Epidemiological and clinical rationale for screening and diagnosis of *Mycoplasma genitalium* infections

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LEARNING OBJECTIVES

1. Discuss the historical and epidemiological background of *Mycoplasma genitalium*
2. Justify the clinical rationale for *M. genitalium testing*
3. Describe the diagnosis of *M. genitalium*
4. Define *M. genitalium* non-gonococcal urethritis and cervicitis
5. Explain the syndromic management and antimicrobial resistance associated with *M. genitalium*

ABBREVIATIONS: CDC - Centers for Disease Control and Prevention, FDA - Food and Drug Administration, HPV - Human Papilloma Virus, LDT - laboratory developed tests, MDx - molecular diagnostics, NAAT - nucleic acid amplification test, NGU - non-gonococcal urethritis, NPV - negative predictive value, PPV - positive predictive value, RUO - research use only, STD - sexually transmitted disease, STI - sexually transmitted infection, ART - anti-retroviral therapy, LOD, limit of detection, LOQ, limit of quantification

INDEX TERMS: Molecular diagnostics, *Mycoplasma genitalium*, sexually transmitted disease, sexually transmitted infections

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Mycoplasma genitalium has been the focus of basic scientific and synthetic biology research as the organism with the smallest genome of all known human bacterial pathogens. As a sexually transmitted organism, substantial clinical and epidemiologic evidence now exists that warrant further consideration of *M. genitalium* as a priority for diagnostic testing. In the early 1980's, *M. genitalium* was first identified from two men with symptomatic non-gonococcal urethritis (NGU) – an inflammatory syndrome most often attributed to infections with *Neisseria gonorrhoeae* or *Chlamydia trachomatis*. Since then, epidemiologic studies of clinical disease, several animal models, and the results of many basic scientific investigations point towards *M. genitalium* as a urogenital pathogen with significant implications for reproductive and sexual health. It is now unequivocally known that *M. genitalium* is found in approximately 15-25% of patients with NGU and in more than one third of non-chlamydial NGU cases.[1] Importantly, *M. genitalium* establishes both acute and chronic infections in the urogenital tract of men and women. This article aims to concisely address the rationale for continued investigation of *M. genitalium* as a sexually transmitted infection (STI) and for the implementation of diagnostic testing paradigms in the USA.

Epidemiology of M. genitalium

As an emerging urogenital pathogen, the vast majority of *M. genitalium* research has been focused on the epidemiologic characteristics and associations with

disease syndromes – first in men, and more recently in women. With regard to the "emergence" of *M. genitalium* infections, it should be clarified that no reports have indicated an increase in prevalence over time but rather a recent expansion in notoriety as a pathogen. Among sexually transmitted disease (STD) clinic attendees and subjects classified as being at high-risk for STI acquisition, the prevalence of *M. genitalium* infection is approximately 7% considering studies form several countries worldwide.[2] Importantly, the prevalence parallels that of other bacterial STIs in that it is tightly linked to characteristic behavioral and demographic risk factors. As such, the urogenital prevalence of *M. genitalium* in high-risk subjects varies from less than 1% to more than 30% depending on the study population.[2] In contrast, study cohorts with a relatively low risk for acquiring STIs show considerably lower rates of infection, ranging from 0 to 4%, with most studies less than 1%. Collectively, *M. genitalium* is present in high- and low-risk populations at levels similar to those of *C. trachomatis* and *N. gonorrhoeae*.

Interest is expanding for understanding the role for *M. genitalium* in enhanced HIV susceptibility and disease progression. African women with *M. genitalium* infection are approximately two and a half times more likely to acquire HIV-1, and co-infection with these two pathogens is common. Positive cross-sectional associations between HIV and *M. genitalium* have been observed in more than 20 studies.[3] The biologic mechanisms for the clinical associations between *M. genitalium* and HIV are completely unknown, but several important lines of evidence provide a rationale for investigation of this co-infection scenario. First, *M. genitalium* has been associated with cervical inflammation in several studies,[1,2,4] whereby microscopic signs of inflammation are often detected in the absence of lower reproductive tract symptoms. Second, urogenital *M. genitalium* infections can be chronic thereby providing the potential for long-term interactions with HIV and/or HIV target cells.[5-8] Importantly, experimental *in vitro* evidence has consistently shown *M. genitalium* to be a cause of mucosal inflammation with a profile consistent with recruitment of lymphocytes and macrophages to the epithelium.[9-14] Considering that macrophages and CD4(+) T lymphocytes are HIV-susceptible cell types, perhaps the association between HIV and *M. genitalium* is not surprising since *M. genitalium* is an inflammatory organism and virtually all STI are associated with HIV. However, the importance of *M. genitalium* as a co-factor for HIV disease progression has not been investigated and very little data exist on management of *M. genitalium* infection in HIV-positive subjects with or without anti-retroviral therapy (ART). To this end, our current understanding of *M. genitalium* does not warrant special recommendations for screening or therapy in HIV-infected individuals.

