Original article

High prevalence of co-infection of azithromycin-resistant *Mycoplasma genitalium* with other sexually transmitted infections: a prospective observational study of London-based symptomatic and STI-contact clinic attendees

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Key words

Mycoplasma genitalium, *Chlamydia trachomatis, Neisseria* gonorrhoeae, azithromycin, antimicrobial resistance, co-infection

Key messages:

- High prevalence of *Mycoplasma genitalium* (MG) infection, and co-infection with *Chlamydia trachomatis* (CT), in symptomatic and STI-contact clinic attendees
- Over 30% MG macrolide resistance-associated mutations in all MG-positive samples
- MG testing should be prioritised for symptomatic and STI-contact female and men-who-have-sex with women clinic attendees, and possibly in those with CT
- Azithromycin should be avoided for the treatment of CT in the absence of MG resistance testing

Abstract

Objectives

Azithromycin treatment of *Chlamydia trachomatis* (CT) may not be adequate to treat concomitant *Mycoplasma genitalium* (MG) infection, and particularly if MG has macrolide resistance associated mutations (MG-MRAM). We estimated prevalence of co-infections of CT with MG carrying MRAM, and risk factors for MG-MRAM amongst a sexual health clinic (SHC) population.

Study Design and Setting

Among symptomatic and STI-contact clinic attendees in London, prevalence of CT-MG co-infection and MG-MRAM were estimated using nucleic acid amplification testing and Sanger sequencing respectively, and their associated risk factors analysed using logistic regression.

Results

MG prevalence was 7.5% (23/307), 17.3% (30/173) and 11.4% (8/70) in females, men-who-have-sex-with-women (MSW) and men-who-have-sex-with-men (MSM); MG co-infection in CT-infected participants represented 28.0% (7/25), 13.5% (5/37), 0.0% (0/0), respectively. Presence of MG-MRAM was in 39.1% (9/23) female swabs, 70.0% (21/30) MSW urine and 83.3% (5/6) MSM rectal swabs. In multivariate analyses, co-infection with another STI was strongly associated with MG-MRAM (OR: 7.19; 95%CI:2.4-21.5).

Conclusion

A significant proportion of CT-positive participants were co-infected with MG, with high rates of MG-MRAM. Our findings suggest MG and MRAM testing should be prioritised for symptomatic and STI-contact females and MSW, and possibly in those with CT. Azithromycin may need to be avoided in CT positive patients for whom these tests are not available.

Introduction

Mycoplasma genitalium (MG), increasingly recognised as an important sexually transmitted infection (STI) and cause of genitourinary discharge(1), is estimated to be responsible for 10-30% of non-gonococcal urethritis (NGU) cases (2,3) and is also associated with cervicitis and pelvic inflammatory disease in women(4). It is unclear if MG causes symptomatic rectal infection, as few data are available, although associations with proctitis have been reported in MSM(5). Despite this, targeted testing for MG in United Kingdom (UK) sexual health clinics (SHCs) is not widely implemented, perhaps due to slow uptake of new commercially available CE-marked diagnostic tests.

Until recently, guidelines recommended single dose therapy azithromycin 1g for treatment of NGU (5), which is associated with high rates of MG treatment failure[1] and selection of 23S rRNA gene mutations(6). Increasingly, data from Asia and Australia detail outbreaks of resistance to a second line treatment for MG, fluoroquinolones(7), resulting primarily from mutations in the quinolone resistance-determining region (QRDR) of the *parC* gene of DNA topoisomerase IV(8), leaving few treatment options available for some patients. Newer treatment guidelines suggest using doxycycline for one week to reduce bacterial load, which by itself has poor efficacy (6), followed by extended dose azithromycin to improve rates of cure(8,9).

UK MG prevalence data are primarily reported at population level(10) and apart from our previous report of MG in symptomatic men-who-have-sex-with-women (MSW) from one London SHC(11), few data are available for symptomatic clinic attendees, particularly females and men-who-have-sex-with-men (MSM). Studies across the USA and Europe have identified high rates of co-infection with CT and NG(15,16) but UK studies are lacking, and there is a global lack of data on prevalence of MG infection and macrolide resistance.

