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REVIEW

Mycoplasma genitalium infection: current treatment options, therapeutic failure, and resistance-associated mutations

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Abstract: *Mycoplasma genitalium* is an important cause of non-gonococcal urethritis, cervicitis, and related upper genital tract infections. The efficacy of doxycycline, used extensively to treat non-gonococcal urethritis in the past, is relatively poor for *M. genitalium* infection; azithromycin has been the preferred treatment for several years. Research on the efficacy of azithromycin has primarily focused on the 1 g single-dose regimen, but some studies have also evaluated higher doses and longer courses, particularly the extended 1.5 g regimen. This extended regimen is thought to be more efficacious than the 1 g single-dose regimen, although the regimens have not been directly compared in clinical trials. Azithromycin treatment failure was first reported in Australia and has subsequently been documented in several continents. Recent reports indicate an upward trend in the prevalence of macrolide-resistant *M. genitalium* infections (transmitted resistance), and cases of induced resistance following azithromycin therapy have also been documented. Emergence of antimicrobial-resistant *M. genitalium*, driven by suboptimal macrolide dosage, now threatens the continued provision of effective and convenient treatments. Advances in techniques to detect resistance mutations in DNA extracts have facilitated correlation of clinical outcomes with genotypic resistance. A strong and consistent association exists between presence of 23S rRNA gene mutations and azithromycin treatment failure. Fluoroquinolones such as moxifloxacin, gatifloxacin, and sitafloxacin remain highly active against most macrolide-resistant *M. genitalium*. However, the first clinical cases of moxifloxacin treatment failure, due to bacteria with coexistent macrolide-associated and fluoroquinolone-associated resistance mutations, were recently published by Australian investigators. Pristinamycin and solithromycin may be of clinical benefit for such multidrug-resistant infections. Further clinical studies are required to determine the optimal therapeutic dosing schedules for both agents to effect clinical cure and minimize the risk of emergent antimicrobial resistance. Continual inappropriate *M. genitalium* treatments will likely lead to untreatable infections in the future.

Keywords: *Mycoplasma genitalium*, non-gonococcal urethritis, macrolide, fluoroquinolone, resistance, treatment failure

Introduction

Mycoplasma genitalium is a cause of acute and chronic non-gonococcal urethritis (NGU) and cervicitis, and is increasingly implicated in upper genital tract infections.[1,2] This minute genital parasite of the Mollicute class grows slowly as it is lacks the genes required for biosynthesis of amino acids and instead relies on host cells for nutrients.[1] Despite its minute size, *M. genitalium* displays features in common with other pathogenic bacteria that enable it to cause disease, evade host immune responses through antigenic variability, and readily develop resistance to antimicrobial agents.[3]

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Antimicrobial resistance is threatening the provision of effective, safe, and convenient treatment for *M. genitalium*, as well as a number of other bacterial sexually transmitted infections (STIs), such as gonorrhea.[4,5] While the gonococcus has a lengthy and well documented evolutionary history in terms of acquisition of new antimicrobial resistance mechanisms, *M. genitalium* is developing resistance to macrolides and fluoroquinolones at a speed belying its small size and, rather unexpectedly, before the introduction of systematic testing and treatment protocols. Clinical and patient factors promoting antimicrobial resistance in STI pathogens are gathering pace, driving the intrinsic propensity of these organisms to acquire antimicrobial resistance determinants or DNA point mutations at alarming rapidity.[4,6–8]

Management issues in the treatment of *M. genitalium* infections

Syndromic treatment of NGU has focused on eradication of *Chlamydia trachomatis*, a well-established cause of reproductive morbidity in women, and is usually instituted at initial presentation before results of investigations to detect specific bacterial causes are made available. In most cases of sexually acquired urethritis and cervicitis, tests are only performed for *Neisseria gonorrhoeae* and *C. trachomatis*.

Few countries offer routine screening for *M. genitalium* and, where this is performed, it typically relies on the use of in-house nucleic acid amplification tests performed on specimens collected at either the initial visit or after failure of first-line therapy. Importantly, there are still no validated and commercially available assays for routine diagnostic testing although these may be available in the near future.[9] While many experts accept current evidence linking *M. genitalium* with upper genital tract infections and infertility, a prospective observational study of morbidity associated with untreated *M. genitalium* infection would not be ethical in the light of current evidence. Doubts about the importance of *M. genitalium* as a reproductive pathogen, along with the lack of an approved diagnostic test, have delayed decisions on testing and treatment protocols.[9,10]

Overview of natural history and prevalence of *M. genitalium* infection

The natural history of *M. genitalium* infection in men with NGU has not been studied, but spontaneous clearance of infection occurred in 55% of a cohort of African women within 3 months.[11] In the absence of systematic screening and on the basis of studies conducted where testing is available, *M. genitalium* is most frequently detected in men who present with urethral symptoms.[12] Prevalence rates of 15%–35% are reported in men with symptomatic non-chlamydial NGU, whereas estimates of population prevalence of *M. genitalium* range from 1.1% to 3.3%.[13] Infections in women and anal infections among men-who-have-sex-with-men (MSM) are largely asymptomatic and therefore remain undiagnosed.[2,14] A study among MSM at a London clinic found *M. genitalium* prevalence rates of 2.7% and 4.4% in first-void urine and rectal samples, respectively, with higher rates in human immunodeficiency virus (HIV)-positive versus HIV-negative MSM, suggesting that asymptomatic rectal infection is relatively common in this risk group.[15] Finally, there is evidence that the prevalence of *M. genitalium* is increasing, at least in Scandinavia. A Danish national survey found that the proportion of those tested who tested positive increased significantly between the periods 2006–2008 and 2009–2010.[13]

Current treatment options

In common with other mycoplasmas, *M. genitalium* lacks a cell wall, and is therefore not susceptible to antibiotics targeting peptidoglycan assembly. Although tetracyclines, in particular doxycycline, have been used to treat NGU for many years, the efficacy of this antimicrobial class is relatively poor and isolates with reduced susceptibility have been reported.[16–18] Azithromycin, a macrolide, is now preferred for the treatment of NGU and related clinical syndromes on account of its long half-life, excellent tissue penetration, and the fact that it can be administered as a single-dose treatment. Clinical studies in which *M. genitalium* testing and treatment results have been reported include observational studies and several randomized clinical trials; these are summarized in Tables 1 and 2. In most cases, research effort has focused on studying the effectiveness of a single 1 g dose of azithromycin.[19–27] Studies have also reported the efficacy of higher doses and longer courses of azithromycin, particularly the extended 1.5 g course, given as 500 mg on day 1 and then 250 mg daily on days 2–5, or less often, two 1 g doses given 5–7 days apart.[17,24,26]

