ORIGINAL ARTICLE

Mycoplasma genitalium: high prevalence of resistance to macrolides and frequent anorectal infection in men who have sex with men in western Sydney

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ABSTRACT

Objectives We aimed to estimate the prevalence of *Mycoplasma genitalium* infection and of mutations linked to macrolide resistance using the ResistancePlus MG assay (SpeeDx, Sydney, New South Wales, Australia) in first-void urine (FVU), anorectal and oropharyngeal samples from men who have sex with men (MSM) attending Western Sydney Sexual Health Centre (WSSHC).

Methods Consecutive symptomatic and asymptomatic MSM attending for STI testing were prospectively enrolled. *M. genitalium* testing using the ResistancePlus MG assay was performed on FVU, anorectal and oropharyngeal samples routinely collected for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* assays.

Results Overall, the prevalence of *M. genitalium* infection in the study group was 13.4% (68/508). Most (79.4%, 54/68) *M. genitalium* harboured macrolide resistance mutations (87.5% of urethral and 75.6% of anorectal infections). The anorectum was the most commonly infected site (45/505, 8.9%), followed by the urethra (24/508, 4.7%). No oropharyngeal *M. genitalium* infections were detected (0/508). Most of the anorectal (93.3%) and urethral (79.2%) infections were asymptomatic. MSM who were taking HIV pre-exposure prophylaxis (PrEP) were twice as likely to be infected with *M. genitalium* compared with MSM who were not on PrEP (OR 2.1, 95% CI 1.3 to 3.6; P=0.0041). Always using condoms for anal sex in the last 3 months was protective of infection (OR 0.8, 95% CI 0.6 to 1.0; P=0.0186).

Conclusions We demonstrated a high prevalence of *M. genitalium* and very high levels of macrolide resistance among MSM attending WSSHC. Our findings support the routine use of an assay to detect macrolide resistance mutations in *M. genitalium* infections. This will ensure, in regions or populations with high rates of macrolide resistance among *M. genitalium* strains, that first-line treatment with azithromycin will only be used if a macrolide-sensitive strain is identified.

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INTRODUCTION

Although *Mycoplasma genitalium* is a well-established cause of non-gonococcal urethritis (NGU), few reports describe asymptomatic or extragenital infections in men who have sex with men (MSM), and evidence is lacking as to the value of screening for *M. genitalium* in asymptomatic populations.[1–7] More information about the pattern of infection, clinical manifestations and biological ramifications of *M. genitalium* infection in MSM would inform testing guidelines. Furthermore, internationally there have been widespread reports of increasing *M. genitalium* resistance to azithromycin, a macrolide antibiotic used for first-line treatment.[8] MSM populations may be particularly vulnerable to acquiring and transmitting macrolide resistant *M. genitalium* due to their higher risk of STIs and the increased likelihood of prior macrolide therapy.

From 2001 to 2014, the overall reported *M. genitalium* prevalence among MSM has ranged from 2.1% to 8.1% (1.6%–5.4% in anorectal infections), but none of these studies examined the prevalence of *M. genitalium* macrolide resistance.[1–6] Oropharyngeal *M. genitalium* has rarely been reported.[9]

Antimicrobial resistance has complicated the treatment of *M. genitalium* infections. Macrolide resistance-associated mutations in region V of 23S rRNA limit effectiveness of initial treatment with azithromycin, and fluoroquinolone resistance is impacting the effectiveness of second-line therapy, leading to ongoing transmission of resistant *M. genitalium* strains.[10 11] In Australia, macrolide resistance mutations were present in 36%–43% of strains detected mainly in men with NGU between 2008 and 2013.[12 13] Macrolide resistance testing when *M. genitalium* is detected could reduce time to cure and thereby limit ongoing transmission of macrolide-resistant strains.[7]

In the present study, we aimed to estimate the prevalence of *M. genitalium* infection as well as the prevalence of mutations linked to macrolide resistance using the ResistancePlus MG assay (SpeeDx Sydney, New South Wales, Australia) in urine, anorectal and oropharyngeal samples from MSM attending Western Sydney Sexual Health Centre (WSSHC).

METHODS

WSSHC provides comprehensive STI testing and treatment for at-risk populations including MSM, sex workers and people living with HIV infection.