Undiagnosed MG co-infection presents challenges to the management of other STIs. For example, a first-line treatment for CT infection is doxycycline(17) for one week, which has poor efficacy as a single agent against MG (6,15). In the treatment of *Neisseria gonorrhoeae* (NG), use of single dose

azithromycin 1g in dual therapy could risk selection of macrolide resistant MG (6,16). To determine clinical management implications of undiagnosed MG infection and resistance using data from a sub-study of a larger programme of work (the "Precise Study: a point of care antimicrobial resistance test for *Neisseria gonorrhoeae* and *Mycoplasma genitalium* infection – ensuring accurate therapy and antibiotic stewardship in sexual health medicine", aimed at to develop and evaluating rapid nucleic acid amplification test (NAAT)-based Point of Care tests (POCTs) for multiple STIs and AMR detection [http://www.preciseresearch.co.uk/]), we aimed to estimate prevalence, co-infections, macrolide and fluoroquinolone resistance associated mutations, and associated risk factors of MG infection in STI-contacts or symptomatic females, MSW and MSM, attending inner London SHCs.

Methods

Study design

Data were collected as part of the larger "Precise Study". The target recruitment numbers for the "Precise Study" were 50 NG positives, 50 MG positives, 30 Trichomonas vaginalis (TV) positives, and 70 CT positives from 500 females and 500 males (100 of which to be MSM). (In order to reach these targets, symptomatic patients and sexual contacts of individuals with CT, NG, TV or NGU were targeted for recruitment. Ethical approval was provided by London Bridge Research Ethics Committee (reference 13/LO/0691).

Recruitment

Participants were prospectively recruited between March 2015 and March 2016. Females and MSW were recruited from one SHC, however, due to initial poor MSM recruitment in that clinic, MSM recruitment was extended to a further two SHCs in order to achieve the 100 MSM participant target for the "Precise Study". Men who reported sex with men and women were classified as MSM. Patient eligibility was determined using triage forms, indicating whether patients met inclusion

criteria to participate in the "Precise Study": aged ≥16 years; attending SHC for routine STI testing including CT and NG (Nucleic acid amplification test [NAAT] testing); symptomatic (itching, genital discharge (all participants), rectal discharge (MSM only), pain/burning when urinating, dysuria, dyspareunia, post-coital bleeding, intermenstrual bleeding, rectal bleeding (MSM only) and pelvic abdominal pain) or being a sexual contact of someone with CT, NG, TV or NGU; and willing to provide appropriate samples (see Specimen Collection, below). Under the eligibility criteria, all MSM were required to be 'willing to provide' additional urine, rectal and pharyngeal samples, however failure to provide one of the above did not result in exclusion from the study.

Patients were approached by research study staff and provided written informed consent to participate in the study prior to seeing a healthcare professional. Study staff populated case report forms capturing basic demographic, clinical and behavioural data.

Specimen collection

Research samples were collected after routine sample collection. Females provided an additional vulvovaginal swab (VVS), either self- or healthcare-collected, in eNAT media (Copan, Italy). All males provided residual first catch urine and MSM provided additional pharyngeal swabs (collected by a healthcare professional) and additional rectal swabs (blind or via proctoscopy). One of each was placed in eNAT.

Research sample processing

DNA was extracted using Virus/Pathogen Midi kit (Qiagen, Germany) with the QIAsymphony instrument (Qiagen). Real-time PCR reactions were run using the Rotor-Gene Q 5plex HRM PCR thermocycler (Qiagen). Samples collected were processed using the FTD Urethritis plus kit (Fast Track Diagnostics, Luxembourg) for the detection of MG, and final resolved sample status determined using a discrepant analysis approach. See Supplementary Material for detailed testing methodology.

Resistance detection

Sanger sequencing was used to determine presence or absence of mutations associated with resistance to azithromycin and fluoroquinolones in MG. The positioning of resistance-associated mutations and primers used for PCR and sequencing can be found in the Supplementary Material along with detailed testing methodology.

Analysis

Analyses included descriptive analysis of participant characteristics. Sample size was determined by the larger “Precise Study”.

Data analysis

Data were analysed using Stata (StataCorp, Texas, USA) for Windows v15.1. Data validation and cleaning was undertaken at both St George’s, University of London and Public Health England independently. Missing data were checked with corresponding clinics and any participants missing one or more sets of results (either clinical NAAT or research results) were excluded from analysis.

Prevalence and 95% confidence intervals (CIs) for MG, CT and NG were derived by gender and anatomical site. Comparison of differences in demographic characteristics and other risk factors for MG macrolide resistance associated mutations (MG-MRAM). (MRAMs) and co-infection was derived using Pearson's chi-squared test. A p-value <0.05 was considered statistically significant.

Odds ratios and 95%CIs of demographic characteristics and other risk factors associated with MG MRAMs were derived from univariate logistic regression. Factors with a $p<0.10$ were further evaluated for independent effect using multivariate analysis, using a forward stepwise approach. The reference group for each category was that with the highest number of participants.

