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Mycoplasma genitalium: Clinical Significance and Diagnosis

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SUMMARY *Mycoplasma genitalium* is considered the smallest self-replicating cell. It was first isolated in 1981, from 2 of 13 men with urethritis. *Mycoplasma genitalium* causes urethritis, cervicitis and pelvic inflammatory disease. Because of difficulties in cultivation, the diagnosis is based exclusively on PCR methodology. The recommended therapy for *Mycoplasma genitalium* infections is azithromycin or doxycycline. Development of macrolide resistance was shown to correlate with treatment failure.

KEY WORDS: *Mycoplasma genitalium*, urethritis, molecular diagnosis, therapy

INTRODUCTION

Mycoplasma genitalium (M.) genitalium was first isolated in 1981, after prolonged incubation from 2 of 13 men with urethritis (1). This fastidious organism is extremely difficult to isolate from clinical specimens with conventional culture technique; its diagnosis requires the use of sensitive nucleic acid amplification tests (NAATs). Commercial kits are becoming available for the detection of *M. genitalium*; however, to date none of the kits has obtained the United States Food and Drug Administration (US FDA) approval certificate (2). The exact mode of infection and pattern of diseases caused by *M. genitalium* still remains to be solved, but the pathogen is presumed to be sexually transmitted, and infections often appear to be chronic and asymptomatic (3-5). Most investigations have been concentrated on male urethritis patients, but *M. genitalium* has also been implicated in pelvic inflammatory disease, pneumonia, arthritis, and acquired immunodeficiency syndrome (AIDS) (6-8).

CLASSIFICATION AND GENERAL CHARACTERISTICS

M. genitalium belongs, along with other mycoplasmas and ureaplasmas, to the family *Mycoplasmatacae,* class *Mollicutes*. They are widely distributed in humans, mammals, birds, reptiles, fish, and other vertebrates, as well as in plants. They differ from other bacteria in that they lack a rigid cell wall (9,10). The mycoplasmas are the smallest prokaryotes capable of self-replication. Genital mycoplasmas represent a complex and unique group of microorganisms that have been associated with a wide array of infectious diseases in adults and infants.

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The family *Mycoplasmatacae* contains two genera: *Mycoplasma* and *Ureaplasma*. The genus *Mycoplasma* contains over 100 species that inhabit a wide variety of plants and animals. From all these species, just 16 species affect genital and respiratory tract in humans; out of them, clinically significant are just few mycoplasmas: *M. pneumoniae* that causes atypical pneumonia and genital mycoplasmas *M. hominis, M. genitalium* and *Ureaplasma (U.) urealyticum* that have been associated with a wide array of infectious diseases in adults and infants.

These organisms are much smaller than most other bacteria; hence, they are able to pass through bacteriologic filters. *M. genitalium* is the smallest one among the mycoplasmas with the cell diameter of only 300 nm and a genome size of only 580 kbp (9,10).

The lack of a typical bacterial cell wall containing peptidoglycan renders these organisms insensitive to cell wall-active antimicrobial agents, such as penicillins and cephalosporins. Because of this, the recovery of these organisms from clinical specimens may have significant therapeutic implications.

PATHOGENESIS

The potential virulence factors in human mycoplasmas have not been studied extensively, but some have been described. *M. pneumoniae* has been the widely most studied human mycoplasma; because of its close genetic relationship with *M. genitalium*, some features can probably be generalized. Both microorganisms are primarily considered surface parasites of mucous membranes and possess polar tip organelles for attachment to host cells like adhesins (11).

The exact molecular pathogenesis of *M. genitalium* is vague, but it has been suggested that tissue damage is just partially caused by mycoplasma toxins and harmful metabolites such as hydrogen peroxide and superoxide.

Mycoplasmas can interact with many components of the immune system, inducing macrophage activation and cytokine production. Some cell components may act as superantigens, and could induce several autoimmune manifestations. *M. genitalium* may invade epithelial cells, which may offer protection from antibiotics (11).

CLINICAL SIGNIFICANCE OF *MYCOPLASMA GENITALIUM*

Genital mycoplasmas are suspected of contributing to a number of pathologic conditions. They are associated with various infections of the genitourinary tract, reproductive failure, and neonatal morbidity and mortality.