MSM accounted for approximately 50% of 8139 visits to the clinic in 2017. Individual patient consent was not required. A sample size of 500 men was calculated assuming prevalence of *M. genitalium* of 5%, test sensitivity of 0.7, specificity of 0.9, at 0.95 confidence and 0.05 precision. Consecutive symptomatic and asymptomatic MSM attending the sexual health centre in western Sydney, Australia, for STI testing, and who had first-void urine (FVU), anorectal and oropharyngeal samples collected, were prospectively enrolled by the attending clinician. *M. genitalium* testing using the ResistancePlus MG assay was performed on FVU, anorectal and oropharyngeal samples routinely collected for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* testing. Men whose tests were positive for *M. genitalium* were treated according to standard clinic protocols, including contact tracing.

The ResistancePlus MG assay (SpeeDx) is a multiplex quantitative (qPCR) assay which uses novel PlexZyme and PlexPrime technology to simultaneously detect *M. genitalium* and five 23S rRNA mutations, A2058C, A2058G, A2058T, A2059C and A2059G (*Escherichia coli* numbering), associated with macrolide resistance.[14 15] The assay incorporates appropriate positive controls to verify the presence of wild-type *M. genitalium* (ie, harbouring no resistance mutations) as well as the presence of strains harbouring the above mutations. Two recent studies evaluated this assay and reported excellent sensitivity and specificity for the detection of *M. genitalium* (97.4%–99.1% and 98.5%–100%, respectively), as well as for the detection of macrolide resistance-associated mutations (97.4%–100% and 96.2%–100%, respectively).[14 15]

Oropharyngeal and anorectal swab specimens consisted of BD ProbeTec dry swabs in diluent (Becton Dickinson, North Ryde, Australia) previously assayed on the BD Viper system (Becton Dickinson) for the routine detection of *C. trachomatis* and *N. gonorrhoeae*. Swabs in diluent were vortex mixed for 10 s. DNA was extracted, from either 750 µL of mixed swab diluent or 1 mL of FVU specimens, by on-board lysis workflow on the NucliSens EasyMag platform (bioMerieux Australia, Baulkham Hills, Australia) and eluted in 100 µL. Five µL of 1 in 10 diluted ResistancePlus MG Internal Control cells (SpeeDx) were added to each specimen prior to lysis addition as described by the manufacturer's evaluation instruction for use document for the manual extraction method.

Real-time detection of *M. genitalium* by ResistancePlus MG assay (SpeeDx) was performed using the LightCycler 480 Instrument II (Roche Diagnostics Australia, North Ryde, Australia) as described by the manufacturer. A 5 µL aliquot of extracted DNA was assayed within a 20 µL final reaction volume in 96-well plates using cycling parameters of 95°C for 2 min, followed by 10 cycles of 95°C for 5 s, 61°C–56°C for 30 s (−0.5°C per cycle), then 40 cycles of 95°C for 5 s, 52°C for 40 s and, finally, cooling at 40°C for 30 s. Negative assay controls consisted of 5 µL PCR grade water (Sigma-Aldrich, Castle Hill, Australia). Data analysis reporting the presence or absence of *M. genitalium*, 23S rRNA mutation and internal control was performed using the ResistancePlus MG Analysis Software beta version LC480_b2.2 (SpeeDx).

Clinical, demographic and behavioural data were retrospectively collected from laboratory and clinic electronic databases and clinical files at WSSHC. Data recorded included age, number and sex of sexual partners in the last 3 months, condom use, HIV serostatus, use of HIV pre-exposure prophylaxis (PrEP), urethral and/or anorectal symptoms, azithromycin treatment in the last 12 months and co-infection with *C. trachomatis* and/or *N. gonorrhoeae*. Urethral symptoms were defined as urethral discharge and/or dysuria, and anorectal symptoms were anorectal pain, discomfort, bleeding and/or discharge. Data were entered into Excel and analysed to examine any associations with *M. genitalium* infection or the presence of macrolide resistance mutations. Prevalence of *M. genitalium* and macrolide resistance were measured for the study population as a whole, and for specific sites of infection (urethral, anorectal or oropharyngeal).

Data were analysed using Stata Statistical Software: Release 12 (StataCorp, College Station, Texas, USA). Bivariate associations were examined using X^2 or Fisher's exact tests, and ORs, 95% CIs and P values calculated using the Mantel-Haenszel method or multiple logistic regression.