A controlled but non-randomized clinical trial recruited STI clinic patients with urethritis or cervicitis from Norway and Sweden from 2002 to 2004.[20] Treatment was initiated with either doxycycline (200 mg on day 1, 100 mg daily on days 2–9) or azithromycin 1 g as a single dose. Those who tested positive for *M. genitalium* were followed up, and if initial treatment failed, were treated with the alternative antibiotic, either azithromycin as an extended 1.5 g regimen (500 mg on day 1, 250 mg daily on days 2–5), or doxycycline

Table 1 Clinical efficacy studies of tetracycline/doxycycline, alone or versus macrolides, for treatment of *Mycoplasma genitalium* infection

Reference	Year	Study type	Population	*M. genitalium* cases (n)	Tetracycline regimen(s) and *M. genitalium* microbiological cure	Macrolide regimen and *M. genitalium* microbiological cure
Horner et al[10]	1993	Prospective case–control study	164 men with/without NGU attending an STI clinic, UK	27 men	DOXY 200 mg d 1, 100 mg d 2–d 9 10/14 cured (71.4%)	Not applicable
Johannisson et al[18]	2000	Uncontrolled observational study	233 men and 85 women attending STI clinics, Sweden	18 men 3 women	TET 500 mg 12 hourly ×10 d 5/13 men cured (38.5%) 0/1 women cured (0%)	Not applicable
Gambini et al[19]	2000	Prospective study with treatment varying by room	201 men with/without NGU attending an STI clinic, Italy	53 men	DOXY 200 mg/day ×7 d 33/35 cured (94.3%)	AZM 1 g stat 14/17 cured (82.4%)
Falk et al[17]	2003	Uncontrolled observational study	519 men and 464 women attending an STI clinic, Sweden	34 men 26 women	DOXY 200 mg d 1, 100 mg d 2–d 9 or LYME 300 mg 12 hourly ×10 d Men: 6/16 cured (37.5%) Women: 4/14 cured (28.6%)	AZM 500 mg d 1, 250 mg d 2–d 5 Men: 16/16 cured (100.0%) Women: 20/20 cured (100.0%)
Björnelius et al[20]	2008	Uncontrolled observational study	152 men with NGU and 60 women with cervicitis attending 6 STI clinics, Norway and Sweden	152 men 60 women	DOXY 200 mg (d 1), 100 mg (d 2–d 9) Men: 13/76 cured (17.1%) Women: 10/27 (37.0%)	AZM 1 g stat Men: 33/39 cured (84.6%) Women: 15/17 cured (88.2%)
Mena et al[21]	2009	Randomized controlled trial	398 men with NGU attending an STI clinic, USA	78 men	DOXY 100 mg 12 hourly ×7 d 14/31 cured (45.2%)	AZM 1 g stat 20/23 cured (87.0%)
Schwebke et al[22]	2011	Randomized controlled trial	305 men with NGU attending 4 STI clinics, USA	94 men	DOXY 100 mg 12 hourly ×7 d (± tinidazole 2 g stat) 12/39 cured (30.8%)	AZM 1 g stat (± tinidazole 2 g stat) 30/45 cured (66.7%)
Manhart et al[23]	2013	Randomized controlled trial	606 men with NGU attending an STI clinic, USA	80 men	DOXY 100 mg 12 hourly ×7 d (+ AZM placebo) 8/27 cured (29.6%, mITT)	AZM 1 g stat (+ DOXY placebo) 15/38 cured (39.5%, mITT)

Abbreviations: *M. genitalium*, *Mycoplasma genitalium*; NGU, non-gonococcal urethritis; STI, sexually transmitted infection; DOXY, doxycycline; TET, tetracycline; LYME, lymecycline; AZM, azithromycin; d, day/days; mITT, modified intention to treat population.

as above. The extended 1.5 g regimen had previously been reported to be very effective as a first-line treatment for *M. genitalium* infection.[17] This extended regimen was generally not used to treat individuals at their first clinic visit but rather reserved to treat individuals with a laboratory-confirmed diagnosis of *M. genitalium* infection or sexual contacts of individuals with recently diagnosed *M. genitalium* urethritis or cervicitis. Single-dose azithromycin 1 g was significantly more effective than doxycycline, curing 85% versus 17% of men, and 88% versus 37% of women. This study did not directly compare the efficacy of the single 1 g dose and the extended 1.5 g regimen of azithromycin, but reported that the extended azithromycin regimen, given after doxycycline had failed, was more effective in eradicating *M. genitalium* (45/47, 96%) compared with an initial single 1 g dose (33/39, 85%).[20] Although this difference was not statistically significant (P=0.133), the findings have substantially influenced clinical practice.

In contrast, a retrospective Norwegian study reported no difference in efficacy of three different azithromycin regimens: 1 g stat, 1 g on day 1 and a repeated 1 g dose on days 5–7, or the extended 1.5 g regimen.[24] Azithromycin efficacy was lower in this retrospective Norwegian study (72%–79%) compared with the non-randomized controlled trial in Swedish and Norwegian clinics. The authors postulated that routine use of azithromycin 1 g in Norway may select for azithromycin-resistant *M. genitalium* strains.[20,24] Additionally, the extended 1.5 g regimen of azithromycin was found to be ineffective once azithromycin 1 g single-dose treatment had failed.[24]

The first randomized clinical trial of *M. genitalium* treatment compared azithromycin 1 g with doxycycline 100 mg twice daily for 7 days, and confirmed the results of previous non-randomized trials and observational studies, ie, that a single 1 g dose of azithromycin was more effective than doxycycline for treatment of *M. genitalium* infection in the USA at the time of the study (2002–2004).[21] However, before the results of this trial were published, a higher rate of azithromycin 1 g treatment failure was reported among *M. genitalium*-infected patients in Australia.[28] In this report, macrolide resistance was identified in strains from patients failing azithromycin treatment. The authors also

Table 2 Clinical efficacy studies of fluoroquinolones, alone or versus macrolides, for treatment of *Mycoplasma genitalium* infection