The lack of conclusive knowledge regarding the pathogenic potential of *M. hominis* and *Ureaplasma* spp. in many conditions is due to their high frequency in healthy persons and the poor design of research studies. Besides, isolation of *M. genitalium* is very difficult. The situation is now changing because of introducing molecular methods in detection of genital mycoplasmas.

Nongonococcal urethritis (NGU) is a common condition in men. Men with NGU can be categorized into those with and without *Chlamydia (C.) trachomatis* infection (12,13). In the studies performed in the early 1990s, it has been reported that *C. trachomatis* was the cause of 35%-50% of NGU cases (14). More recently, it was observed that *C. trachomatis* caused approximately one-third of NGU cases (15). So, in the majority of cases, the clinical syndrome is referred to as nonchlamydial nongonococcal urethritis (NCNGU). However, in a significant percentage of urethritis cases, the causative agent could not be found (16). In 1981, *M. genitalium* was first isolated from the urethras of 2 of 13 men with urethritis (1). Studies that attempted to assess its association with disease were hampered by the difficulty of growing the organism in culture (1). In more recent years, more reliable detection has become possible after the development of specific polymerase chain reaction (PCR) assays. In the 1990s, large numbers of papers on the role of *M. genitalium* in male non-gonococcal urethritis were published. Although different criteria were used to define patient and control groups, all the studies demonstrated a higher prevalence of *M. genitalium* in the groups of patients with NGU (17,18). In those studies from different countries, the prevalence of *M. genitalium* in patients with NGU ranged from 13% to 42%, and in asymptomatic men from 0% to 15% (19). In Croatia, the prevalence in symptomatic men is 2.3% (unpublished data, Plečko, Žele).

Moreover, *M. genitalium* appears to be detected with highest prevalence in men with *C. trachomatis* negative urethritis. Several studies have shown that men with *M. genitalium* positive NGU have symptoms at least as often as those with chlamydial urethritis. Systematic studies linking *M. genitalium* to the complications such as epididymitis and prostatitis are lacking. However, *M. genitalium* DNA has been found both in the urethra of men with epididymitis, and in the prostatic tissue of men with prostatitis (20,21). Detection of *M. genitalium* in men with acute NGU also was associated significantly with balanitis and/or posthitis (22).

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Like *U. urealyticum* and *M. hominis*, *M. genitalium* attaches to spermatozoa, but there is no evidence of its role in sperm quality (21).

In women, the presence of *M. genitalium* is associated with cervicitis and urethritis. *M. genitalium* can be detected in the endometrium of women with pelvic inflammatory disease, and, on a single occasion, was found in the fallopian tube (23-25). It has also been confirmed that *M. genitalium* is associated with pelvic inflammatory disease, independently of gonococcal or chlamydial infection. Several PCR-based studies from geographically diverse populations confirm that *M. genitalium* is associated with clinical pelvic inflammatory disease. In these infections, *M. genitalium* is diagnosed in 13%-16% of cases. Serologic studies suggest a strong association between past infection with *M. genitalium* and tubal factor infertility (26).

DIAGNOSIS

Genital mycoplasmas (*M. hominis* and *U. urealyticum*) can be cultivated in the enriched broth that contains arginine, urea and phenol red indicator. This broth is inoculated with the specimen and is incubated aerobically at 35 °C. The medium is observed for changes in the color of the indicator. Namely, the growth of the mycoplasmas changes pH; subculture to solid media and subsequent incubation allows for recovery and identification of both *M. hominis* and *U. urealyticum*. The culture of *M. genitalium* is extremely difficult, time-consuming and lasts up to 8 weeks. This is the reason why cultivation of *M. genitalium* is used for research purposes only (11).

From the early 1990s, detection of *M. genitalium* is dependent mainly on PCR methods (1). These assays were based on the MgPa gene (encoding the major surface protein MgPa) and 16S rRNA gene sequence (11). First assays were based on the MgPa DNA sequence, but in further studies genetic variability of the MgPa gene was observed, so there was a risk of false negative reactions using these primers. Later, the MgPa-1/MgPa-3 assay was validated as a confirmatory assay as part of the development of 16S rRNA gene PCR. Briefly, 16S rRNA positive result is further confirmed by MgPa-1/MgPa-3 PCR (11).