Results

Participant overview

Of the 786 patients approached, 308 females, 173 MSW and 88 MSM provided clinic and research test results. Of the 88 MSM who consented to have samples taken from all three anatomical sites, 70 participants provided samples from all three sites, and a total of 79 urine, 79 rectal and 85 pharyngeal samples were received. Reasons samples were not collected from MSM included: accidental disposal at clinic, inadvertent neglect of sample taking by participant, or failure of collection by clinician during examination. One female participant also had an unresolved discrepant result and was removed from analysis, resulting in a total of 550 participants (307 females, 173 MSW and 70 MSM) providing a full set of samples along with routine NAAT and research test results. The proportion of females, MSW and MSM who were symptomatic was: 98.7% (304/308), 97.1% (168/173), and 62.9% (44/70), respectively. The corresponding proportions who were sexual contacts of an individual with CT, NG, TV or NGU were 6.8% (21/308), 12.7% (22/173), and 41.4% (29/70).

CT, MG and NG infection and co-infection prevalence by population group

Of the total 723 samples, discrepant analysis was needed for 4 CT diagnoses, 20 NG diagnoses, and 5 MG diagnoses. Prevalence of any infection (CT, MG, NG) was 13.6% (42/307) in females, 39.3% (68/173) in MSW, and 45.7% (32/70) in MSM (all sample types combined). Among those positive for any of these infections, co-infection (≥1 CT, MG or NG within a sample) was present in 19.0% (8/42), 13.2% (9/68) and 15.6% (5/32), respectively. There was no difference between rates of co-infections in males and females (p=0.124).

In MSM, prevalence of any infection by anatomical site was 21.5% (17/79), 40.5% (32/79) and 18.8% (16/85) in urine, rectal and pharyngeal samples, respectively. Among positives, co-infection was present in 5.9% (1/17), 6.3% (2/32) and 0.0% (0/16), respectively.

Prevalence estimates of individual infections and co-infections are shown in Table 1. Prevalence of CT and MG was highest in MSW, whereas NG was most prevalent in MSM. Co-infection was present

in all population groups, although differences existed by pathogen. There were no MG-NG co-infections detected in any participants.

Table 1: Resolved CT, NG and MG prevalence and co-infection in females, MSW and MSM

Total participants	Females 307		MSW 173		MSM 70	
	No. positive (%)	**95%CI**	**No. positive (%)**	**95%CI**	**No. positive (%)**	**95%CI**
Overall CT infections	25 (8.1)	5.6-11.7	37 (21.4)	16.0-28.1	9 (12.9)	6.9-22.7
Overall MG infections	23 (7.5)	5.0-11.0	30 (17.3)	12.4-23.7	8 (11.4)	5.0-11.0
Overall NG infections	4 (1.3)	0.5-3.3	10 (5.8)	3.2-10.3	27 (38.6)	28.1-50.3
CT Mono infections	17 (5.5)	3.5-8.7	28 (16.2)	11.4-22.4	4 (5.7)	2.2-13.8
MG Mono infections	16 (5.2)	3.2-8.3	25 (14.5)	10.0-20.5	8 (11.4)	5.0-11.0
NG Mono infections	1 (0.3)	0.0-0.2	6 (3.5)	1.6-7.4	22 (31.4)	21.8-43.0
CT-MG co-infection	5 (1.6)	0.7-3.8	5 (2.9)	1.2-6.6	0 (0)	-
CT-NG co-infection	1 (0.3)	0.0-0.2	4 (2.3)	0.9-5.8	5 (7.1)	3.1-15.7
CT-MG-NG co-infections	2 (0.7)	0.2-2.3	0 (0)	-	0 (0)	-
MG-NG co-infection	0 (0)	-	0 (0)	-	0 (0)	-

CT, MG and NG infection and co-infection prevalence by anatomical site in MSM

As shown in table 2, in MSM that provided any sample, there were no MG co-infections at any anatomical site. CT-NG co-infections were identified in urine and rectal samples, but there were no pharyngeal co-infections.