Reference	Year	Study type	Population	*M. genitalium* cases (n)	Fluoroquinolone regimen(s) and *M. genitalium* microbiological cure	Macrolide regimen(s) and *M. genitalium* microbiological cure
Yasuda et al[45]	2005	Uncontrolled observational study	97 men with NGU attending a urology clinic, Japan	97 men	LVFX 100 mg 8 hourly ×7 d or 14 d TFLX 150 mg 8 hourly ×14 d GFLX 200 mg 12 hourly ×7 d or 14 d LVFX (7 d): 5/16 cured (31.3%) LVFX (14 d): 9/18 cured (50.0%) TFLX: 5/7 cured (71.4%) GFLX (7 d): 22/24 cured (91.7%) GFLX (14 d): 6/6 cured (100.0%)	Not applicable
Jernberg et al[24]	2005	Uncontrolled observational study	5,423 men and 4,683 women attending an STI clinic, Norway Patients were either symptomatic for STIs or contacts of patients diagnosed with urethritis, cervicitis, or *M. genitalium* infection	234 men 218 women	OFX 200 mg 12 hourly ×10 d MXF 400 mg daily ×7 d OFX: 25/45 cured (55.6%) MXF: 27/27 cured (100.0%)	AZM 1 g stat AZM 1 g on d 1 and d 5–d 7 AZM 500 mg daily d 1, 250 mg daily d 2–d 5 AZM 1 g stat: 144/183 cured (78.7%) AZM 1 g + 1 g: 28/38 cured (73.7%) AZM 1.5 g (first-line): 76/98 cured (77.6%) AZM 1.5 g (second/third-line): 8/23 cured (37.8%)
Bradshaw et al[28]	2006	Prospective case–control study	636 men with/without NGU attending an STI clinic, Australia	34 men	MXF 400 mg daily ×10 d (following AZM 1 g stat on basis of persistent urethral symptoms) 9/9 cured (100.0%)	AZM 1 g stat or AZM 1 g per week ×3 weeks 23/32 cured (71.9%)
Bradshaw et al[25]	2008	Uncontrolled observational study	1,538 men and 313 women attending an STI clinic, Australia Patients had NGU/epididymo-orchitis, cervicitis/PID, or were contacts of patients infected with *M. genitalium*	161 men 30 women	MXF 400 mg daily ×10 d (following AZM 1 g stat on basis of persistent urethral symptoms) 11/11 cured (100.0%)	AZM 1 g stat 101/120 cured (84.2%)
Takahashi et al[44]	2011	Uncontrolled observational study	87 men with NGU attending a urology clinic, Japan	5 men	LVFX 500 mg daily ×7 d 3/5 cured (60.0%)	Not applicable
Hamasuna et al[49]	2011	Uncontrolled clinical trial	169 men with NGU attending urology clinics, Japan	18 men	GFLX 200 mg 12 hourly ×7 d 15/18 cured (83.3%)	Not applicable
Ito et al[47]	2012	Uncontrolled clinical trial	89 men with NGU attending a urology clinic, Japan	14 men	STFX 100 mg 12 hourly ×7 d 11/11 cured (100.0%)	Not applicable
Terada et al[26]	2012	Uncontrolled retrospective study	257 women with *M. genitalium* positive cervicitis attending a gynecology clinic, Japan	257 women	MXF 400 mg daily ×7 d MXF 400 mg daily ×14 d LVFX 500 mg daily ×7 d LVFX 500 mg daily ×14 d STFX 200 mg daily ×7 d STFX 200 mg daily ×14 d MXF (7 d): 38/42 cured (90.5%) MXF (14 d): 42/42 cured (100.0%) LVFX (7 d): 12/22 cured (54.5%)	AZM 1 g stat AZM SR 2 g CAM 400 mg daily ×7 d CAM 400 mg daily ×14 d AZM 1 g stat: 36/42 (85.7%) AZM SR 2 g stat: 19/21 (90.5%) CAM (7 d): 13/20 (65.0%) CAM (14 d): 17/20 (85.0%)

						LVFX (14 d): 15/21 cured (71.4%)	
						STFX (7 d): 11/14 cured (78.6%)	
						STFX (14 d): 12/13 cured (92.3%)	
Takahashi et al[48]	2013	Uncontrolled clinical trial	208 men with NGU attending a urology clinic, Japan	16 men		STFX 100 mg 12 hourly ×7 d 15/16 cured (93.8%)	Not applicable
Bissessor et al[27]	2015	Prospective cohort study	160 patients with *M. genitalium* infections attending an STI clinic, Australia	112 men	43 women	MXF 400 mg daily ×10 d 53/60 cured (88.3%)[a]	AZM 1 g stat 95/155 cured (61.3%)

Notes: [a]Six of the seven patients who failed MXF therapy were treated with pristinamycin at a dose of 1 g 6 hourly ×10 d; these six patients were *M. genitalium* polymerase chain reaction-negative at 28 d post-pristinamycin therapy.
Abbreviations: NGU, non-gonococcal urethritis; STI, sexually transmitted infection; PID, pelvic inflammatory disease; LVFX, levofloxacin; TFLX, tosufloxacin; GFLX, gatifloxacin; STFX, sitafloxacin; OFX, ofloxacin; MXF, moxifloxacin; AZM, azithromycin; CAM, clarithromycin; *M. genitalium*, *Mycoplasma genitalium*; SR, slow release; d, day/days.

reported that moxifloxacin eradicated all cases of persistent infection.[28]

By 2009, experts had become concerned about the suboptimal effectiveness of the azithromycin 1 g single-dose regimen, given the premise that treatment should cure at least 95% of uncomplicated STIs.[29] The comparative efficacy of the extended 1.5 g azithromycin regimen has never been assessed in a randomized controlled trial and, unfortunately, it was not included in the design of two large NGU treatment trials that were taking place in the USA at the same time.[22,23]

Anagrius et al have shed further light onto the question of choice of azithromycin regimen.[30] Consistent with previous observational clinical studies, they did not find a significant difference in treatment efficacy between the single 1 g and extended 1.5 g doses. However, seven patients who had macrolide-susceptible *M. genitalium* infection prior to treatment with azithromycin 1 g, and who failed initial treatment, had emergent macrolide resistance. In contrast, the single man who failed the extended 1.5 g course of azithromycin was infected with a macrolide-resistant strain of *M. genitalium*, and 77/77 individuals without pre-existing macrolide resistance were cured by this regimen as either first-line or second-line treatment.[30]

A strong and consistent association between presence of 23S rRNA gene mutations and failure of azithromycin treatment began to emerge when clinical outcomes and *M. genitalium* resistance testing results were correlated (Table 3).[7,31,32] However, it should be noted that epidemiological studies have the potential to overestimate population prevalence of resistance when clinical information about previous antibiotic treatment is unavailable.[33] In addition, patients with macrolide resistance mutations may still test negative after treatment with single-dose azithromycin.[34] This outcome may reflect failure to detect persistent infection due to low bacterial loads associated with *M. genitalium* infection or to natural resolution of infection.[31,35,36]

An alarming trend is now apparent, with macrolide-resistant *M. genitalium* being widely reported as the underlying cause for the increasing rates of treatment failure with the azithromycin 1 g single-dose regimen. Although suboptimal macrolide dosage appears to be the main driver of the observed trend, the role of socioepidemiological factors, for example importation of antimicrobial-resistant *M. genitalium* strains or transmission of these within defined sexual networks, remains uncertain and requires more research.[28,31,37]