The first real-time PCR assay for *M. genitalium* was published by Jensen *et al*. in 2002 (27). Later, numerous studies used quantitative methods (28,29). These assays are very sensitive and specific. The MgPa gene and 16S rRNA encoding-genes of *M. genitalium* are most commonly used as targets in *M. genitalium* qPCR. In a recently published study, Müller *et al*. have developed a quantitative real-time Rotor-Gene PCR (qPCR) assay targeting the *pdhD* gene of *M. genitalium* (29).

Quantitative PCR is a useful tool for detecting *M. genitalium* bacterial loads in a range of samples, including vaginal and cervical swabs, urethral swabs, and first-void urine (30). Based on published literature, first-pass urine sample appears to be a better sample for men than urethral swab (31). Several qPCR assays are able to detect and quantify *M. genitalium* as low as 2 copies/reaction (29).

The commercial assays for detection of *M. genitalium* are available, such as the AmpliSens® *Mycoplasma genitalium*, Eph PCR kit, Bio-Rad Dx CT/NG/MG assay, Euroclone DUPLICa kit, and Gen-Probe; however, none has yet obtained the US FDA approval. At present, most laboratories use kits for research, so it is important that laboratories actively engage in external quality assurance programs using real clinical specimens before they introduce molecular methods in routine (2).

ANTIBIOTIC THERAPY

The current recommended treatment for *M. genitalium* infection is the macrolide antibiotic azithromycin. A comparative trial and one observational study have both shown that a single 1 g dose of azithromycin is significantly more effective than multi-dose doxycycline, with cure rates of 86%-87% *versus* 22%-45% (2). Development of macrolide resistance has been shown to correlate with subsequent azithromycin treatment failure. The genetic basis for drug resistance was shown to be mutations in region V of the 23S rRNA gene, which is well described in other Mollicutes. The genetic basis for drug resistance was established by sequencing parts of the 23S ribosomal RNA gene and the genes encoding the L4 and L22 proteins (31-33). These findings raise concern about the use of single-dose azithromycin treatment of nongonococcal urethritis of unknown etiology. In case of persistent symptoms of *M. genitalium* urethritis or detection of macrolide resistance, the recommended therapy is moxifloxacin 400 mg daily for 7-10 days (2).

References

1. Jensen JS, Uldum SA, Sondergard-Andersen J, Vuust J, Lind K. Polymerase chain reaction for detection of *Mycoplasma genitalium* in clinical samples. J Clin Microbiol 1991;29:46-50.
2. Twin J, Jensen JS, Bradshaw CS, Garland SM, Fairley C, Yi Min L, *et al.* Transmission and selection of macrolide resistant *Mycoplasma genitalium* infections detected by rapid high resolution melt analysis. PloS ONE 2012; 7(4):e35593.doi:10.1371/journal.pone.0035593.