178

179 *Table 2: CT, NG and MG prevalence and co-infection by anatomical site in MSM*

Total samples	URINE 79		RECTAL 79		PHARYNGEAL 85	
	No. positive (%)	**95%CI**	**No. positive (%)**	**95%CI**	**No. positive (%)**	**95%CI**
Any infection of CT/NG/MG	17 (21.5)	13.9-31.8	32 (40.5)	30.4-51.2	16 (18.8)	11.9-28.4
Overall CT infections	5 (6.3)	2.7-14.0	6 (7.6)	3.5-15.6	1 (1.2)	0.2-6.4
Overall NG infections	11 (13.9)	8.0-23.2	22 (27.8)	19.2-38.6	15 (17.6)	11.0-27.1
Overall MG infections	2 (2.5)	0.7-8.8	6 (7.6)	3.5-15.6	0 (0)	-
CT-MG co-infection	0 (0)	-	0 (0)	-	0 (0)	-
CT-NG co-infection	1 (1.3)	0.2-6.8	2 (2.5)	0.7-8.8	0 (0)	-
CT-NG-MG co-infections	0 (0)	-	0 (0)	-	0 (0)	-
MG-NG co-infection	0 (0)	-	0 (0)	-	0 (0)	-

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181 **MG macrolide and fluoroquinolone resistance by population group**

182 Among females and MSM, there were no mutations in either *gyrA* or *parC* associated with
183 fluoroquinolone resistance for MG. In MSW, one MG (mono-infection) (3.3%, 1/30 95%CI 0.6-16.7)
184 had mutations in *parC* at position S83. No resistance towards macrolides or fluoroquinolones was
185 detected in MG-positive MSM urogenital or pharyngeal samples. As shown in table 3, MRAM was
186 detected in female swabs, MSW urine and MSM rectal samples, for both mono- and co-infections.

187

188 *Table 3: Macrolide resistant samples as determined by the presence of A2058 and A2059 mutations*
189 *in 23S rRNA*

190 **There were no MG-positive pharyngeal samples*

191 R^+: macrolide resistant; n=number of MG positives; MG: *Mycoplasma genitalium*; CT: *Chlamydia*
192 *trachomatis; MSW: Men-who-have-sex-with-women; MSM: men-who-have-sex-with-men*

	Females		MSW		MSM Rectal samples*		MSM Urine samples*	
	R^+/n	**% (95%CI)**	**R^+/n**	**% (95%CI)**	**R^+/n**	**% (95%CI)**	**R^+/n**	**% (95%CI)**
Overall MG infections	9/23	39.1 (22.2-59.2)	21/30	70.0 (52.1-83.3)	5/6	83.3 (43.7-97.0)	0/2	0 (0-84.2)
MG mono infection	6/16	37.5 (18.4-61.4)	17/25	68.0 (48.4-82.8)	5/6	83.3 (43.7-97.0)	0/2	0 (0-84.2)
CT-MG co-infection	3/5	60.0 (23.1-88.2)	4/5	80.0 (37.6-96.4)	-	-	-	-

193

194 **Risk factors associated with MG macrolide resistance**

195 Risk factors included in the logistic regression model for association with MG MRAMs are shown in
196 Table 4. In univariate analysis, risk factors with strong evidence of association with MG MRAMs
197 were sexual orientation, age, ethnicity, recent STI diagnosis and co-infection with another STI.

198 In multivariable analysis, compared to MSW, females were less likely to have MG MRAMs (AOR (95%
199 CI): 0.23 (0.09- 0.58)). Being of Black ethnicity (2.64 (1.06-6.56)) increased the odds of having
200 MRAMs in MG samples compared to those of White ethnicity. Co-infection with another STI was
201 associated with MG MRAMs (7.19 (2.41-21.46)).

202

203 *Table 4: Univariate and multivariable logistic regression analysis of factors associated with MG*

204 *macrolide resistance (MRAM)*

		Univariate		Multivariable	
	Prevalence of MG MRAMs	Odds ratio (95% confidence interval)	P value	Adjusted odds ratio (AOR) (95% confidence interval)	P value
Sexual orientation					
MSW	12.1	1	-	1	-
MSM	4.3	0.32 (0.09-1.12)	0.076	0.25(0.05-1.37)	0.309
Females	**2.9**	**0.22 (0.10-0.49)**	**<0.001**	**0.23 (0.09-0.58)**	**<0.05**
Age group (years)					
16-19	13.0	1	-	1	-
20-24	6.5	0.46 (0.15-1.42)	0.178	0.41 (0.11-1.48)	0.174
25-34	**5.1**	**0.35 (0.14-1.03)**	**<0.05**	0.30 (0.09-1.07)	0.063
35+	4.8	0.34 (0.10-1.01)	0.072	0.34 (0.08-1.38)	0.131
Ethnicity					
White	2.8	1	-	1	-
Asian	10.0	3.83 (0.77-19.03)	0.101	3.16 (0.52-19.30)	0.213
Black	**11.5**	**4.49 (1.97-10.25)**	**<0.001**	**2.64 (1.06-6.56)**	**<0.05**
Other	7.1	2.65 (0.79-8.92)	0.116	2.18 (0.53-8.91)	0.278
Co-infection*					
No	4.7	1	-	1	-
Yes	**32.0**	**9.03 (3.39-24.05)**	**<0.001**	**7.19 (2.41-21.46)**	**<0.001**
Recent STI					
No	4.9	1	-	1	-
Yes	9.6	2.03 (0.91-4.57)	0.080	1.62 (0.64-4.11)	0.305