The presence of macrolide resistance-associated mutations has been highly associated with failure to eradicate *M. genitalium* in several Australian clinical studies.[28,31,36]

Table 3 Laboratory studies of *Mycoplasma genitalium* antimicrobial susceptibility and genotypic resistance testing

Reference	Year	Study type	Population	*M. genitalium* DNA extracts or isolates examined (n)	Macrolide resistance (MIC data/resistance mutations[a])	Fluoroquinolone resistance (MIC data/resistance mutations[b])	Comments
Bradshaw et al[28]	2006	Prospective case–control study	9 men with NGU attending an STI clinic, Australia	4 isolates	All 4 isolates had raised MICs to macrolides (AZM >8 mg/L, ERY >32 mg/L, CAM >32 mg/L)	Not applicable	8/9 men experienced improvement/ resolution of symptoms before NGU recurrence; one was persistently asymptomatic
Jensen et al[32]	2008	Laboratory analysis	*M. genitalium* DNA extracts and isolates from 12 men with NGU who failed AZM therapy in Australia, Norway, and Sweden 7 AZM^S genetically distinct control strains, Sweden	12 AZM^R DNA extracts 7 clinical isolates 7 control strains	7/7 isolates persisting after azithromycin therapy had raised MICs to macrolides (AZM ≥8 mg/L, ERY ≥16 mg/L, CAM ≥16 mg/L) 12 23S rRNA gene mutations reported: 5 at position 2059 (all A2059G); 5 at position 2058 (4 A2058G, one A2058C)	Not applicable	None
Chrisment et al[40]	2012	Uncontrolled retrospective study	136 patients with *M. genitalium* infection attending STI clinics, general practice clinics, and hospitals, France	115 DNA extracts	13 23S rRNA gene mutations reported: 9 at position 2059 (6 A2059G, 2 A2059T, 1 A2059C); 2 at position 2058 (2 A2058G); 1 at position A2062T; 1 at position C2038T	Not applicable	None
Shimada et al[60]	2010	Uncontrolled retrospective study	308 men with NGU attending a urology clinic, Japan	28 DNA extracts	Not applicable	Single substitutions reported in the *gyrA* gene at position 321 (T321A) in 2 specimens Single substitutions reported in the *parC* gene of 4 specimens: G248A, G259T, A260T, A290G	None
Shimada et al[34]	2011	Uncontrolled retrospective study	308 men with NGU attending a urology clinic, Japan	25 DNA extracts	4 23S rRNA gene mutations reported: 1 at position 2059 (A2059G); 3 at position 2185 (T2185G)	Not applicable	The A2158G mutation is not associated with macrolide resistance in other bacteria. Amino acid substitutions reported in the L4 and L22 ribosomal proteins of unknown significance. The strain with the A2059G mutation was cured with AZM 1 g
Ito et al[7]	2011	Laboratory analysis	7 men with *M. genitalium* related NGU which failed AZM therapy at a urology clinic, Japan	7 DNA extracts	4 23S rRNA gene mutations reported: 2 at position 2059 (A2059G); 2 at position 2058 (A2058G)	Not applicable	All 7 men had no AZM^R mutations in pretreatment *M. genitalium* DNA extracts. One of the *M. genitalium* strains with the A2058G mutation also had a L4 protein mutation

Walker et al[35]	2013	Longitudinal study	1,100 women attending 29 primary care clinics, Australia	33 DNA extracts	Unspecified 23S rRNA gene mutations were reported in 2/27 pretreatment samples from patients cured with AZM 1 g stat, and also in the test-of-cure samples of 3/3 patients who failed AZM 1 g stat therapy	Not applicable	Women were recruited in the Chlamydia Incidence and Re-infection Rates Study. There were 27 baseline, 14 follow-up, and 3 positive test-of-cure specimens
Twin et al[31]	2012	Laboratory analysis	82 pretreatment and 20 post-treatment samples from patients with clinical treatment failure attending an STI clinic, Australia	102 DNA extracts	16/82 pretreatment samples had 23S rRNA gene mutations (A2058G, A2059G, A2059C) 20/20 post-treatment samples from patients failing AZM therapy had 23S rRNA gene mutations (12 A2059G, 7 A2058G, 1 A2059C)	Not applicable	Mutations detected by high resolution melt analysis that may not have been able to detect the type 4 SNP (A-to-T) at position 2058
Gesink et al[39]	2012	Uncontrolled observational study	314 participants recruited through telephone and community initiatives, Greenland	26 DNA extracts	Single 23S rRNA gene mutations reported in 26/26 *M. genitalium* cases tested: 17 at position 2058 (A2058G); 9 at position 2059 (A2059G)	Not applicable	None
Tagg et al[33]	2013	Laboratory analysis	143 initial and 43 follow-up *M. genitalium*-positive samples from 167 patients attending STI clinics, Australia	186 DNA extracts	62/143 (43.4%) initial DNA extracts had 23S rRNA gene mutations at either position 2058 (21 A2058G, 2 A2058T, 1 A2058C), or 2059 (38 A2059G) Follow-up DNA extracts of 23/24 (95.8%) patients had 23S rRNA gene mutations	1/143 (0.7%) had *gyrA* mutations (G285C) resulting in an amino acid changes 22/143 (15.4%) initial DNA extracts had *parC* mutations (11 G248T, 3 G259A, 2 G259T, 1 G241T, 1 G244A, 1 A247C, 1 G259C, 1 A260G, 1 G307A) resulting in amino acid changes Follow-up DNA extracts from 3/24 (12.5%) patients had *gyrA*/*parC* mutations	The matched initial DNA extract for 8/23 follow-up specimens with 23S rRNA mutations did not have evidence of these same AZM^R mutations
Couldwell et al[36]	2013	Uncontrolled observational study	33 patients attending a STI clinic with *M. genitalium* infections, either as NGU cases (30 men) or their sexual partners (2 women, 1 man), Australia	32 DNA extracts	15/32 (46.9%) had 23S rRNA gene mutations at position 2058 (A2058G, A2058T) or position 2059 (A2059G)	6/32 (18.8%) had *gyrA* mutations (G285C) or *parC* mutations (G248T, G259A, A260G)	Transmitted macrolide resistance was reported in 4/20 (20.0%) of samples from patients who had not received AZM pretreatment. None of the study group reported prior fluoroquinolone use
Yew et al[8]	2011	Laboratory analysis	11 *M. genitalium* DNA extracts from men with recurrent NGU, New Zealand	9 DNA extracts	4/9 (44.4%) had A2059G mutations in the 23S rRNA gene	Not applicable	Unable to amplify 23S rRNA genes of 2/11 known *M. genitalium*-positive DNA extracts

(*Continued*)

Table 3 (*Continued*)

