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Clinical and diagnostic challenges of antimicrobial resistance in *Mycoplasma genitalium*

By Litty Tan, BSc, PhD

M*ycoplasma genitalium* is a small bacterium from the Mollicute class that was first isolated from the human urogenital tract in the 1980s.¹ It has taken some time to gain traction as an accepted sexually transmitted infection (STI); however, the U.S. Centers for Disease Control and Prevention's (CDC) most recent guidelines for sexually transmitted diseases (STDs)² have highlighted *M. genitalium* as an emerging issue. *M. genitalium* has been strongly and consistently associated with non-gonococcal urethritis (NGU) and cervicitis, and is also implicated in pelvic inflammatory disease in women.³⁻⁶

Table 1 summarizes the diseases associated with *M. genitalium* and the level of evidence for this association, as published in the World Health Organization (WHO) manual, *Laboratory Diagnosis of Sexually Transmitted Infections, Including Human Immunodeficiency Virus*.⁵

Disease in men

M. genitalium was first discovered in men with NGU, and most of the subsequent studies focus in the same manner.⁴ Urethritis is an inflammation of the urethra and may be characterized by symptoms such as dysuria, urethral pruritus, and purulent or non-purulent urethral discharge. Urethritis can have many infectious etiologies. When it is not caused by a gonorrheal infection it is known as non-gonococcal urethritis or NGU. *C. trachomatis* is the primary cause of NGU, responsible for 15 percent to 40 percent of cases,⁷ and *M. genitalium* is now recognized as the second most common cause, responsible for 15 percent to 25 percent of cases in men with symptomatic NGU.⁴ U.S. studies on *M. genitalium* prevalence in men with NGU range from 13 percent to 31 percent. (**Table 2**)

The strong association of *M. genitalium* with NGU has led to its inclusion in diagnostic considerations by the Centers for Disease Control and Prevention's Sexually Transmitted Diseases Treatment Guidelines,² European guidelines for management of NGU,⁸ and the European guideline on *Mycoplasma genitalium* infections.⁹ *M. genitalium* infection is also strongly correlated with persistent or recurrent NGU, found in 19 percent to 41 percent of men with persistent or recurrent disease.^{4,10,11} These high levels may be a consequence of current treatment practices resulting in inadequate syndromic treatment of NGU.

Disease in women

Mucopurulent cervicitis has also been described as the female counterpart of NGU in men,⁸ and non-gonococcal mucopurulent cervicitis is caused by similar etiologic agents.

C. trachomatis is responsible for 20 percent to 40 percent of cases, and *M. genitalium* is responsible for five percent to 20 percent of cases.⁸

Disease	Level of disease association*
Urethritis	++++
Cervicitis	+++
Bacterial vaginosis	-
Endometritis and/or Pelvic Inflammatory Disease (PID)	+++
Preterm birth	+/-
Infertility (women)	+

* ++++ strong association, +++ association in most studies, + association from a few studies, +/- conflicting results (adapted from WHO 2013)

Table 1. Disease associations of *Mycoplasma genitalium*.⁵

Meta-analysis studies on the disease association of *M. genitalium* infection and female reproductive tract syndromes have shown significant association with increased risk of cervicitis, pelvic inflammatory disease (PID), pre-term birth, spontaneous abortion, and risk of infertility.^{1,6} U.S. studies on *M. genitalium* prevalence in women with cervicitis range from 11.2 percent to 28.6 percent. (**Table 3**)

Prevalence of *M. genitalium*

From the limited data available, the prevalence of *M. genitalium* in the general population is reported at between 1.1 percent and 3.3 percent (**Table 4, pg. 10**). In comparison to other causes of sexually transmitted infections, this is higher than the prevalence of *Neisseria gonorrhoeae* at 0.4 percent, but lower than *Chlamydia trachomatis* at 4.2 percent and *Trichomonas vaginalis* at 2.3 percent.^{4,2} Similar to *C. trachomatis*, *M. genitalium* can also be found in asymptomatic infection^{12,13}; thus the true prevalence may be underestimated.