Sexual contact of individual with an STI					
No	6.3	1	-	-	-
Yes	4.2	0.64 (0.19-2.17)	0.479	-	-
Sex Abroad (within the past 3 months)					
No	5.5	1	-	-	-
Yes	4.9	0.89 (0.20-3.91)	0.873	-	-
Sex with someone from outside the UK (within the past 3 months)					
No	6.3	1	-	-	-
Yes	3.4	0.53 (0.18-1.58)	0.252	-	-
Regular partner					
No	3.4	1	-	-	-
Yes	5.9	1.76 (0.51-6.08)	0.368	-	-

MRAM: MG macrolide resistance; MSW: Men who have sex with women; MSM: men who have sex with men; STI: sexually transmitted infection; AOR: adjusted odds ratio

**Co-infection: ≥1 CT, MG or NG within a sample*

Discussion

This study confirms that in an inner London sexual health clinical setting among STI-contacts and symptomatic patients, MG prevalence is high overall, and in our sample set particularly those diagnosed with CT infection. MG-MRAM infections were present in nearly 40% of MG-positive

212 samples from women, two-thirds of MSW MG-positive samples, and were more likely to be found in
213 those with a co-infection than in those with a mono-infection.

214 These findings have implications for clinical management of STI-contacts and symptomatic patients
215 in SHCs. Although the UK first line treatment for CT, doxycycline, is a poorly effective monotherapy
216 for MG infection(16,17), recent evidence suggests pre-treatment with doxycycline can significantly
217 reduce bacterial load ($p<0.001$) (8). Additionally, increasing evidence suggests 1g azithromycin, the
218 UK's second line CT therapy, may not be as effective for CT treatment as previously thought(18,19)
219 and is associated with a high rate of MG treatment failure, commonly due to selection or presence
220 of 23S rRNA mutations (17,20). Importantly, high rates of MRAMs in our study data and macrolide
221 resistance worldwide (30-80%)(12,21,22) suggest azithromycin monotherapy should not be used, at
222 any dose, without appropriate resistance testing.

223 Our study also highlights the potential need for MG testing in those clinically indicated, as already
224 recommended in a number of treatment guidelines (9,23,24). Despite MG testing being adopted in
225 some UK SHCs, it is still far from universally implemented. Implications of our findings on clinical
226 management would very much depend on availability of both MG and macrolide resistance tests.
227 Such tests are now commercially available (25). In situations where it is not feasible to use such tests
228 on all indicated patients, our findings suggest testing could be directed at symptomatic and STI-
229 contact patients with CT infection; others have demonstrated utility targeting testing at those
230 diagnosed with NGU (9,24). Thus, in all scenarios where symptoms may suggest a CT or MG infection
231 (with or without access to a routine MG test), treating with doxycycline at baseline followed by
232 either test of cure (TOC) or further treatment directed at MG infection may be sensible approaches
233 to management, and reduce potential macrolide resistance selection pressure (6,26). However,
234 based on our dataset, testing only CT positives who are STI-contacts or symptomatic would still miss
235 high numbers of MG infections (72% in our study), and would not be adequate. Cost-effectiveness of
236 deploying macrolide resistance tests would depend in-part on there being sufficient numbers of

susceptible strains circulating and effective alternative treatment options available. Given the high rate of macrolide resistance, and emerging fluoroquinolone resistance worldwide (6,22,27,28), investigating the public health impact and cost-effectiveness of these tests is important.

Our study included results from three population groups (females, MSW and MSM). Recruitment was restricted to those with symptoms or who were sexual contacts of an individual with CT, NG, TV, or NGU, to inform management of this patient group, in-line with evidence that MG testing should not be expanded to asymptomatic individuals(29). For MSM, participants were sampled from three anatomical sites and from three London locations, providing a better overall representation of individual infection status. Finally, testing was performed in a robust manner with all three clinics using the same CT/NG routine NAAT.

