79%) had notably higher rates of resistance, with almost identical patterns emerging in the first pilot. Few individuals of Asian or Asian British, Mixed and other ethnicity were included in the sample across pilots, limiting the generalisability of any findings in these population groups (Appendix 1). Additionally, few specimens with fluoroquinolone resistance were identified in 2019 (21 out of 251) and 2020 (26 out of 233). Data from both years, however, suggested an increased likelihood of fluoroquinolone-resistant infection in those with a previous STI diagnosis in the past year compared to those without, and with increasing age (Appendix 2).

Almost all individuals infected with macrolide-susceptible *M. genitalium* were successfully treated with azithromycin, although only one in 4 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, 35 out of 49 (71%) *M. genitalium* infections with mutations associated with macrolide resistance were confirmed or assumed to be clinically cured using azithromycin as a component of the first treatment; this is consistent with findings from the first pilot report. Among the 35 individuals who were given azithromycin, had a macrolide-resistant *M. genitalium* infection and were known or assumed to be successfully treated, 86% (30 out of 35) also received doxycycline, with a median interval of one week between different treatments. Therefore, it is possible that doxycycline contributed

towards the successful treatment of macrolide resistant infections. However, of the 14 individuals who were infected with a macrolide resistant strain and who had a positive test-of-cure, 13 (93%) were given doxycycline in addition to azithromycin, yet still failed treatment. Among these, the median time interval between doxycycline and azithromycin treatments was also one week.

Among the 6 specimens that displayed a fluoroquinolone-resistant genotype and were managed with moxifloxacin, 6 had a ParC S83I substitution and one also had a GyrA M95I substitution, all of which were assumed to be clinically cured as no test-of-cure data were reported. Four individuals were also given doxycycline as pre-treatment, mostly under 2 weeks prior to treatment with moxifloxacin. Doxycycline may therefore have a role in combination therapy with moxifloxacin, but more data are needed.

In addition to the need for a larger sample size to improve AMR prevalence estimates, there were several limitations of this pilot. National guidelines do not recommend asymptomatic screening for *M. genitalium*. Results reported here are therefore not representative of all individuals with infection with *M. genitalium*. Additionally, we are unable to assess the representativeness of the sample of people included in the MARS pilots because all SHCs in England are not yet reporting *M. genitalium* diagnoses through routine national STI surveillance. The lack of national *M. genitalium* data therefore impacts our ability to discern whether sentinel sites in MARS are representative of all individuals diagnosed with infection with *M. genitalium* in England. Moreover, in addition to a small sample size, 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. Data on correlations between genotypic resistance denoted by the presence of mutations in key genes and clinical treatment failure are also lacking, especially for the genes involved in fluoroquinolone resistance (*parC* and *gyrA*), making interpretation of results difficult. Finally, data regarding sex abroad were poorly completed for the second successive year, limiting our ability to assess the impact of travel on AMR in *M. genitalium*.

If current macrolide and fluoroquinolone resistance estimates are accurate, a sample size of approximately 1,000 specimens per surveillance round – 4 times the sample size of this pilot – is required to detect a change within 5% of current resistance figures. Scaling up surveillance of AMR in *M. genitalium* amid the COVID-19 pandemic and reduced laboratory capacity will prove challenging and partly explains the smaller sample size in the second MARS pilot compared to the first. Nevertheless, required improvements identified from the first pilot were successfully implemented in the second, including adapting the data collection form to include additional questions regarding treatment(s), repeat testing and test-of-cure.

Conclusion

The results of the second pilot of MARS were consistent with those observed in the first, demonstrating evidence of widespread macrolide resistance and increasing dual-drug resistance. Additional data on population groups with higher likelihood of resistant infection were collected which, while adding to the existing evidence collected from the first pilot, must still be improved to better characterise those most at risk of AMR infection and mutations predictive of treatment failure.

Establishing MARS as a regular surveillance programme will mitigate the limitations of the first and second pilot studies, by increasing the sample size to improve statistical power for determining AMR risk factors and determining treatment outcomes, which will subsequently provide stronger evidence to inform clinical management guidelines.