Reference	Year	Study type	Population	*M. genitalium* DNA extracts or isolates examined (n)	Macrolide resistance (MIC data/resistance mutations[a])	Fluoroquinolone resistance (MIC data/resistance mutations[b])	Comments
Anagrius et al[30]	2013	Uncontrolled retrospective study	11 patients testing positive for *M. genitalium* after treatment with azithromycin 1 g single-dose (n=10) or extended azithromycin 1.5 g (n=1) therapy, Sweden	8 DNA extracts	1/8 (12.5%) pretreatment and 8/8 (100.0%) post-treatment samples had non-specified macrolide-associated 23S rRNA gene mutations	Not applicable	2/10 pretreatment samples were missing and 1/10 pretreatment samples had insufficient DNA for amplification. The patient failing the extended azithromycin had macrolide mutations in the pretreatment DNA extract
Pond et al[41]	2014	Uncontrolled observational study	217 men with urethritis-related symptoms, UK	22 DNA extracts	23S rRNA gene mutations reported in 9/22 (40.9%) samples: 5 at position 2058 (A2058G); 9 at position 2059 (3 A2059G, 1 A2059C)	1/22 (4.5%) had a *parC* mutation (A247C)	None
Kikuchi et al[38]	2014	Laboratory analysis	90 *M. genitalium* DNA extracts from men with NGU, Japan	68 DNA extracts (macrolide resistance testing) 51 DNA extracts (fluoroquinolone resistance testing)	23S rRNA gene mutations reported in 5/68 (7.4%) samples: 4 at position 2058 (A2058G); 1 at position 2059 (A2059G)	5/51 (9.8%) had *gyrA* mutations (4 C267T, 1 C270T); 18/51 (35.3%) had *parC* mutations (12 G248A, 3 G248T, 2 G259A, 1 C356A)	The significance of the reported C356A mutation is unclear as it is outside the fluoroquinolone resistance-determining region
Salado-Rasmussen and Jensen[13]	2014	Uncontrolled retrospective survey	1,008 patients from general practice, private specialists and hospitals with *M. genitalium* infection, Denmark	1,085 DNA extracts	385/1,008 (35.5%) patients had macrolide resistance; A2058G (61%) and A2059G (35%) were the most common mutations	Not applicable	None
Hay et al[42]	2015	Laboratory analysis	601 women attending primary health care clinics, South Africa	41 DNA extracts	A2058G mutations reported in the 23S rRNA gene of 4/41 (9.8%) DNA extracts tested	Not applicable	None

Notes: [a]Mutation positions are according to *Escherichia coli* numbering; [b]mutation positions are according to *M. genitalium* G37 genome (GenBank accession number NC000908.2).

Abbreviations: *M. genitalium, Mycoplasma genitalium*; NGU, non-gonococcal urethritis; STI, sexually transmitted infection; AZM, azithromycin; CAM, clarithromycin; ERY, erythromycin; AZM^S, azithromycin-susceptible; AZM^R, azithromycin-resistant; SNP, single nucleotide polymorphism; MIC, minimum inhibitory concentration.

In Melbourne, Australia, azithromycin efficacy has declined from 84% between 2005 and 2007, and to 69% from 2007 to 2009 ($P<0.001$).[31] Elsewhere in the Pacific region, macrolide resistance mutations were not detected in a small number of *M. genitalium*-positive urethral samples from Japanese men tested in 2011–2012, whereas five (29.4%) of 17 screened *M. genitalium* DNA extracts had 23S rRNA gene mutations in 2013.[38] A similar trend has been observed in the USA where, by 2011, only 40% of infections were cured by single-dose azithromycin 1 g, compared with 87% in 2002–2004.[21,23]

In Scandinavia, a retrospective case study in Sweden tracked the trajectory of macrolide resistance from 2006 to 2007, when no macrolide resistance was detected, through to 2011, when 21% of *M. genitalium*-positive samples harbored 23S rRNA gene mutations associated with macrolide resistance.[30] A Danish national survey reported a 38% prevalence of macrolide resistance-associated mutations in first *M. genitalium* test samples from 2007 to 2010.[13] The lowest rate of resistance was found in samples from private specialists, mostly gynecologists who were conducting screening for STIs including *M. genitalium*. The highest rate occurred among STI clinic patients where *M. genitalium* testing was generally restricted to persistently symptomatic patients with negative results for other pathogens; these patients were likely to have received azithromycin treatment prior to their first *M. genitalium* test. In an alarming report from Greenland, 100% of *M. genitalium* strains detected in 2008–2009 carried macrolide resistance mutations, resulting in replacement of azithromycin with tetracyclines in the recommended syndromic treatment guideline for urethritis and cervicitis.[39]

Elsewhere in Europe, the rate of macrolide resistance varied in France from 10% to 15% each year from 2006 to 2010, whereas no resistance mutations were detected in the small number of available samples from 2003 to 2005.[40] There was, however, no significant trend observed between 2003 and 2006 or between 2007 and 2010. In the UK, *M. genitalium* was detected in five asymptomatic and 17 symptomatic men with and without urethritis in a London clinic.[41] Among these 22 initial samples, nine harbored macrolide-associated resistance mutations, and phylogenetic analysis of 18 samples revealed two main clusters within which strain types were not closely related. None of the men with urethritis and with macrolide-resistant strains of *M. genitalium* returned for follow-up, despite having received treatment with either doxycycline or azithromycin 1 g that would have been unlikely to cure their infections.

There is a lack of data on the prevalence of macrolide resistance-associated mutations among the *M. genitalium* strains circulating in African, Asian, and Latin American countries. Many countries within these continental regions rely on syndromic management for STI control, and laboratory diagnostic capability is generally absent or very minimal. In addition, tetracyclines are preferred to macrolides for syndromic management of genital discharges due to the differential cost and limited budget for STI control. Accordingly, it remains very unclear as to what role *M. genitalium* plays in reproductive tract morbidity in resource-poor settings and to what extent *M. genitalium* strains have acquired resistance mutations. The only reported macrolide resistance data from Africa has been laboratory-based using remnant specimens collected over 4 months in 2011–2012 in a limited geographic area in rural South Africa.[42] The authors reported a prevalence of 23S rRNA gene mutations in four (9.8%) of 41 DNA extracts screened. We were unable to find any studies reporting macrolide-associated mutations in *M. genitalium* strains from Latin America or resource-poor countries in Asia.