Table 1 Characteristics of MSM enrolled (n=508) and not enrolled (n=47)

Characteristic	Enrolled	Not enrolled	OR	95% CI	P value
Mean age (years)	33.2	38.2	N/A	N/A	0.0030
HIV-positive n (%)	30 (5.9)	13 (27.7)	0.2	0.1 to 0.4	<0.0001
CD4 Mean (median)	775 (791)	862 (751)	N/A	N/A	0.3798
On PrEP n (%)	169 (33.3)	15 (31.9)	1.1	0.6 to 2.0	0.8506
Urethral symptoms n (%)	36 (7.1)	2 (4.3)	1.7	0.4 to 7.4	0.4625
Anorectal symptoms n (%)	12 (2.4)	1 (2.1)	1.1	0.1 to 8.8	0.9191
CT/NG urethral n (%)	25 (4.9)	2 (4.3)	1.2	0.3 to 5.1	0.8393
CT/NG anorectal n (%)	67 (13.2)	7 (15.2)	0.9	0.4 to 2.0	0.6988

CD4, CD4 cells/µL; CT/NG anorectal, *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* anorectal infection; CT/NG urethral, *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* urethral infection; MSM, men who have sex with men; N/A, not available; PrEP, HIV pre-exposure prophylaxis.

RESULTS

The study group included 508 consecutive MSM who attended WSSHC from February to May 2017. Enrolled men represented 91.5% of all eligible men who attended the clinic for STI testing of FVU, anorectal and oropharyngeal samples during the study period. Compared with HIV-negative MSM, HIV-positive MSM were significantly less likely to be enrolled, and were older (P=0.002). However, there was no significant difference in mean CD4 cell count, which was >750 cells/µL in HIV-positive MSM enrolled and not enrolled, and rates of urethral or anorectal chlamydial and/or gonococcal infections were similar for MSM with and without HIV infection. Characteristics of men enrolled and not enrolled are detailed in table 1.

Prevalence of *M. genitalium*, macrolide resistance and co-infections

Overall prevalence of *M. genitalium* infection in the study group was 13.4% (68/508). The anorectum was the most commonly infected site in 45/505 (8.9%), followed by the urethra in 24/508 (4.7%). There was no result for three men whose anorectal samples were inhibited on testing. No oropharyngeal *M. genitalium* infections were detected (0/508). Only one man had both urethral and anorectal *M. genitalium* infection. Among asymptomatic men, the prevalence of *M. genitalium* was 8.5% (42/493) in the anorectum, and 4.0% (19/472) in the urethra. Overall, 79.4% (54/68) of infections were macrolide-resistant, with 21/24 (87.5%) of urethral and 34/45 (75.6%) of anorectal infections harbouring macrolide resistance-associated mutations

Table 2 Associations with *M. genitalium* infection

	MG positive n (%)	MG negative n (%)	OR (95% CI)	P value
Age group (years)			0.9 (0.7 to 1.2)	0.4261
≤29	34 (13.4)	219 (86.6)	0.2 (0.1 to 0.2)	
30–39	20 (14.1)	122 (85.9)	0.2 (0.1 to 0.3)	
40–49	12 (19.4)	50 (80.7)	0.2 (0.1 to 0.5)	
≥50	2 (3.9)	49 (96.1)	0.0 (0.0 to 0.2)	
On PrEP			2.1 (1.3 to 3.6)	0.0041
Yes	33/169 (19.5)	136/169 (80.5)		
No	35/339 (10.3)	304/339 (89.7)		
Anorectal CT			3.9 (1.8 to 8.5)	0.0002
Yes	11/45 (24.4)	35/460 (7.6)		
No	34/45 (75.6)	425/460 (92.4)		
Anorectal NG			1.5 (0.5 to 4.3)	0.5036
Yes	4/45 (8.9)	29/460 (6.3)		
No	41/45 (91.1)	431/460 (93.7)		
Urethral CT			2.2 (0.5 to 10.2)	0.2902
Yes	2/24 (8.3)	19/484 (3.9)		
No	22/24 (91.7)	465/484 (96.1)		
Urethral NG			3.0 (0.4 to 25.2)	0.2966
Yes	1/24 (4.2)	7/484 (1.5)		
No	23/24 (95.8)	477/484 (98.6)		
Male partners last 3/12			1.2 (0.9 to 1.5)	0.30
0	1/8 (12.5)	7/8 (87.5)	0.1	
1	12/86 (14.0)	74/86 (86.1)	0.2	
2–5	29/252 (11.5)	223/252 (88.5)	0.1	
6–10	16/96 (16.7)	80/96 (83.3)	0.2	
>10	10/57 (17.5)	47/57 (82.5)	0.2	
Female partners last 3/12			1.8 (0.8 to 4.2)	0.1511
Yes	8/68 (11.8)	30/439 (6.8)		
No	60/68 (88.2)	409/439 (93.2)		
Condom use			0.8 (0.6 to 1.0)	0.0186
Never	16/94 (17.0)	78/94 (83.0)	0.2	
<50%	19/117 (16.2)	98/117 (83.8)	0.2	
≥50%	24/168 (14.3)	144/168 (85.7)	0.2	
Always	7/108 (6.5)	101/108 (93.5)	0.1	
N/A	1/15 (6.7)	14/15 (93.3)	0.1	
HIV infection			0.7 (0.2 to 2.4)	0.5749
Yes	3/30 (10.0)	27/30 (90.0)		
No	65/478 (13.6)	413/478 (86.4)		

