Frequency and correlates of *Mycoplasma genitalium* antimicrobial resistance mutations and their association with treatment outcomes: findings from a national sentinel surveillance pilot in England

Helen Fifer[1], Rachel Merrick[1], Rachel Pitt[1], Mandy Yung[1], Hester Allen[1], Michaela Day[1], Katy Sinka[1], Neil Woodford[1], Hamish Mohammed[1], Colin Brown[1], Gwenda Hughes[1], Michelle Cole[1] on behalf of the MARS Collaborators Group*

***Collaborators Group:**

Achyuta Nori[2], Amy Evans[3], Ania Richardson[4], Ann Sullivan[5], Bruce Macrae[6], Clive Graham[7], Debra Padgett[7], Emma Page[3], Eva Jungmann[4], Jara Phattey[8], Joanna Glascodine[9], Jonathan Ross[8], Lucy Garvey[10], Matthew Phillips[11], Michael Clarke[12], Michael Rayment[5], Monica Rebec[5], Nicola Fearnley[13], Nimal Wickramasinghe[8], Paddy Horner[12], Penelope Cliff[2], Peter Muir[12], Rita Browne[4], Roberta Brum[5], Victoria Tittle[5]

[1] National Infection Service, Public Health England, 61 Colindale Avenue, London, UK

[2] Guy's and St Thomas' NHS Foundation Trust, London, UK

[3] Leeds Teaching Hospitals NHS Trust, Leeds, UK

[4] Central and North West London NHS Foundation Trust, London, UK

[5] Chelsea and Westminster Healthcare NHS Foundation Trust, London, UK

[6] UCLH NHS Foundation Trust, London, UK

[7] North Cumbria University Hospitals NHS Trust, Cumbria, UK

[8] University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK

[9] Bradford Teaching Hospitals NHS Foundation Trust, Bradford, UK

[10] Imperial College Healthcare NHS Trust, London, UK

[11] North Cumbria Integrated Care NHS Foundation Trust, Cumbria, UK

[12] North Bristol NHS Trust, Bristol, UK

[13] Locala Community Partnerships CIC, Yorkshire, UK

Corresponding author: Helen Fifer, Blood Safety, Hepatitis, STI &HIV Division, National Infection Service, Public Health England, 61 Colindale Avenue, London NW9 5EQ; email helen.fifer@phe.gov.uk.

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Article summary: *M. genitalium* AMR surveillance in England showed that over two-thirds of *M. genitalium* specimens had mutations associated with macrolide resistance, and that resistance was associated with MSM and previous STIs.

Abstract

Background

Mycoplasma genitalium infection is a public health concern due to extensive antimicrobial resistance (AMR). Using data from a pilot of *M. genitalium* AMR surveillance, we determined the prevalence and risk factors for resistance among specimens from sexual health clinic attendees and assessed treatment outcomes.

Methods

Seventeen sexual health clinics in England sent consecutive *M genitalium*-positive specimens to the national reference laboratory from January to March 2019. Regions of the 23S rRNA,*parC* and *gyrA* genes associated with macrolide and fluoroquinolone resistance, respectively, were amplified and sequenced where appropriate. Fisher's exact tests, univariate and multivariable logistic regression models were used to determine associations between demographic, clinical and behavioural factors and resistance-associated mutations.

Results

Over two-thirds (173/249, 69%) of *M. genitalium* specimens had mutations associated with macrolide resistance, while predicted fluoroquinolone (21/251, 8%) and dual-drug (12/237, 5%) resistance were less prevalent. No specimens had both *gyrA* and *parC* resistance associated mutations. Macrolide resistance was more common in specimens from men who have sex with men (MSM) compared to heterosexual men (aOR: 2.64; 95% CI: 1.09-6.38;

p=0.03). There was an association between both macrolide and fluoroquinolone resistance and having a previous STI (*p*=0.06).

Only 19% of individuals returned for a test-of-cure. Of those infected with a macrolide-resistant genotype who were given azithromycin, 57/78 (73%) were known or assumed to be clinically cured; however, 43/57 (75%) of these also received doxycycline. Of the 21 with a macrolide-resistant genotype who failed treatment, 18/21 (86%) also received doxycycline.

Conclusions

While macrolide resistance was widespread, particularly among specimens from MSM and those with a previous STI diagnosis in the past year, resistance-associated mutations in *M. genitalium* did not appear to be unequivocally predictive of treatment failure.

Key words: *Mycoplasma genitalium*, antimicrobial resistance, macrolides, fluoroquinolones.

Introduction

Mycoplasma genitalium is a sexually transmitted pathogen associated with non-gonococcal urethritis (NGU) in men 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). Limited availability of diagnostics and syndromic management have led to the emergence of multidrug-resistant infections worldwide (3).

Current management guidelines in the United Kingdom recommend *M. genitalium* testing for people presenting with NGU or PID, and their current sex partners (2). Azithromycin, a macrolide antibiotic, is recommended as the first-line treatment, ideally when genotypic susceptibility has been confirmed. Doxycycline is given as pre-treatment to lower bacterial load. Moxifloxacin, a broad-spectrum fluoroquinolone, is the second-line treatment. It is recommended that all patients attend for a test-of-cure (TOC) five weeks after the start of treatment (2).