The most recent data on macrolide resistance is from a prospective cohort of *M. genitalium*-infected patients with NGU, cervicitis, or pelvic inflammatory disease, as well as their sexual contacts, enrolled in Melbourne, Australia, between June 2012 and July 2013.[27] Only 3% of patients were lost to follow-up; 95 (61%) of 155 were microbiologically cured by single-dose azithromycin 1 g. Baseline macrolide resistance was detected in 56 (36%) patients (transmitted resistance) and most (87%) of these failed azithromycin therapy. In addition, eleven (11%) of the 99 patients without baseline macrolide resistance also developed signature 23S rRNA gene mutations (induced resistance) and failed therapy. Overall, a high azithromycin 1 g treatment failure rate (39%) was reported in this study.[27] This study provided the first definitive evidence for timing of test of cure; all patients who tested negative for *M. genitalium* at day 28 by polymerase chain reaction (PCR) assay also tested negative by day 14.

Fluoroquinolones such as moxifloxacin, gatifloxacin, and sitafloxacin remain highly active against most macrolide-resistant *M. genitalium* isolates.[43] Although demonstrated to have high activity against *M. genitalium* in vitro, the newer fluoroquinolones, including gemifloxacin, sparfloxacin, grepafloxacin, trovafloxacin, and garenoxacin, have yet to be evaluated in clinical trials.[43] In contrast, ciprofloxacin has poor activity, and both ofloxacin and levofloxacin are less active against *M. genitalium* than moxifloxacin and the newer fluoroquinolones mentioned above.

Ofloxacin and levofloxacin have been used to treat NGU in the past, particularly in Japan, although neither are ideal

drugs to treat *M. genitalium* infection.[43,44] Levofloxacin, given as 100 mg 8-hourly for 7 days or 14 days has been shown to produce low *M. genitalium* eradication rate of 31% or 50%, respectively, and has been associated with a high prevalence of recurrence of urethral discharge.[45,46] In a small study with nine evaluable patients, a 10-day course of ofloxacin 200 mg 12 hourly failed to clear *M. genitalium* in 56% of cases.[24]

Moxifloxacin 400 mg once daily for 7–10 days generally cures *M. genitalium* infections that have failed azithromycin therapy.[25,32] As a result, moxifloxacin is currently the treatment of choice for macrolide-resistant *M. genitalium* infections. Based on the results of in vitro susceptibility testing, sitafloxacin appears to be as active as moxifloxacin. Two recent small clinical studies in Japan, where moxifloxacin is not available, reported that a 100 mg 12-hourly regimen of sitafloxacin for 1 week eradicated *M. genitalium* in 11/11 and 15/16 patients, respectively, including five patients with persistent or recurrent NGU.[47,48] Although no longer available, gatifloxacin, given at a dosage of 200 mg 12-hourly for 1 or 2 weeks, also resulted in high eradication rates for *M. genitalium* in men with NGU.[45,49]

The first clinical report of moxifloxacin treatment failure associated with fluoroquinolone-associated resistance mutations in *M. genitalium* strains emerged in 2013 from Sydney, Australia.[36] A recent study from Melbourne found that moxifloxacin cured only 53 (88%) of 60 macrolide-resistant *M. genitalium* infections; the seven that failed moxifloxacin had fluoroquinolone-associated resistance mutations in *gyrA* and *parC*.[27] Accordingly, it is strongly recommended that clinicians avoid low-efficacy fluoroquinolones, such as levofloxacin or ofloxacin, to treat NGU cases for fear of driving a rise in the prevalence of fluoroquinolone resistance among *M. genitalium* strains. While most *M. genitalium* strains remain susceptible to moxifloxacin and sitafloxacin, there is increasing concern about how best to treat dual macrolide-resistant and fluoroquinolone-resistant *M. genitalium* infections.

A new fluoroketolide antibiotic, solithromycin, has shown superior in vitro activity against *M. genitalium* compared with macrolides, fluoroquinolones, and tetracyclines.[50] When tested against macrolide-resistant strains, solithromycin was more active in vitro than azithromycin, although there was evidence of some cross-resistance.[50] Mutations in the *M. genitalium* 23S rRNA gene at position 2058 (*Escherichia coli* numbering) led to higher solithromycin minimum inhibitory concentrations (MICs) than those in position 2059 and were the only changes explaining solithromycin resistance. In Denmark, where 40% of *M. genitalium* strains are azithromycin-resistant, the authors postulate that 85% of these resistant strains, or 94% of all *M. genitalium* strains, would be susceptible to solithromycin. Superior activity is thought to be due to solithromycin having three ribosomal binding sites, compared with only one in the case of azithromycin. Solithromycin also showed good activity against five strains from patients who had failed both azithromycin and moxifloxacin treatment.[50] This antimicrobial agent was recently shown to be highly effective against *C. trachomatis* and *N. gonorrhoeae* in vitro and against uncomplicated urogenital gonorrhea in a Phase II clinical trial, suggesting it could treat several STIs simultaneously.[51–53] Should the efficacy of solithromycin be demonstrated in further clinical trials, it may be an option for the syndromic management of urethritis and related clinical syndromes in the future.

Pristinamycin, a streptogramin antimicrobial generally used to treat vancomycin-resistant *Enterococcus faecium* bacteremia and complicated skin infections caused by methicillin-resistant *Staphylococcus aureus*, has also been used to treat *M. genitalium* infections. Bissessor et al administered pristinamycin in a regimen of 1 g 6 hourly for 10 days to six patients who failed both azithromycin (1 g as a single dose) and moxifloxacin (400 mg daily for 10 days).[27] All six patients remained PCR-negative for *M. genitalium* 28 days after receiving the pristinamycin. As this study represents the first reported use of pristinamycin among a small group of patients infected with multi-drug resistant *M. genitalium*, further clinical evaluations are required in order to better evaluate the effectiveness, optimal dosage, and potential for acquisition of antimicrobial resistance determinants. Even if pristinamycin continues to prove effective, its currently limited availability and high cost do not support wider use, particularly in resource-poor settings.

Antimicrobial resistance testing in *M. genitalium*

M. genitalium was first cultured by direct inoculation of urethral swab material onto SP4 Mycoplasma medium and subsequently by coculture of urethral specimens with Vero cell cultures grown in supplemented serum-free medium.[54,55] *M. genitalium* has now been successfully isolated from urethral swabs, urinary sediments, and cervical swabs.[56] In vitro antimicrobial susceptibility testing traditionally requires isolation of a single strain through multiple passages in culture (axenic culture). This has proven difficult due to the fastidious nutritional and environmental requirements of *M. genitalium* as well as its slow growth; indeed, it can take up to 6 months to isolate a single colony. This propensity of *M. genitalium* culture to fail has impeded studies reliant

on observations of bacterial growth following addition of serial dilutions of antimicrobial agents to SP4 medium-based axenic cultures.[56]

In an attempt to overcome the challenges of strain loss with subsequent subcultures, the growth of *M. genitalium* in inoculated Vero cell cultures has been monitored by use of a quantitative TaqMan 5′ nuclease real-time PCR, which in turn relies on detection of the single-copy *mgpB* adhesion gene.[57] In this assay, growth inhibition due to the presence of antimicrobial agents can be expressed as a proportion of the DNA load of *M. genitalium* controls grown in the same culture system. Whichever method is used, phenotypic resistance testing for *M. genitalium* remains a laborious and time-consuming process. Consequently, there are relatively few antimicrobial susceptibility studies reported in the literature. The data that do exist may not be representative of the larger number of untested *M. genitalium* strains circulating on a global level.