Condom use, condom use for anal sex last 3 months: N/A, no anal sex in the last 3 months (data missing for six men); female partners last 3/12, any female sexual partner/s in the last 3 months (data missing for one man); male partners last 3/12, number of male sexual partners in the last 3 months (data missing for nine men).
CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; N/A, not available; NG, *Neisseria gonorrhoeae*; PrEP, HIV pre-exposure prophylaxis.

(no significant difference; P=0.25). Apart from being less common among men aged 50 years and older, presence of *M. genitalium* infection was not related to age (table 2). In this cohort, *C. trachomatis* was detected in 65/508 (12.8%) men overall, in 21/508 (4.1%) FVU samples, in 46/508 (9.1%) anorectal samples and in 10/508 (2.0%) oropharyngeal samples. Likewise, *N. gonorrhoeae* were detected in 62/508 (12.2%) overall, in 8/508 (1.6%) FVU samples, in 33/508 (6.5%) anorectal samples and in 43/508 (8.5%) oropharyngeal samples.

Association between macrolide resistance and previous azithromycin treatment

Information on azithromycin treatment in the last 12 months was available for 65/68 of the men with *M. genitalium* infection. Among 52 men with macrolide-resistant strains, 46.2% had been treated with azithromycin within 12 months. While 24/25 (96.0%) of men who had received azithromycin had macrolide-resistant *M. genitalium* infection, 28/40 (70.0%) of those who had not also harboured resistant strains. Men who had received azithromycin treatment in the last 12 months were significantly more likely to have a macrolide-resistant *M. genitalium* infection (OR 10.3, 95% CI 1.1 to 96.9, P=0.0114). No other study factor was associated with presence of macrolide-resistance mutations in *M. genitalium*, including presence of urethral (P=0.9606) or anorectal (P=0.3706) symptoms.

Associations between *M. genitalium,* STI co-infections, HIV and symptoms

Associations between *M. genitalium*, symptoms and other infections are presented in table 2. Co-infections with *C. trachomatis* were more common than with *N. gonorrhoeae*. Two men had anorectal infection with all three microorganisms. Anorectal *C. trachomatis* was independently associated with anorectal *M. genitalium* (OR 5.0, 95% CI 2.1 to 11.8, P<0.001) after controlling for condom use, number of male sexual partners in the last 3 months, age, anorectal *N. gonorrhoeae*, use of PrEP and HIV infection. HIV-infected men were not more likely than men without HIV infection to test positive for *M. genitalium* or to have infection with *C. trachomatis* or *N. gonorrhoeae* (P=0.26).

M. genitalium was detected in 3 of the 12 men with anorectal symptoms, of whom one had co-infection with *C. trachomatis*. Very few anorectal infections were associated with symptoms, and a consistently significant association was found only for anorectal gonococcal infection (table 3). Urethral gonococcal or chlamydial co-infection was detected in two of the five men with urethral symptoms who tested positive for *M. genitalium*. For all three pathogens, urethral infection was independently associated with presence of urethral symptoms (table 3).

Associations with PrEP, partners and condom use

Men who were on PrEP were twice as likely to be infected with *M. genitalium* compared with men who were not on PrEP (table 2). There was no difference in the prevalence of macrolide resistance-associated mutations in *M. genitalium* infections among men on PrEP (78.8%) compared with men not on PrEP (80.0%), OR 0.9 (95% CI 0.3 to 3.0, P=0.9024). The number of recent male sexual partners did not predict *M. genitalium* positivity; however, always using condoms for anal sex in the last 3 months was protective of infection (table 2). Men who always used condoms for anal sex in the last 3 months were less likely to be on PrEP (OR 0.6, 95% CI 0.5 to 0.7, P<0.001). On multivariate analysis, controlling for condom use, number of male partners in the last 3 months, age, other urethral or anal infections and HIV infection, *M. genitalium* positivity remained significantly associated with use of PrEP (OR 2.5, 95% CI 2.0 to 5.2, P=0.015).