There are some limitations to this study. Firstly, these data were collected as part of a larger study (the "Precise Study"), the aim of which was the development and evaluation of a NAAT-based POCT for NG and MG infection and resistance. Consequently, symptomatic patients and sexual contacts of individuals with CT, NG, TV or NGU were targeted for recruitment in order to increase the likelihood of STI-positive individuals. This however means we are unable to comment on the prevalence of MG infection or resistance in asymptomatic patients, and the consequent importance of testing (and treating) these individuals for MG.

Secondly, participants were recruited on the basis of self-reported symptoms, which may vary between females and males. For example, physiological vaginal discharge is not uncommonly reported as a symptom in females, and pathological discharge often includes non-STIs such as candidiasis and bacterial vaginosis. Another limitation is that, common to many studies with heterosexual women, extra-genital testing was not offered despite recent evidence detailing high rates of rectal CT infection in this population group (19,30,31) . Therefore, it is possible prevalence and co-infections in female participants may have been underestimated. Additionally, the absence of NG-MG co-infections warrants further investigation as this could be related to the relatively small

sample size of the MSM participants. Samples were only collected from patients attending London clinics and data collected from MSW and females from one clinic, so may not be representative of the wider symptomatic population. Finally, although low numbers, TV results were excluded from this analysis due to testing only with the laboratory test without confirmatory testing, and for the original purpose of this evaluation within the "Precise Study", CT, MG and NG were tested using a discrepant analysis approach.

Our risk factor analysis demonstrated that men, co-infection with another STI and black ethnicity were all independent risk factors associated with macrolide resistant MG. Having a co-infection was the strongest independent risk factor, perhaps indicating these participants were at a higher risk of previous STIs or, as MG is not routinely tested for, could be due to historic missed infection. Alternatively, these data may be a surrogate for previous azithromycin exposure and may represent and emphasise a need for vigilance in clinical history taking, particularly for subjective factors such as patient recall of previous antibiotic use or an STI diagnosis. We did not have data on previous exposure to azithromycin or prevalence of recent diagnoses of non-specific genital infection. History of azithromycin therapy around the time of the study may have helped explain these findings. This further emphasises the need for MG testing, suggesting that in the presence of CT or NG infection, use of azithromycin to treat any MG co-infection should be avoided unless specifically testing for MG resistance.

We found a low prevalence of genotypic fluoroquinolone resistance in our sample set (0.18%) compared to other prevalence studies reporting 8.6-53.1% (22,32) supporting the development of new diagnostic fluoroquinolone resistance tests to help with resistance-guided therapy.

In summary, our data show high prevalence of co-infection of MG with CT, and high prevalence of macrolide resistant MG, particularly in CT co-infections, amongst symptomatic patients and contacts of STIs. The findings suggest the need for MG testing, in particular for the management of STI-contact and symptomatic females and MSW, and possibly in those with CT. Our MSM dataset had

few MG positives with MRAMs, which combined with the current lack of evidence for the role of MG in MSM sexual health, means recommendations for this population cannot be made. For management of CT infections, data support an approach of doxycycline as first-line therapy to avoid azithromycin for patients whose MG status and resistance profile are unknown. In those subsequently testing positive for MG, azithromycin should only be used following the demonstration that the infection strain is genotypically sensitive.

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Author contributions

E.H.E and S.T.S conceived the study, and S.S.F contributed to overall study concept. M.H, M.J.P and N.K.T planned and performed laboratory work. Data collection, extraction and analysis was performed by C.E.B., S.O., M.F. and E.H.E. Manuscript was prepared by C.E.B, M.F and S.T.S.

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Transparency declaration

STS reports on behalf of himself and colleagues of the SGUL ADREU grants from National Institute for Health Research, during the conduct of the study; other from Binx Health Limited, non-financial support from Oxford Nanopore Technology, non-financial support from Cepheid, other from Alere, other from SpeeDx, grants from Innovate UK, outside the submitted work. EHE also reports grants from Becton Dickinson, grants from UKCRC, outside the submitted work. SSF also reports grants from National Institute of Health Research (NIHR), during the conduct of the study; grants from Innovate UK, grants from European Commission, outside the submitted work. The other authors report no conflict of interest relevant to this article.

References

1. **Horner PJ**, Martin DH. Mycoplasma genitalium Infection in Men. J Infect Dis. 2017 Jul;216(suppl_2):S396–405.

2. **Taylor-Robinson D**, Horner PJ. The role of Mycoplasma genitalium in non-gonococcal urethritis. Sex Transm Infect. 2001 Aug;77(4):229–31.

3. **Bradshaw CS**, Tabrizi SN, Read TRH, Garland SM, Hopkins CA, Moss LM, et al. Etiologies of Nongonococcal Urethritis: Bacteria, Viruses, and the Association with Orogenital Exposure. J Infect Dis. 2006 Feb;193(3):336–45.