Advances in techniques to detect putative resistance mutations in initial culture specimens without the need for axenic culture, and more recently, directly from clinical samples, have facilitated epidemiological studies of *M. genitalium* resistance, as well as correlation of clinical outcomes with results of genotypic resistance testing.[31,33,56,58] Rapid high resolution melt analysis (HRMA) now allows detection of macrolide resistance-associated mutations at the time of initial detection of *M. genitalium*. This dramatically reduces the time needed to perform resistance testing, which may be as long as 2–3 months for previously described in vitro MIC determination based on the Vero cell culture system and quantitative TaqMan 5′ nuclease real-time PCR determination of growth inhibition.[32,57] However, the rapid HRMA assay was unable to detect type IV single nucleotide polymorphisms within the 23S rRNA gene at position 2058 (ie, A2058T, *E. coli* numbering).[31] This is an important limitation of the HRMA assay as A2058T mutations do comprise a small proportion of macrolide resistance-associated mutations in some reports.[13,33,38] A real-time PCR assay based on fluorescence resonance energy transfer coupled with melting curve analysis was reported to be more discriminatory and reproducible in clinical specimens when compared with the rapid HRMA assay.[58] Use of such rapid assays on specimens collected prior to treatment avoids the wait for a test-of-cure result before instituting second-line treatment for patients with persistent NGU. However, treatment would not be expedited for those azithromycin-treated men who developed emergent macrolide resistance following therapy.

Overview of mutations associated with resistance and treatment failure

Tetracyclines

In vitro antimicrobial susceptibility testing of recent clinical isolates has demonstrated the emergence of some strains with decreased susceptibility to doxycycline (1 μg/mL) and tetracycline (4 μg/mL).[16] Although tetracycline resistance-associated mutations have not so far been identified in *M. genitalium*, *tetM* gene mutations conferring tetracycline resistance have been identified in *M. hominis* and *Ureaplasma urealyticum* isolated from genital specimens.[43]

Macrolides

Macrolide antibiotics, including azithromycin, prevent bacterial replication by binding to the 50S ribosomal subunit, inhibiting translation of mRNA and thus interfering with protein synthesis. Mutations at positions 2058 and 2059 (*E. coli* numbering) in region V of the 23S rRNA gene alter ribosomal structure, thereby preventing macrolide binding, and have been associated with macrolide resistance in a number of pathogenic bacteria, including *M. genitalium* and two other sexually acquired pathogens, *N. gonorrhoeae* and *Treponema pallidum*.[59] While the latter two sexually transmitted pathogens have multiple copies of 23S rRNA genes, *M. genitalium* has only a single rRNA gene operon encoding for the 23S, 16S, and 5S rRNA subunits. It has been hypothesized that this relative deficiency in the number of 23S rRNA gene copies may increase the susceptibility of *M. genitalium* to develop high-level macrolide resistance.[38] In addition, the ability of *M. genitalium* to exist intracellularly, together with its very slow growth, could favor selection of macrolide-resistant strains, given that azithromycin has a much longer intracellular than extracellular half-life.[3]

The first study to demonstrate macrolide resistance in azithromycin treatment failure in *M. genitalium* urethritis was reported in 2006.[28] The authors performed phenotypic antimicrobial drug susceptibility testing on four specimens, collected after azithromycin 1 g single-dose treatment had failed, and reported increased MICs to azithromycin (>8 mg/L), erythromycin (>32 mg/L), and clarithromycin (>32 mg/L). All four isolates were sensitive to moxifloxacin, with MICs in the range of 0.031–0.125 mg/L, and retained in vitro susceptibility to doxycycline (MICs 0.125–0.25 mg/L).[28]

In an attempt to determine the genetic mechanism underlying the observed macrolide resistance, these four isolates and three macrolide-resistant *M. genitalium* isolates from

Scandinavian patients, who had also failed azithromycin, were further studied along with several distinct azithromycin-susceptible *M. genitalium* strains.[32] The genetic basis for drug resistance was determined by sequencing the 23S rRNA gene, as well as genes encoding L4 and L22 proteins, as mutations with these genes were already associated with macrolide resistance in other Mollicutes.

The authors identified three different mutations at positions 2058 and 2059 (*E. coli* numbering) in region V of the 23S rRNA gene which were deemed responsible for the macrolide resistance phenotype.[32] Although some point mutations were found in the L4 and L22 genes, most of them did not result in amino acid changes, and their effect was thought to be minor or non-existent in terms of the expression of the macrolide-resistant phenotype. Only one strain possessed an amino acid substitution, ie, the H69R mutation in L4, known to be associated with macrolide resistance in Mollicutes. The authors subsequently developed and validated a PCR assay to detect macrolide resistance-associated mutations.[32] Nine paired pretreatment and post-treatment samples from patients who failed a single dose 1 g dose of azithromycin were further analyzed with this assay. Macrolide resistance-associated 23S rRNA gene mutations were present in two of the pretreatment DNA extracts and all of the nine post-treatment DNA extracts, suggesting that azithromycin resistance had emerged during treatment. Induced macrolide resistance has subsequently been reported by others.[7,8]

Researchers in Melbourne, Australia, reported that rapid HRMA detected sexually transmitted macrolide resistance mutations in 16 (20%) of 82 pretreatment samples, while selection of macrolide resistance-associated mutations occurred in eleven (55%) of 20 of those with initial wild-type infections who failed initial treatment.[31] Elsewhere in Australia, macrolide resistance-associated mutations were detected by sequencing of PCR amplicons in 62 (43%) of 143 initial *M. genitalium*-positive samples collected in Sydney from 2008 to 2011.[33] Sexually transmitted macrolide resistance was present in four (20%) of a small subset of 20 samples collected from patients who had never received azithromycin prior to their first test.[36]

Fluoroquinolones

Fluoroquinolone antibiotics bind to the DNA gyrase and topoisomerase IV enzymes, blocking DNA replication. Mutations in defined regions of the DNA gyrase genes, *gyrA* and *gyrB*, and the topoisomerase IV genes, *parC* and *parE*, have been linked to high-level fluoroquinolone resistance in various bacteria, including *N. gonorrhoeae* and *M. genitalium*.[5,33]

As mentioned above, the first clinical reports of *M. genitalium* infection failing therapy with moxifloxacin as a result of fluoroquinolone-associated resistance mutations emerged in 2013.[36] Fluoroquinolone resistance-associated mutations in the *parC* and/or *gyrA* genes were detected in eleven (15%) of 143 initial *M. genitalium* PCR-positive samples from Sydney and in six (19%) of 32 of these samples from patients at one clinic.[33,36] In this population, fluoroquinolone antibiotics are not used for treatment of any STIs or widely in the community for the treatment of other infectious diseases. Despite this, fluoroquinolone resistance-associated mutations were significantly associated with failure of moxifloxacin treatment (P=0.005).[36] Patients infected with *M. genitalium* strains containing both macrolide and fluoroquinolone resistance-associated mutations failed therapy with both azithromycin and moxifloxacin, raising concerns about untreatable *M. genitalium* infection in the future.