Diagnosis of M. genitalium infection

Due to the fastidious nature of *M. genitalium*, culture-based isolation of the organism is time-consuming, labor intensive and, as such, has no diagnostic utility. Despite high rates of isolation from nucleic acid amplification test (NAAT)-positive men, culture-based isolation procedures currently involve co-culture of the specimen with Vero cells for weeks to months before reaching a titer suitable for sub-culture or adaptation to axenic (cell-free) growth medium. In turn, virtually most contemporary clinical studies have relied upon NAATs for diagnosis. Commercially developed testing kits have entered the European market but, to date, no *M. genitalium* test has acquired FDA approval for use in the USA. A research use only (RUO) NAAT developed by GenProbe-Hologic, Inc. has been utilized by select collaborating laboratories. In recent years, this test has been utilized extensively for male and female urogenital specimens and compared to several laboratory developed tests (LDTs) despite not being available commercially. The optimized LDT platform developed by Jensen and colleagues in 2004[15] has been widely employed in clinical research settings worldwide, and serves as a validated reference laboratory test for STI surveillance at the Staten Serum Institut in Copenhagen, Denmark.

Given the quality of PCR reagents currently available to researchers and the expansion of molecular diagnostics (MDx) methods into research and clinical laboratories, several LDTs have been utilized for investigation of *M. genitalium*. However, without an established gold standard for which to validate these tests, the results from epidemiologic studies that employ LDTs should be interpreted with caution. Ma and colleagues exemplify this notion in a 2010 study where the authors note considerable variability in targeted genomic loci for several previously published NAATs.[16] Such variability in the primer/probe target sequence could impact assay

sensitivity thereby rendering false-negative results and inaccurate interpretation of prevalence and disease associations. In lieu of an FDA-approved test, researchers and reference labs have pushed forward using LDTs with anticipation of an approved test marketed in the USA soon. Since organism culture is the current 'gold standard' for *M. genitalium* detection, it is recognized that the clinical trial(s) for FDA submission will be lengthy and costly for the first NAAT submitted for approval.

In the mean time, as LDTs are validated within clinical laboratories for internal use, it is imperative to implement only thoroughly scrutinized tests where strict and accurate criteria are used in development. Performance characteristics that must be assessed and defined include specificity, sensitivity, positive predictive value (PPV) (the probability that those testing positive are indeed positive), negative predictive value (NPV) (accurately identifying uninfected individuals), and assay reproducibility. Without a gold-standard NAAT comparator, the value of some of these performance points are limited, but accurately defining the limit of detection (LOD) and limit of quantification (LOQ) for each assay system is imperative for interpreting the validity of the test. In short, the current lack of a standardized and FDA-approved NAAT is an impediment to our continued investigation of *M. genitalium* disease. Filling this gap in the diagnostic testing menu in the USA would aid directly in providing more informed and appropriate therapy to the enormous number of patients with urogenital disease for which *M. genitalium* is a plausible etiology.