A recent multicenter clinical study cohort in the U.S. investigated the prevalence of *M. genitalium* infections in

urogenital specimens collected from 946 male and female subjects.¹⁴ *M. genitalium* prevalence rates were 16.1 percent for females and 17.2 percent for males. Significant risk factors for *M. genitalium* infections were African descent, younger age, non-Hispanic ethnicity, and female symptomatic status.

Management of NGU and cervicitis

Different etiologic agents, such as *C. trachomatis* and *M. genitalium*, can have similar clinical presentations, as seen in urethritis and cervicitis. Therefore these STIs are commonly managed as syndromes grouped by similar symptoms. In syndromic management, treatment is targeted at the most common causes at the time of diagnosis and before the etiologic agent is identified.

Urethritis in symptomatic men is first determined to be gonococcal or non-gonococcal using microscopy. In the U.S., the Sexually Transmitted Diseases Treatment Guidelines, 2015, recommend presumptive treatment of NGU with doxycycline, a tetracycline antibiotic, or azithromycin, a macrolide antibiotic.² Doxycycline and azithromycin are highly effective against *C. trachomatis* (67 percent to 87 percent for men with NGU); however, doxycycline regimens have poor cure rates for *M. genitalium* (30 percent to 45 percent).^{15,16} Therefore, if doxycycline was given as the first line therapy for NGU where *M. genitalium* was the causative agent, treatment is more likely to fail. Of further concern is the high rate of *M. genitalium* macrolide resistance appearing in studies world-wide.^{14,17-29,31} The most recent and comprehensive U.S. study found macrolide-resistant *M. genitalium* phenotypes in 51 percent of females and 42 percent of males,¹⁴ indicating that a significant portion of the tested population would not respond to current frontline treatment recommendations.

The practice of syndromic treatment and subsequent use of doxycycline and/or azithromycin without appropriate and targeted diagnostics is likely contributing to the high prevalence of *M. genitalium* in persistent or recurrent urethritis.

Currently available tests for *M. genitalium*

Although *M. genitalium* has been in the literature for several decades, it has only recently been more broadly recognized as an STI¹ and is currently not listed as a notifiable disease;

Study cohort	Study period	Population size (N)	MG prevalence
STD clinic (Baltimore, MD)	no info	286	13.9% ⁴⁴
STD clinic (New Orleans, LA)	Feb 2001 - Apr 2004	398	20% ¹⁵
2 STD clinics (Baltimore, MD)	2004	290	22.2% ⁴⁵
4 STD clinics (Birmingham, AL; New Orleans, LA; Durham, NC; Baltimore, MD)	Nov 2006 - Apr 2009	305	31% ¹⁶
STD clinic (Seattle, WA)	May 2007 - Oct 2009	370	12.5% ⁴⁶
STD clinic (Seattle, WA)	Jan 2007 - Jul 2011	606	13% ³¹

Table 2. Prevalence of MG in men with NGU in the U.S.

Study cohort	Study period	Population size (N)	MG prevalence
STD clinic (Baltimore, MD)	no info	286	13.9% ⁴⁴
STD clinic (New Orleans, LA)	Feb 2001 - Apr 2004	398	20% ¹⁵
2 STD clinics (Baltimore, MD)	2004	290	22.2% ⁴⁵
4 STD clinics (Birmingham, AL; New Orleans, LA; Durham, NC; Baltimore, MD)	Nov 2006 - Apr 2009	305	31% ¹⁶
STD clinic (Seattle, WA)	May 2007 - Oct 2009	370	12.5% ⁴⁶
STD clinic (Seattle, WA)	Jan 2007 - Jul 2011	606	13% ³¹

Table 3. Prevalence of MG in women with cervicitis in the U.S.