Macrolide resistance is conferred by a single base mutation, primarily at position A2058 or A2059 in region V of the 23S rRNA gene (*Escherichia coli* numbering). 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). However, there are only limited data to support correlations between mutations in *parC* and clinical moxifloxacin resistance (4). Mutations in the QRDR of the *gyrA* gene in isolates where the *parC* has a fluoroquinolone-resistant genotype have been suggested to increase the likelihood of decreased susceptibility to fluoroquinolone antimicrobials (4,5) however without phenotypic data this is difficult to confirm.

The national reference laboratory offers a service for *M. genitalium* AMR testing. Retrospective analysis of data from this service 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). However, these data are biased as they are from a charged service and lack important behavioural and clinical information. We piloted a sentinel surveillance system to prospectively collect epidemiological data linked to AMR results to address this gap and inform management guidelines.

Materials and Methods

Seventeen SHCs geographically dispersed across England sent all consecutive *M. genitalium*-positive specimens (specimen types included swabs or urine in residual nucleic acid amplification buffers and neat urine) to the reference laboratory from January to March 2019.

M. genitalium-positivity was confirmed using an in-house multiplex real-time PCR targeting two intrinsic *M. genitalium* genes, *MgPa* and *gap* (7,8). Region V of the 23S rRNA gene (9) and the QRDR of the *parC* gene (10) were amplified, followed by Sanger sequencing. Where resistance mutations were detected in the *parC* QRDR, the QRDR of the *gyrA* gene was also amplified and sequenced (10). Predicted antibiotic resistance was inferred from the detection of known mutations in these genes.

Demographic, behavioural, clinical and treatment information were reported by the SHCs for every individual with a confirmed positive specimen using a secure online questionnaire. Individuals who did not have a subsequent test result were assumed to have been clinically cured.

Univariate and multivariable logistic regression models were used to test for associations between demographic, behavioural and clinical factors and macrolide resistance-

associated mutations. Bivariable associations with fluoroquinolone resistance were determined using Fisher's exact tests due to small cell sizes. Data analysis was carried out using STATA v15.1 (StataCorp LP, College Station, TX, USA).

Results

Among 352 *M. genitalium*-positive specimens, 188 (53%) were from heterosexual men, 95 (27%) were from women, and 66 (19%) were from men who have sex with men (MSM). Most specimens were from people aged 25- to 34-years-old (150/352, 43%) and those of white (144/352, 41%) or black or black British (104/352, 30%) ethnicity. Macrolide sequence data were available for 249/352 (71%) specimens, fluoroquinolone sequence data were available for 251/352 (71%), and data for both were available for 237/352 (67%). Sequencing failed in specimens with low DNA load. Overall, only 66/352 (19%) individuals returned for a TOC.

Macrolide Resistance

173/249 (69%) sequenced specimens had a macrolide resistance mutation in the 23S rRNA gene; A2059G (84/173, 49%) and A2058G (81/173, 47%) were the most common mutations. Factors associated with macrolide resistance are shown in Table 1. After adjusting for confounding, there was evidence of increased odds of macrolide resistance among specimens from MSM compared to heterosexual men (aOR: 2.64; 95% CI: 1.09-6.38; p=0.03). When compared to those without a previous STI diagnosis in the past year, specimens from those with an STI diagnosis in the previous year were weakly associated with macrolide resistance (aOR: 2.29; 95% CI: 0.95-5.49; p=0.06).

Fluoroquinolone Resistance

Twenty-one (8%) specimens had a mutation predictive of fluoroquinolone resistance; encoding ParC substitutions of S83I (10/21, 48%), D87N (9/21, 43%), D87Y (1/21, 5%) and

S83R (1/21, 5%). Predicted dual-drug resistance was detected in 12/237 (5%) of specimens. *gyrA* sequencing data was available for 17/21 (81%) of *parC*-resistant specimens. All sequences were wild-type. On bivariable analyses of *parC*-resistant specimens, resistance prevalence differed among people of white (10/101, 10%), black or black British (1/72, 1%) and other (including Mixed, Asian or Asian British) (8/43, 19%) ethnicities (p=0.008). There was weak evidence of an association between resistance mutations and having an STI diagnosis in the previous year (7/43, 16%) compared to not having an STI diagnosis in the previous year (14/208, 7%) (p=0.06).

Treatment Outcomes

195 people were prescribed azithromycin, either alone or as a component of their first treatment for *M. genitalium*; no sequence data were available for 56/195 (29%) of them. Of those with sequence data available, 61/139 (44%) had a macrolide-susceptible infection. All were assumed to be successfully treated as repeat tests were only available for five of these individuals, all of whom retested negative. A further 78/139 (56%) people prescribed azithromycin were infected with a macrolide-resistant genotype. Of these, 57/78 (73%) were known or assumed to be clinically cured (three negative TOCs, 54 individuals did not return for TOC). The majority, 45/57 (79%), of these individuals were symptomatic at the time of their first positive test. 43/57 (75%) were also treated with doxycycline (with an average of 16 days between treatment with doxycycline and azithromycin). However, 21/78 (27%) people prescribed azithromycin and with a macrolide-resistant genotype had a positive TOC; 18/21 (86%) of these were given doxycycline in addition to azithromycin. The average time interval between treatment with doxycycline and azithromycin for this group was 19 days. 18/21 (86%) were symptomatic at the time of their first positive test, and 13/21 (62%) were symptomatic at their second test.