M. genitalium NGU and cervicitis

Virtually all studies of both symptomatic and asymptomatic men support the fact that *M. genitalium* is a common etiology of NGU independent of *C. trachomatis*.[1] In the pooled analysis of more than 35 independent studies of *M. genitalium* conducted by Taylor-Robinson and Jensen, the combined odds ratio was 5.5 (95% CI: 4.3-7.0) for NGU and 7.6 (95% CI: 5.5-10.5) for non-chlamydial NGU. In this light, the US Center for Disease Control and Prevention (CDC) recognizes *M. genitalium* as an etiology of NGU despite the absence of an FDA-approved diagnostic test. Current STD treatment guidelines can be found at http://www.cdc.gov/std/treatment/2010/. Urethritis is most often characterized by acute and/or chronic inflammation defined microscopically by urethral leukocytosis. Symptoms manifest typically as dysuria or localized itching, with urethral discharge being the most common clinical sign of urethritis. As the most common urogenital syndrome in men, NGU is strongly associated with infection by *C. trachomatis*, *M. genitalium*, *T. vaginalis* and Herpes Simplex Virus. However, no infectious or non-infectious etiology can be identified in up to 40% of cases,[17] and thus underscores the current misunderstandings of the exceedingly common syndrome in men.

Cervicitis, an inflammatory syndrome of the uterine cervix, has several parallel characteristics with male urethritis and diagnosis similarly relies upon signs of purulent discharge and/or microscopic leukocytosis. It is important to note that cervicitis is always diagnosed on clinical exam since few symptoms exist unless cervical mucopus is severe and results in vaginal discharge. Unlike for male urethritis, a standardized clinical definition has yet to be established for cervicitis and, as such, variable combinations of overt and microscopic signs have been employed. Despite the heterogeneity and some conflicting results from previous studies, the evidence for *M. genitalium* as a cause of cervical inflammation is stronger than for any other female reproductive tract syndrome. In studies where microscopic criteria were considered independent of non-microscopic criteria, all studies have shown a positive association with cervicitis.[2] In contrast, studies using non-microscopic criteria (e.g. mucopus, edema, erythema, post-sample bleeding) less frequently demonstrated an association between *M. genitalium* and cervicitis.[4] Many of these discrepancies are attributed to the variable clinical definition of cervicitis. When diagnosed based on microscopic criteria alone, the true importance and pathological consequences of cervicitis as a syndrome is generally misunderstood regardless of the etiology. In contrast, cervicitis with purulent discharge (mucopurulent cervicitis) clearly indicates an inflammatory disease state requiring intervention. Although cervical infection by *M. genitalium* could lead to purulent discharge, it is important to remember that cervical discharge may be secondary to upper reproductive tract inflammation or pelvic inflammatory disease (PID) for which *M. genitalium* has been implicated in several studies.[1,2,4,18] Taken together, continued clinical and laboratory investigation of lower genital tract inflammation is imperative for

understanding the role of STI pathogens like *M. genitalium* in reproductive health.

Syndromic management and antimicrobial resistance

Implementation of a widespread screening and treatment program for *M. genitalium* in the USA is a distant reality with insufficient rationale and data to compute the necessary cost-effectiveness analyses. This is owed in part to the lack of a commercially available testing platform, and also because additional randomized treatment trials are needed to accurately establish the most effective treatment paradigms. With these shortcomings, this begs the question of whether *M. genitalium* testing is even warranted if syndromic management using CDC-recommended paradigm is effective and routinely utilized. In the STD clinic setting, management of men with urethritis is among the top services provided to attendees. Common for male urethritis and lower reproductive tract inflammation in women, syndromic management is the practice of directing therapeutic intervention based solely on syndrome-related signs and symptoms in the absence of STI test results. This practice is nearly universal in STD clinics because NAAT results have turn-around times of hours to days, and it is imperative to begin treatment at the initial visit since the patients do not readily follow up. Stat dosing, that is providing antibiotics at the clinic based on syndromic interpretation, is a widely utilized component of this treatment paradigm because 1) compliance is assured; 2) STD clinic attendees often have no financial means to fill a prescription; and 3) in the absence of diagnostic test results, several potential etiologic agents can be managed with a single antibiotic.

Arguably the most important step in syndromic management of urogenital disease in men and women is determining whether *N. gonorrhoeae* is present during the initial clinic visit. Gram staining of urethral smears for microscopic identification of *N. gonorrhoeae* is a widely used point of care procedure that, despite some concerns about sensitivity, remains an essential practice for discerning gonococcal urethritis or cervicitis from NGU or non-gonococcal cervicitis. This test is generally regarded as the preferred means for concurrently documenting inflammation and the presence of Gram-negative intracellular diplococci. Subjects without signs of *N. gonorrhoeae* infection are typically managed syndromically.