DISCUSSION

Key findings of our study were the high frequency of *M. genitalium* infection and very high prevalence of *M. genitalium* macrolide resistance in MSM attending our clinic in western Sydney. Our study also confirms that the oropharynx is not an important site for *M. genitalium* infection.

Table 3 Associations between anogenital symptoms and STIs

Site of infection	Pathogen	Symptoms* n (%)	OR (95% CI)	aOR (95% CI)
Anorectal	*Mycoplasma genitalium*	3/45 (6.7)	3.6 (0.9 to 13.8)	4.0 (1.0 to 16.3)
	Chlamydia trachomatis	1/46 (2.2)	0.9 (0.1 to 7.2)	0.3 (0.0 to 2.8)
	Neisseria gonorrhoeae	4/33 (12.1)	8.1 (2.3 to 28.9)	10.2 (2.7 to 39.1)
Urethral	*M. genitalium*	5/24 (20.8)	3.9 (1.3 to 11.1)	3.5 (1.0 to 11.8)
	C. trachomatis	12/21 (57.1)	25.7 (9.0 to 73.3)	19.0 (6.7 to 53.4)
	N. gonorrhoeae	6/8 (75.0)	47.0 (8.2 to 269.0)	21.7 (3.3 to 143.1)

*Anorectal symptoms in the case of anorectal infection, urethral symptoms in the case of urethral infection.
aOR, adjusted OR after controlling for the other two anorectal or urethral infections.

Almost one in eight men was infected with *M. genitalium*, most commonly in the anorectum. In this cohort, the overall prevalence of *M. genitalium* was similar to that of both *C. trachomatis* and *N. gonorrhoeae*. More than three-quarters of all urethral and anorectal *M. genitalium* infections harboured macrolide resistance-associated mutations. We used a new multiplex qPCR assay, ResistancePlus MG (SpeeDx), that simultaneously detects the presence of *M. genitalium* and macrolide resistance-associated mutations.[14 15] A realistic laboratory procedure turnaround time for this assay is up to 72 hours from laboratory receipt of the sample. Results can be available within five working days of sample collection, enabling initial treatment with moxifloxacin for men with macrolide-resistant *M. genitalium* infection, and facilitating appropriate management of sexual contacts. While moxifloxacin is likely to be effective in the majority of these cases, further treatment options for men whose *M. genitalium* infections are also resistant to moxifloxacin remain incompletely evaluated.[13]

Macrolide resistance in anorectal infections has not previously been systematically investigated, but was recently detected in 7/7 (100%) anorectal samples in Melbourne, Australia.[15] Selection of macrolide-resistant strains is likely among groups with high rates of partner change and frequent episodes of STI treatment. In our study, presence of macrolide resistance in *M. genitalium* strains was associated with azithromycin treatment in the last 12 months, and this finding was also recently reported by investigators in Spain.[16] Although we now routinely use doxycycline for initial treatment of NGU and anorectal chlamydial infection, azithromycin is still used in combination with ceftriaxone for the treatment of gonococcal infections. Nevertheless, there was no history of prior azithromycin in over half of the men with macrolide-resistant infections, suggesting that resistant infections are being commonly transmitted and acquired.

Only a small proportion of both urethral and anorectal infections were symptomatic in this cohort, providing some information regarding the symptomatic proportion of *M. genitalium* infections among MSM; only one-fifth of MSM with urethral, and 6.7% with anorectal *M. genitalium* infections were symptomatic. However, it is difficult to determine if the presence of symptoms was due to *M. genitalium* in all cases as another pathogen was detected in almost half of urethral and in a third of anorectal symptomatic infections.