Among the 139 individuals who were prescribed moxifloxacin, either alone or as a component of their first treatment, sequence data was not available for 39/139 (28%) infections. Of those with sequence data available, 92/100 (92%) were infected with a fluoroquinolone-susceptible genotype and nearly all (91/92, 99%) of these were known or assumed to be successfully treated (one positive TOC, one negative TOC, 90 individuals did not return for TOC). A further 8/100 (8%) were infected with a fluoroquinolone-resistant genotype. Three of these, all with the S83I substitution in ParC, failed treatment with moxifloxacin only. One individual with the D87Y mutation had a negative TOC, and the remaining four individuals with a fluoroquinolone-resistant genotype (two with the S83I

mutation and two with the D87N mutation) did not return for TOC. All five of these were symptomatic at the time of their first positive test and were given doxycycline as well as moxifloxacin, on an average of 22 days apart. Three of them also received azithromycin as a component of their treatment, all of whom had mutations associated with macrolide resistance.

Discussion

Using data from the first national pilot of sentinel surveillance of *M. genitalium* AMR, we have corroborated earlier reports of extensive macrolide resistance in *M. genitalium* and demonstrated fluoroquinolone and dual-drug resistance in England (6). Macrolide resistance was significantly more common in specimens from MSM and, despite a small sample size, there was some evidence of an association between having a previous STI diagnosis and both macrolide and fluoroquinolone resistance.

The association between MSM, a previous STI diagnosis and AMR may reflect antibiotic treatment exerting a selection pressure for the development of resistance in those who experience disproportionately high rates of STIs (11). Until recently, UK guidelines recommended the use of azithromycin for treating NGU and chlamydia, and as a component of dual therapy for gonorrhoea. Therefore, undiagnosed co-infection with *M. genitalium* could be exposed to sub-therapeutic azithromycin levels, potentially selecting for the development of *de novo* resistance. A study in the United States of men with urethritis found that the prevalence of macrolide resistance was higher among *M. genitalium* specimens from people with recent NGU than those who did not report NGU (12). Furthermore, MSM may also belong to sexual networks with high levels of transmitted resistance: disproportionately high rates of *Neisseria gonorrhoeae* AMR are seen in MSM (13).

Resistance-associated mutations were not unequivocally predictive of treatment failure as almost three-quarters of people with a macrolide-resistant infection who were treated with azithromycin were assumed to have cleared their infection. This is consistent with another study which found that 35% of men with macrolide-resistant *M. genitalium* infection reported persistent symptoms 15 days after azithromycin treatment (12) While it is possible that doxycycline contributed towards successful treatment in some cases in our study, potentially by reducing bacterial load (14), the impact of prior doxycycline treatment on macrolide treatment efficacy was inconsistent.

The number of specimens with mutations associated with fluoroquinolone resistance was too low to draw firm conclusions about the clinical significance of different ParC mutations, and no *parC*-resistant specimens also had *gyrA* mutations. However, the D87N and S83I mutations have the strongest published evidence for being predictive of treatment failure (6) and, in our study, three of five people whose infection had the S83I mutation failed moxifloxacin treatment. Interestingly, those who were assumed to be clinically cured also received doxycycline, whereas those who received moxifloxacin alone did not. Those whose infections had the D87N and D87Y mutations also were assumed to have cleared infection with moxifloxacin and doxycycline. These findings suggest that doxycycline may have a role in combination therapy with moxifloxacin.

A major limitation of this study was that a very low proportion of individuals (19%) returned for a TOC. It was assumed that people were less likely to return if their symptoms had resolved and therefore the absence of a repeat positive test was used as a proxy for successful treatment; this was a pragmatic decision in order to provide real-world data for treatment outcomes. It is likely to have over-estimated clinical cure rates and gives no indication of microbiological clearance. Additionally, repeat positive tests may have been due to reinfection or persistent infection resulting from poor compliance with treatment.

Furthermore, as repeat *M. genitalium* testing after receiving doxycycline and before starting either azithromycin or moxifloxacin is not recommended, we cannot exclude the possibility that doxycycline alone cleared some infections. Finally, it was not possible to sequence nearly a thrid of the specimens, and these may have had different rates of resistance to those which were sequenced successfully.

We have demonstrated that continued sentinel surveillance is essential to inform updates to national management guidelines and the addition of epidemiological data provided a rich data source for understanding the risk factors for AMR. However, further research is needed to understand and quantify the relationship between the presence of resistance mutations and treatment failure, and the potential mitigating role of pre-treatment with doxycycline.

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