Appendices

1. Macrolide resistance by individuals' characteristics

Table 2. Number of all individuals with *M. genitalium* macrolide resistance among sentinel sexual health clinics in England, by individuals' characteristics (N=230), January to March 2020*

Characteristics	Resistant (n)	Susceptible	Total (N)	Resistant (n of N, %)
Specimens	159	71	230	69%
Age group (years)				
15 to 19	7	6	13	54%
20 to 24	51	18	69	74%
25 to 34	74	26	100	74%
35 to 44	23	14	37	62%
45 to 64	3	4	7	43%
Unknown	1	3	4	25%
Gender and sexual orientation				
MSM	44	6	50	88%
Heterosexual men	83	38	121	69%
Women	30	25	55	55%
Unknown	2	2	4	50%
Ethnicity				
White	69	43	112	62%
Mixed	14	5	19	74%
Asian or Asian British	9	2	11	82%
Black or Black British	44	11	55	80%
Other Ethnic Groups	8	2	10	80%
Unclassified	15	8	23	65%
HIV status				
HIV negative	136	59	195	70%

Characteristics	Resistant (n)	Susceptible	Total (N)	Resistant (n of N, %)
HIV positive	7	4	11	64%
Unknown	16	8	24	67%
Total UK partners (past 3 months)				
0 to 1	81	42	123	66%
2 to 5	58	22	80	73%
6 or more	5	1	6	83%
Unknown	15	6	21	71%
Number of sexual partners while abroad (past 3 months)				
0	75	40	115	65%
1 or more	8	7	15	53%
Unknown	76	24	100	76%
Symptoms (at first test)				
No symptoms	37	16	53	70%
Symptoms	122	55	177	69%
Tests per *M. genitalium* episode				
Urethral	13	11	24	54%
Urine	113	34	147	77%
Cervical, rectal, other	2	0	2	100%
Vaginal	27	21	48	56%
Unknown	4	5	9	44%
Grouped individuals with previous STI diagnosis (past year)†				
None	136	65	201	68%
Yes	23	6	29	79%
Previous STI diagnosis (past year)				
Chlamydia	11	2	13	85%
Gonorrhoea	10	0	10	100%
M. genitalium	3	3	6	50%
Other STI	7	4	11	64%
None	136	65	201	68%

* Excludes specimens which could not be tested for mutations associated with macrolide resistance (n=21).
† For grouped previous STI, STI episodes are counted only once for individuals.

2. Fluoroquinolone resistance by individuals' characteristics

Table 3. Number of all individuals with *M. genitalium* fluoroquinolone resistance among sentinel sexual health clinics in England, by individuals' characteristics (N=233), January to March 2020*

Characteristics	Resistant (n)	Susceptible	Total (N)	Resistant (n of N, %)
Specimens	26	208	233	11%
Age group (years)				
15 to 19	0	12	12	0%
20 to 24	3	65	68	4%
25 to 34	11	94	104	11%
35 to 44	9	29	38	24%
45 to 64	2	5	7	29%
Unknown	1	3	4	25%
Gender and sexual orientation				
MSM	12	38	50	24%
Heterosexual men	11	112	122	9%
Women	2	55	57	4%
Unknown	1	3	4	25%
Ethnicity				
White	9	106	114	8%
Mixed	3	16	19	16%
Asian or Asian British	1	9	10	10%
Black or Black British	7	49	56	13%
Other Ethnic Groups	5	5	10	50%
Unclassified	1	23	24	4%
HIV status				
HIV negative	21	178	198	11%
HIV positive	2	9	11	18%
Unknown	3	21	24	13%

Characteristics	Resistant (n)	Susceptible	Total (N)	Resistant (n of N, %)
Total UK partners (past 3 months)				
0 to 1	8	120	127	6%
2 to 5	12	67	79	15%
6 or more	0	6	6	0%
Unknown	6	15	21	29%
Number of sexual partners while abroad (past 3 months)				
0	7	110	117	6%
1 or more	5	10	15	33%
Unknown	14	88	101	14%
Symptoms (at first test)				
No symptoms	8	49	56	14%
Symptoms	18	159	177	10%
Tests per *M. genitalium* episode				
Urethral	3	21	24	13%
Urine	19	130	148	13%
Cervical, rectal, other	1	2	3	33%
Vaginal	2	47	49	4%
Unknown	1	8	9	11%
Grouped individuals with previous STI diagnosis (past year)†				
None	19	187	205	9%
Yes	7	21	28	25%
Previous STI diagnosis (past year)				
Chlamydia	5	8	13	38%
Gonorrhoea	3	6	9	33%
M. genitalium	1	5	6	17%
Other STI	2	9	11	18%
None	19	187	205	9%

* Excludes specimens which could not be tested for mutations associated with fluoroquinolone resistance (n=18).
† For grouped previous STI, STI episodes are counted only once for individuals.

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