There is currently no international consensus on screening of asymptomatic MSM for *M. genitalium*. Among high-risk MSM, asymptomatic anorectal *M. genitalium* represents a reservoir of transmissible infection, while antibiotic treatment for other STIs is probably contributing to rising *M. genitalium* antimicrobial resistance. Consequently, it is likely that transmission of macrolide-resistant infections will continue unchecked. Nevertheless, the public health value of testing asymptomatic men for *M. genitalium* has not been established.[7]

Although recent European guidelines advise that regular *M. genitalium* testing including anal sampling could be considered due to the risk of increased HIV transmission, we lack evidence of synergy between *M. genitalium* and HIV in asymptomatic MSM, as the majority of data supporting this interaction relate to women[7 17–20] Additionally, there is only limited evidence that *M. genitalium* causes proctitis. No significant association with anorectal symptoms was found in three observational studies.[1–3] However, in one study of 154 men with proctitis, 12% tested positive for *M. genitalium*, with significantly higher organism loads among cases compared with men with asymptomatic rectal infection.[21] Stronger evidence implicating *M. genitalium* in proctitis and/or HIV transmission/acquisition in MSM would support expanding *M. genitalium* screening for asymptomatic MSM. However, we still lack clarity on whether or not all *M. genitalium* strains are pathogenic, which makes interpretation of positive screening test results difficult in the absence of symptoms or disease. Of more practical importance, the lack of reliable treatment alternatives in cases where moxifloxacin treatment fails, and the level of patient anxiety that ensues, must also be considered in the screening risk-benefit equation.

The increased prevalence of *M. genitalium* infection in men taking PrEP is largely but not entirely explained by lower rates of condom use. In this cohort, *M. genitalium* infection was not associated with HIV infection per se. Several previous studies have reported that *M. genitalium* was more common in MSM who were HIV-positive compared with those HIV-negative.[3 21] Reasons underlying differences between studies may reflect behavioural factors, the degree of HIV-associated immunosuppression and density of sexual networks.

Our study had several limitations. First, some eligible men, particularly HIV-positive patients, were not recruited due to clinicians failing to enrol them. However, analysis of recruited patients showed that there was no difference in prevalence of *M. genitalium* infection related to HIV status, and comparison of HIV-positive men enrolled and not enrolled did not show any difference in prevalence of other STIs. Secondly, the sample size was designed to measure prevalence of infection, but may not have been large enough to accurately measure the proportion of symptomatic *M. genitalium* infection. Thirdly, use of azithromycin in the last 12 months may have been underestimated if men had received azithromycin elsewhere and that was not recorded in the clinical files. Lastly, the study was conducted in a single clinic and results may not be applicable to other clinical settings in Australia or internationally.

In conclusion, we have demonstrated a high prevalence of *M. genitalium* among MSM attending our service, and highlighted therapeutic challenges posed by high levels of macrolide resistance. By prolonging duration of infection and infectivity, antimicrobial resistance-related treatment failure is likely implicated in rising *M. genitalium* prevalence. Our findings support the

routine use of an assay to both detect *M. genitalium* infection as well as identify the presence of any macrolide resistance mutations, as recommended in the recent European guidelines on *M. genitalium*.[7] Such an assay could ensure that first-line treatment with azithromycin is only used if a macrolide-sensitive *M. genitalium* strain is identified in regions or populations with high rates of macrolide resistance. Finally, this new diagnostic tool could assist with ongoing efforts to strengthen *M. genitalium* antimicrobial resistance surveillance as well as inform local and regional *M. genitalium* treatment guidelines.

Key messages

- 13.4% of men who have sex with men in western Sydney have *Mycoplasma genitalium* infection, most commonly anorectal (8.9%), followed by urethral (4.7%); no oropharyngeal infections were detected.
- Most anorectal (93.3%) and urethral (79.2%) infections were asymptomatic.
- Nearly 80% of *M. genitalium* infections harboured macrolide resistance-associated mutations.
- A diagnostic assay that can simultaneously detect *M. genitalium* and macrolide resistance-associated mutations will help to limit inappropriate azithromycin treatment.

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Contributors All of the authors contributed to study design. MP, DLC and DAL planned and conducted the clinical aspects, and DJ, NJJ and SC planned and conducted the laboratory aspects, of the study. DJ performed most of the laboratory assays and laboratory data collection, and NJJ and SC had oversight of laboratory procedures. DLC collected clinical data, performed statistical analyses and wrote the initial manuscript draft, apart from laboratory procedures, which were written by DJ. All of the authors reviewed and made contributions to the final manuscript.

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Competing interests SpeeDx personnel assisted with specimen extraction and provided laboratory technical advice, but were not involved in performing the ResistancePlus MG assays, or in the design, conduct or reporting of this study.

Ethics approval This study was approved by Western Sydney Local Health District Human Research Ethics Committee, HREC reference number: HREC/16/WMEAD/280; SSA reference number SSA/16/WMEAD/304.

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