thus, testing patterns vary widely throughout the U.S. and globally. Whereas both chlamydia and gonorrhea are widely tested and reported on, there are no official guidelines on screening for *M. genitalium* in sexually active individuals, and many clinics do not currently request testing for this pathogen in initial consultations. More recent publications and discussions are now calling for the monitoring of *M. genitalium* in high-risk populations.^{14, 27-29}

When testing does occur, laboratories have limited options. *M. genitalium* is a fastidious organism and grows very slowly in culture, taking up to six months to detect.¹ Only a few labs globally have been able to isolate *M. genitalium* from clinical samples. Additionally, due to closely related species, serology lacks adequate specificity.³⁰ Therefore, culture and serology, which are the traditional diagnostic methods for bacteria, are not suitable for routine testing of *M. genitalium*. The only viable diagnostic option is detection using a nucleic acid amplification test (NAAT).

There are currently no U.S. Food and Drug Administration (FDA)-cleared tests for detection of *M. genitalium*. For that reason, in-house NAATs have been extensively used along with available CE-marked or research use only (RUO) assays. The majority of in-use tests allow only for detection of *M. genitalium*, providing no information on potential resistance status of an infection.²⁹ The rise in prevalence of antibiotic-resistant strains, however, has influenced the latest European guidelines for the management of *M. genitalium* infection, recommending the addition of resistance testing for effective management of NGU and other *M. genitalium* infections.^{8,9}

Mutations associated with azithromycin resistance

Macrolide resistance in *M. genitalium* has been strongly and consistently associated with mutations occurring at positions 2058 and 2059 (Escherichia coli numbering) of region V of the 23S rRNA gene.^{4,32} The five most common mutations are A2058G (adenine transition to guanine base at position 2058), A2059G, A2058T, A2058C, and A2059C,³² and those are the only mutations found in the majority of studies.^{14,17-27} Isolated *M. genitalium* strains with four of the most common mutations have been shown to confer high-level azithromycin resistance in vitro,³³ and all five mutations have been found in

Country of study	Age	Population size (N)	MG prevalence	
			Men % (CI%)	Women % (CI%)
Denmark	21-23	1652	1.1% (0.3-1.9%)	2.3% (1.3-3.2%) ⁴⁷
N.America	18-27	2932	1.1% (0.5-2.4%)	0.8% (0.4-1.6%) ⁴⁸
Australia	16-25	1116	n/a	1.6% (0.7-2.6%) ⁴⁹
UK	15-27	2378	n/a	3.3% (2.6-4.1%) ⁴²
UK	16-44	4507	1.2% (0.7-1.8%)	1.3% (0.9-1.9%) ⁴³

CI – Confidence interval

Table 4. Prevalence of MG in the general population

samples from patients exhibiting azithromycin treatment failure.⁴

There have been limited U.S. studies investigating 23S rRNA mutation prevalence. One study investigating *M. genitalium* prevalence also analyzed a subset of subjects for macrolide-resistant mutations and found rates of 50.8 percent [95 percent CI, 42.2 percent to 59.3 percent] in females (65 of 128) and 42 percent [95 percent CI, 29.4 percent to 55.8 percent] in males (21 of 50), with an overall prevalence of 48.3 percent.¹⁴

Testing for antibiotic resistance in *M. genitalium*

Given the high failure rates of the now standard azithromycin treatment, a detection-only assay for *M. genitalium* may have limited use in informing effective patient management. The European guidelines for management of NGU⁸ now recommend screening of male patients with urethritis for macrolide resistance mutations in order to improve clinical outcomes.