4. **Wiesenfeld HC**, Manhart LE. Mycoplasma genitalium in Women: Current Knowledge and Research Priorities for This Recently Emerged Pathogen. J Infect Dis. 2017 Jul;216(suppl_2):S389–95.

5. **Edlund M**, Blaxhult A, Phd M, Bratt G. The spread of Mycoplasma genitalium among men who have sex with men. Int J STD AIDS. 2012 Jun;23(6):455-6. doi: 10.1258/ijsa.2009.009411.

6. **Read TRH**, Fairley CK, Tabrizi SN, Bissessor M, Vodstrcil L, Chow EPF, et al. Azithromycin 1.5g Over 5 Days Compared to 1g Single Dose in Urethral *Mycoplasma genitalium* : Impact on Treatment Outcome and Resistance. Clin Infect Dis . 2017 Feb 1;64(3):250–6.

7. **Manhart LE**, Jensen JS, Bradshaw CS, Golden MR, Martin DH. Efficacy of Antimicrobial Therapy for *Mycoplasma genitalium* Infections. Clin Infect Dis. 2015 Dec;61(suppl 8):S802–17.

8. **Kikuchi M**, Ito S, Yasuda M, Tsuchiya T, Hatazaki K, Takanashi M, et al. Remarkable increase in fluoroquinolone-resistant Mycoplasma genitalium in Japan. J Antimicrob Chemother. 2014 Sep;69(9):2376–82.

9. **Tagg KA**, Jeoffreys NJ, Couldwell DL, Donald JA, Gilbert GL. Fluoroquinolone and macrolide resistance-associated mutations in Mycoplasma genitalium. J Clin Microbiol. 2013 Jul;51(7):2245–9.

10. **Read TRH**, Fairley CK, Murray GL, Jensen JS, Danielewski J, Worthington K, et al. Outcomes of Resistance-guided Sequential Treatment of Mycoplasma genitalium Infections: A Prospective Evaluation. Clin Infect Dis. 2019 Feb 1;68(4):554-560. doi: 10.1093/cid/ciy477.

11. **Australian Sexual Health Alliance**. Mycoplasma genitalium - Australian STI Management Guidelines. 2018. Available from: http://www.sti.guidelines.org.au/sexually-transmissible-infections/mycoplasma-genitalium.

12. **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 Dec;44(6):1982–94.

13. **Pond MJ**, Nori A V., Witney AA, Lopeman RC, Butcher PD, Sadiq ST. High Prevalence of Antibiotic-Resistant Mycoplasma genitalium in Nongonococcal Urethritis: The Need for Routine Testing and the Inadequacy of Current Treatment Options. Clin Infect Dis. 2014 Mar;58(5):631–7.

14. **Getman D**, Jiang A, O'Donnell M, Cohen S. Mycoplasma genitalium Prevalence, Coinfection, and Macrolide Antibiotic Resistance Frequency in a Multicenter Clinical Study Cohort in the United States. J Clin Microbiol. 2016 Sep;54(9):2278–83.

15. **Gratrix J**, Plitt S, Turnbull L, Smyczek P, Brandley J, Scarrott R, et al. Prevalence and antibiotic resistance ofMycoplasma genitaliumamong STI clinic attendees in Western Canada: a cross-sectional analysis. BMJ Open. 2017 Jul;7(7):e016300.

16. **Nwokolo NC**, Dragovic B, Patel S, Tong CW, Barker G, Radcliffe K. 2015 UK national guideline for the management of infection with *Chlamydia trachomatis*. Int J STD AIDS. 2016 Mar 4;27(4):251–67.

17. **Wikstrom A**, Jensen JS. Mycoplasma genitalium: a common cause of persistent urethritis among men treated with doxycycline. Sex Transm Infect. 2006 Aug;82(4):276–9.

18. **Anagrius C**, Loré B, Jensen JS. Treatment of Mycoplasma genitalium. Observations from a Swedish STD Clinic. Coenye T, editor. PLoS One. 2013 Apr 8;8(4):e61481.

19. **Jensen JS**, Bradshaw C. Management of Mycoplasma genitalium infections - can we hit a moving target? BMC Infect Dis. 2015 Aug 19;15:343. 6

20. **Lau A**, Kong F, Fairley CK, Donovan B, Chen M, Bradshaw C, et al. Treatment efficacy of azithromycin 1 g single dose versus doxycycline 100 mg twice daily for 7 days for the treatment of rectal chlamydia among men who have sex with men - a double-blind randomised controlled trial protocol. BMC Infect Dis. 2017;17(1):35.