An ideal diagnostic and treatment paradigm would include point of care (POC) testing for STIs thereby eliminating syndromic management and presumptive antibiotic therapy of NGU and non-gonococcal cervicitis. Unfortunately, with exception to HIV and *Trichomonas vaginalis* (discussed in an accompanying article in this series), POC diagnostics are still far outnumbered by high-throughput hospital and reference laboratory testing platforms with longer turn-around times. The current CDC-recommended treatment strategy of male NGU and non-gonococcal cervicitis indicates stat dosing of azithromycin or a seven-day doxycycline regimen, which is tailored to eradicating *C. trachomatis* infection. The rationale for this is seemingly clear since *C. trachomatis* is responsible for approximately 25% of non-gonococcal cervicitis and NGU.[17] However, *M. genitalium* is also associated with 15-25% of male NGU cases and thus is an important consideration in presumptive treatment.[1]

Several contemporary studies have shown that a single 1 gram dose of azithromycin is markedly more effective than doxycycline for clinical cure of *M. genitalium* infection.[1] A recent study of NGU showed only a 67% clearance rate for *M. genitalium* using the single 1 gram dose.[19] In addition, several studies have highlighted the potential for stat dosing to induce drug resistance associated with treatment failure.[20-27] It seems that the single 1 gram dose is not sufficient for clearance of infection in many individuals because extending the dosing paradigm to five days substantially increases cure rates as discussed below. Therefore, knowing that up to 25% of NGU is associated with *M. genitalium* infection, and that microbiologic cure rate in a recent double-blind treatment trial was 40% for 1 g of azithromycin, it is estimated that approximately 10% of men with NGU could potentially benefit from testing and modified therapy regimen. This estimate is likely a conservative one because many clinics still utilize doxycycline as the first line therapy with even higher rates of treatment failure.[1] Rather than stat azithromycin, one such regimen would be extended azithromycin dosing such as 500 mg on day one followed by 250 mg daily on days two through five; this has cure rates between 85-100%.[1] Patients failing extended azithromycin therapy should be treated with moxifloxacin for which few cases of treatment failure have been reported.[28] It should be noted that the superiority of moxifloxacin (400 mg for up to 10 days)

is accepted in the field, but has not been evaluated in clinical trials and is based primarily on observational studies.

With the absence of reliable and differential signs/symptoms or biomarkers predictive of *M. genitalium* infection, differential therapy as outlined above would require a POC test to circumvent the turn-around times of high-throughput testing. Unfortunately, very limited data exists on the efficacy of a second 1 gram dose of azithromycin several days after the initial visit, which would extend the azithromycin regimen if test results from the initial visit indicate *M. genitalium*. In the one study to address this, similar cure rates were observed between the regimens of two 1 gram doses (five to seven days apart) compared to the single 1 gram dose (78 vs 79%, respectively).[29] The distinct advantage to having a *M. genitalium* testing platform would be 1) as a screening tool in high and low risk populations; and 2) as a diagnostic test in complicated treatment failure cases of NGU or non-gonococcal cervicitis. Incorporating *M. genitalium* testing in combination with *C. trachomatis* and *N. gonorrhoeae* would be ideal for screening men and women, particularly in low-risk populations, since these subjects are less like to be symptomatic and requiring immediate therapy, and more likely to be available for follow up and subsequent antibiotics. This would also facilitate the identification and treatment of subjects with chronic asymptomatic infection. Since *M. genitalium* infections in women are often asymptomatic, together with the fact that several studies have shown associations with more severe upper tract sequelae, differential diagnosis of *M. genitalium* could have substantial impact of women's health when used as a screening test.

In conclusion, although the true extent to which *M. genitalium* impacts reproductive and sexual health remains to be seen, the need for a diagnostic test is strong and will directly address this gap in knowledge. Substantial evidence has been gathered from study of human subjects, *in vitro* experimental investigations, and from inoculation of laboratory animals that collectively highlight the need to understand *M. genitalium* as a prevalent and emerging urogenital pathogen. Much like for *C. trachomatis*, it is predicted that the testing market will follow the availability of a FDA-approved commercial testing platform. The full implications of chlamydial infection could not be assessed without clinicians and researchers having access to a validated diagnostic test, and this is true for *M. genitalium* as well.

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