There are currently no FDA-cleared tests for detection of *M. genitalium* or for the determination of resistance status. With resistance linked to multiple mutations in the 23S rRNA gene, many studies rely on sequencing methods to determine resistant phenotypes^{14,27} This approach is not readily conducive to clinical practice due to high cost and increased sample processing times. Some alternative in-house approaches include the use of high resolution melting (HRM)³⁴ or qPCR based on fluorescence resonance emission transfer (FRET) coupled with melting curve analysis.²⁰ Clinical practices rarely incorporate HRM methodologies, as results may vary due to DNA concentration and can be difficult to reproduce in other labs, and melt curve analysis requires skilled interpretation. The example of the FRET assay is also not suitable for clinical purposes, as researchers only achieved a sensitivity of 76.7 percent.²⁰

NAAT diagnostics for allelic discrimination typically utilize qPCR with allele-specific primers or detection with allele-specific fluorescent probes.³⁵ This approach works well for conditions involving a single nucleotide polymorphism (SNP); however, design costs and complexity increase when multiple SNPs are involved. Often there are multiple variations within the same location, and/or SNPs are clustered within areas associated with specific structural or functional importance, leading to difficulties with cross priming or primer competition that affects specificity and sensitivity of the assay.³⁶ The mutations associated with azithromycin resistance in *M. genitalium* represent such complexity.

The one available CE marked test for both detection of

M. genitalium and resistance markers incorporates a novel priming technology to overcome these diagnostic challenges. This technology introduces an insert sequence during amplification, creating amplicons that are distinctly different from the original sequence.³⁶ Advantages to this approach may include increased stringency and subsequent selective amplification of mutant alleles, as well as the ability to multiplex a number of different SNP targets.³⁶ The assay combines detection of *M. genitalium* with five azithromycin-associated mutations, multiplexing the detection of multiple

resistance markers within a single-well reaction.³⁷ Tested on 400 stored clinical samples of positive *M. genitalium* patients, *M. genitalium* detection sensitivity was 99.1 percent, specificity 98.5 percent, while sensitivity and specificity of mutation detection was 97.4 percent and 100 percent respectively.³⁷

Treatment of *M. genitalium*

Mycoplasma lack a cell wall and thus are unaffected by many common antibiotics, leaving limited treatment options. Although the CDC STD guidelines recommend either doxycycline or azithromycin for the presumptive treatment of NGU,² due to the rising recognition of *M. genitalium* in NGU, and the known ineffectiveness of doxycycline, many countries (Australia, New Zealand, United Kingdom, Denmark, Japan) use only azithromycin.²¹ The European guidelines on *Mycoplasma genitalium* infections⁹ specifically recommend azithromycin for treatment of uncomplicated *M. genitalium* infections.

While azithromycin is more effective than doxycycline in the treatment of *M. genitalium*, cure rates have been declining. Three clinical trials across a decade in the U.S. showed declining cure rates from 77 percent in 2002-2004¹⁵ to 67 percent in 2006-2009¹⁶ to 40 percent in 2007-2011.³¹ Lau et al³⁸ recently published a meta-analysis of 21 studies on the efficacy of azithromycin for *M. genitalium* treatment, demonstrating declining cure rates since 2009. The pooled microbial cure for the 12 studies conducted prior to 2009 was 85.3 percent (95 percent confidence interval [CI], 82.3 percent to 88.3 percent). For the nine studies conducted since the beginning of 2009, the pooled microbial cure was 67.0 percent (CI, 57.0 percent to 76.9 percent).³⁸

M. genitalium treatment failures can be addressed with moxifloxacin. Initial reports showed moxifloxacin to be highly effective,^{1,11} yet its use is limited due to cost, side effects, availability, and potential for development of resistance. Resistance to moxifloxacin has been observed over the last decade, often in conjunction with macrolide resistance, resulting in persistent infection that is very challenging to manage.²⁹

M. genitalium is exhibiting alarming capabilities of developing antimicrobial resistance (AMR), and the widespread use of azithromycin as front-line treatment appears to be driving even higher rates of resistance.^{28,29,38} There is a real clinical need to accompany *M. genitalium* testing with surveillance of macrolide resistance-mediating mutations to enhance patient management and control the spread of AMR.^{8,29,37,39,40} Dr. Margaret Chan, the

WHO director, summarized perfectly the larger AMR issue facing us when she said, "Today, antibiotics are rarely prescribed based on a definitive diagnosis. Having rapid, low-cost, and readily available diagnostics is an essential part of the solution to this urgent problem."⁵⁰

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