Subsequently, fluoroquinolone resistance was also reported from a London clinic.[41] In addition, approximately one-third of 51 Japanese men with NGU were infected with *M. genitalium* and had fluoroquinolone resistance-associated mutations in *parC*, but 9/9 were cured by sitafloxacin 100 mg prescribed twice daily for 7 days.[38] The relatively high prevalence of fluoroquinolone resistance in this patient group may be a consequence of the common use of fluoroquinolones in STI treatment in Japan.[60]

Future directions

Despite mounting evidence of increasing failure of azithromycin 1 g as a single-dose treatment for *M. genitalium*-associated NGU, this regimen continues to be used as first-line treatment for NGU in many parts of the world. This is in part because NGU treatment remains focused on treating chlamydial infections, which are deemed to have more serious sequelae. While *C. trachomatis* is universally accepted as an STI, the pathogen status of *M. genitalium* is not so prominent, which has in turn led to recent calls for *M. genitalium* to be regarded more seriously and to be recognized as a significant STI with associated morbidity.[2,10] Once this happens, there will be enhanced efforts to introduce commercial assays for *M. genitalium* detection, ideally multiplexed with *C. trachomatis* and *N. gonorrhoeae*. In resource-poor settings, more effort is required to validate genital discharge syndromic management protocols that could adequately treat both *C. trachomatis* and *M. genitalium* infections.

STI treatments are devised according to the local epidemiology of antimicrobial susceptibility, but generating such data for *M. genitalium* strains would be a major and ongoing challenge for laboratories. Diagnostic testing for *M. genitalium* has not been widely available, and antimicrobial susceptibility testing remains available in only a few laboratories worldwide.[9] Consequently, the issue of macrolide treatment failure in *M. genitalium* infection was unrecognized until relatively recently. It is clear, in retrospect, that the choice of treatment for *M. genitalium* infections within the context of NGU has always been inadequate. By the time that randomized trials were designed to investigate *M. genitalium* treatment, macrolide resistance among *M. genitalium* strains was entrenched and rising. Evidence of increasing failure of azithromycin in the treatment of NGU re-emphasizes the ease with which antibiotic resistance can accelerate where suboptimal treatment is provided for a common infection or syndrome.

There are now calls to abandon single-dose azithromycin 1 g treatment for *M. genitalium* and related clinical syndromes.[10] One suggested strategy is to revert to use of doxycycline for treatment of NGU, and to then use the extended regimen of azithromycin 1.5 g for those who fail initial therapy, with a 10-day course of moxifloxacin as third-line therapy, and to treat contacts with the same regimen(s).[10] This approach could be used in settings with or without availability of *M. genitalium* testing, and would potentially slow the rate of resistance development. Its success relies on three premises: firstly, that the extended 1.5 g azithromycin regimen is sufficiently effective, for which there is limited evidence to date; secondly, that patients who fail therapy will continue to return for follow-up, and lastly that macrolide resistance is not already present.[24,30] Epidemiological studies have detected circulating macrolide resistance in up to 100% of local strains in some populations.[39] In addition, there may be consequences for treatment of other pathogens. For example, suboptimal adherence to doxycycline occurred in 28% of men in a prospective randomized controlled trial of NGU treatment, and was associated with 9-fold higher risk of microbiological failure among men infected with *C. trachomatis*.[61]

The current practices of performing *M. genitalium* testing primarily in men with NGU and failure to provide systematic screening recommendations for asymptomatic individuals contribute to the selection pressure generating macrolide resistance, especially among groups with high rates of partner change. Given published prevalence data, it is likely that many MSM who receive the single-dose azithromycin 1 g treatment, either for chlamydial infection or as dual therapy with ceftriaxone for treatment of gonorrhea, are also asymptomatically infected with rectal *M. genitalium*.[15] Some infections may be cured, but macrolide resistance probably emerges with high frequency in this scenario, leading to pathogen persistence and onward transmission to sexual partners. In the case of *M. genitalium* infection in women, more than one-third of a cohort of African female sex workers received syndromic treatment for other STIs during follow-up, without any effect on clearance of *M. genitalium*, even though some of these infections would have been expected to respond to fluoroquinolones and doxycycline given as syndromic management for vaginal discharge and lower abdominal pain syndromes.[11] This finding has led to speculation of widespread *M. genitalium* antimicrobial resistance in sub-Saharan Africa, where in some cohorts and particularly among HIV-infected patients, the prevalence of *M. genitalium* infection exceeds that of gonorrhea and chlamydial infection.[62,63]

Antimicrobial susceptibility surveillance should be instituted more widely, particularly in resource-limited settings where data are either very few or non-existent, to inform treatment guidelines. New molecular technologies have shortened the many months formerly required for antimicrobial susceptibility testing through use of axenic culture systems. It is now possible to test patients' specimens directly for the presence of signature resistance mutations for macrolide and fluoroquinolone resistance.[31,57,58] Ideally, future *M. genitalium* detection assays would incorporate detection of macrolide resistance mutations, which could improve treatment effectiveness and help limit the spread of resistance.[9,13]

Conclusion

In conclusion, the minimalist nature of *M. genitalium*, encompassing its error-prone genome, parasitic lifestyle, and slow replication, has ironically proved to be its greatest strength, giving this organism the ability to evade detection and readily develop treatment resistance. Effective management of *M. genitalium* infection, within the context of broader STI control, will ideally require a number of new interventions including: the development and validation of a commercial multiplex assay to detect *N. gonorrhoeae*, *C. trachomatis*, and *M. genitalium* incorporating detection of key resistance mutations; systematic screening of high-risk groups, including screening among MSM for rectal infection; establishment of local and regional surveillance networks to monitor prevalence of infection and antimicrobial resistance; and development and clinical evaluation of new treatments.

Solithromycin is a promising option, offering a higher barrier to resistance and potential efficacy in syndromic STI treatment in *M. genitalium*-associated clinical syndromes such as NGU, as well as in resource-limited settings.

Disclosure

The authors report no conflicts of interest in this work.

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