21. **Chandra NL**, Broad C, Folkard K, Town K, Harding-Esch EM, Woodhall SC, et al. Detection of *Chlamydia trachomatis* in rectal specimens in women and its association with anal intercourse: a systematic review and meta-analysis. Sex Transm Infect. 2018 Aug;94(5):320–6.

22. **Shimada Y**, Deguchi T, Nakane K, Yasuda M, Yokoi S, Ito S, et al. Macrolide Resistance–associated 23S rRNA Mutation in Mycoplasma genitalium, Japan. Emerg Infect Dis. 2011 Jun; 17(6): 1148–1150. doi: 10.3201/eid1706.101055

23. **Martens L**, Kuster S, de Vos W, Kersten M, Berkhout H, Hagen F. Macrolide-Resistant *Mycoplasma genitalium* in Southeastern Region of the Netherlands, 2014–2017. Emerg Infect Dis . 2019 Jul;25(7):1297–303.

24. **Deguchi T**, Ito S, Yasuda M, Sato Y, Uchida C, Sawamura M, et al. Surveillance of the prevalence of macrolide and/or fluoroquinolone resistance-associated mutations in Mycoplasma genitalium in Japan. J Infect Chemother. 2018 Nov;24(11):861-867. doi: 10.1016/j.jiac.2018.08.009

25. **Soni S**, Horner P, Rayment M, Pinto-sander N, Naous N, Parkhouse A, et al. 2018 BASHH UK national guideline for the management of infection with *Mycoplasma genitalium*. International Journal of STD and AIDS. 2019; 30(10) 983-950.

26. **Jensen JS**, Cusini M, Gomberg M, Moi H. 2016 European guideline on Mycoplasma genitalium infections. J Eur Acad Dermatology Venereol. 2016;30(10):1650–6.

27. **Harding-Esch EM**, Nori A V, Hegazi A, Pond MJ, Okolo O, Nardone A, et al. Impact of deploying multiple point-of-care tests with a “sample first” approach on a sexual health clinical care pathway. A service evaluation. Sex Transm Infect. 2017 Sep;93(6):424–9.

28. **Jensen JS**, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin treatment failure in Mycoplasma genitalium-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. Clin Infect Dis. 2008;47(12):1546–53.

29. **Kikuchi M**, Ito S, Yasuda M, Tsuchiya T, Hatazaki K, Takanashi M, et al. Remarkable increase in fluoroquinolone-resistant Mycoplasma genitalium in Japan. J Antimicrob Chemother. 2014 Sep 1;69(9):2376–82.

30. **Murray GL**, Bradshaw CS, Bissessor M, Danielewski J, Garland SM, Jensen JS, et al. Increasing Macrolide and Fluoroquinolone Resistance in Mycoplasma genitalium. Emerg Infect Dis. 2017;23(5):809–12.

31. **Baumann L**, Cina M, Egli-Gany D, Goutaki M, Halbeisen FS, Lohrer G-R, et al. Prevalence of Mycoplasma genitalium in different population groups: systematic review andmeta-analysis. Sex Transm Infect. 2018;94(4).

32. **van Liere GA**, Hoebe CJ, Wolffs PF, Dukers-Muijrers NH. High co-occurrence of anorectal

chlamydia with urogenital chlamydia in women visiting an STI clinic revealed by routine universal testing in an observational study; a recommendation towards a better anorectal chlamydia control in women. BMC Infect Dis. 2014 Dec;14(1):274.

33. **Tao G**, Hoover KW, Nye MB, Peters PJ, Gift TL, Body BA. Infrequent Testing of Women for Rectal Chlamydia and Gonorrhea in the United States. Clin Infect Dis. 2018 Feb;66(4):570–5.

34. **Murray GL**, Bradshaw CS, Bissessor M, Danielewski J, Garland SM, Jensen JS, et al. Increasing Macrolide and Fluoroquinolone Resistance in Mycoplasma genitalium. Emerg Infect Dis. 2017;23(5):809–12.

35. **Zhou W,** Du W, Cao H, et al. Detection of gyrA and parC Mutations Associated with Ciprofloxacin Resistance in Neisseria gonorrhoeae by Use of Oligonucleotide Biochip Technology. *J Clin Microbiol*. 2004;42(12):5819-5824. doi:10.1128/JCM.42.12.5819-5824.2004

36. **Jensen JS**, Björnelius E, Dohn B, Lidbrink 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;42(2):683-692. doi:10.1128/JCM.42.2.683-692.2004