3. Mena L, Wang X, Mroczkowski TF, Martin DH. *Mycoplasma genitalium* infections in asymptomatic men and men with urethritis attending a sexually transmitted diseases clinic in New Orleans. CID 2002;35:1167-73.
4. Yu THT, Tang W, Lau KH, Chong LY, Lo KK, Wong CKH, *et al.* Role of *Mycoplasma genitalium* and *Ureaplasma urealyticum* in non-gonococcal urethritis in Hong Kong. Hong Kong Med J 2008;14:125-9.
5. Manhart LE, Critchlow CW, Holmes KK, Dutro SM, Eschenbach DA, Stevens CE, *et al.* Mucopurulent cervitis and *Mycoplasma genitalium*. J Infect Dis 2003;187:650-7.
6. Haggerty CL, Taylor BD. *Mycoplasma genitalium*: an emerging cause of pelvic inflammatory disease. Infect Dis Obstet Gynecol 2011;959816.
7. Haggerty CL, Totten PA, Astete SG, Ness RB. *Mycoplasma genitalium* among women with nongonococcal nonchlamydial pelvic inflammatory disease. Infect Dis Obstet Gynecol 2006;30184.
8. Ross JDC, Jensen JS. *Mycoplasma genitalium* as a sexually transmitted infection: implications for screening, testing, and treatment. Sex Transm Infect 2006;82:269-71.
9. Taylor-Robinson D, Furr PM. Genital mycoplasma infections. Klin Wochenschr 1997;109:578-83.
10. Tully JG, Taylor-Robinson D, Cole RM, Rose DL. A newly discovered mycoplasma in the human urogenital tract. Lancet 1981;1288-91.
11. Jensen JS. *Mycoplasma genitalium* infections (dissertation). Dan Med Bull 2006;53:1-27.
12. Horner PJ, Taylor-Robinson D. Association of *Mycoplasma genitalium* with balanoposthitis in men with non-gonococcal urethritis. Sex Transm Infect 2010. doi:10.1136/sti.2010.044487.
13. Uuskula A, Kohl PK. Genital mycoplasmas, including *Mycoplasma genitalium*, as sexually transmitted agents. Int J STD AIDS 2002;13:79-85.
14. Hooton TM, Roberts MC, Kenny GE. *Mycoplasma genitalium* and non-gonococcal urethritis. Lancet 1994;343(8889):69.
15. Taylor-Robinson D, Horner P. *Mycoplasma genitalium* and asymptomatic chlamydia-negative nongonococcal urethritis revisited. Int J STD AIDS 2005;16:768-9.
16. Maeda S, Tamaki M, Kubota Y, Nguyen PB, Yasuda M, Deguchi T. Treatment of men with urethritis negative for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum* and *Ureaplasma urealyticum*. Int J Urol 2007;14:422-5.
17. Jensen JS. *Mycoplasma genitalium* infections. Diagnosis, clinical aspects and pathogenesis. Dan Med Bull 2006;53:1-27. Review.
18. Jensen AJ, Kleveland CR, Moghaddam A, Haaheim H, Hjemevoll SO, Skogen V. *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum* among students in northern Norway. J Eur Acad Dermatol Venereol 2012 Mar 26. doi: 10.1111/j.1468-3083.2012.04528.x. [Epub ahead of print] PubMed PMID: 22449180.
19. Falk L, Fredlund H, Jensen JS. Symptomatic urethritis is more prevalent in men infected with *Mycoplasma genitalium* than with *Chlamydia trachomatis*. Sex Transm Infect 2004;80:289-93.
20. Mandar R, Raukas E, Turk S, Korrovits P, Punab M. *Mycoplasamas* in semen of chronic prostatitis patients. Scand J Urol Nephrol 2005;39:479-82.
21. Gdoura R, Kchaou W, Chaari C, Znazen A, Keskes L, Rebai, T, *et al. Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis* and *Mycoplasma genitalium* infections and semen quality of infertile men. BMC Infect Dis 2007;7:129.
22. Horner PJ, Taylor-Robinson D. Association of *Mycoplasma genitalium* with balanoposthitis in men with non-gonococcal urethritis. Sex Transm Infect 2011;87:38-40.
23. Korte JE, Baseman JB, Cagle MP, Herrera C, Piper JM, Holden AEC, *et al.* Cervicitis and genitourinary symptoms in women culture positive for *Mycoplasma genitalium*. Am J Reprod Immunol 2006;55:265-75.
24. Kataoka S, Yamada T, Chou K, Nishida R. Association between preterm birth and vaginal colonization by *Mycoplasmas* in early pregnancy. J Clin Microbiol 2006:44:51-5.
25. Wroblewski JKH, Manhart LE, Dickey KA, Hudspeth MK, Totten PA. Comparison of transcription-mediated amplification and PCR assay results for various genital specimens types for detection of *Mycoplasma genitalium*. J Clin Microbiol 2006;44:3306-12.
26. Clausen HF, Fedder J, Drasbek M, Nielsen PK, Toft B, Ingerslev HJ, *et al.* Serological investigation of *Mycoplasma genitalium* in infertile women. Hum Reprod 2001;16:1866-74.
27. Jensen JS, Borre MB, Dohn B. Detection of *Mycoplasma genitalium* by PCR amplification of the 16S rRNA gene. J Clin Microbiol 2003;41:261-6.
28. Stellrecht KA, Woron AM, Mishrik NG, Venezia RA. Comparison of MUltiplex PCR assay with culture for detection of genital mycoplasmas. J Clin Microbiol 2004;42:1528-33.

29. Müller EE, Venter JME, Magooa MP, Morrison C, Lewis DA, Mavdzenge SN. Development of a rotor-gene-real-time PCR assay for the detection and quantification of *Mycoplasma genitalium*. J Microbiol Methods 2012;88:311-5.
30. Blaylock MW, Musatovova O, Baseman JG, Baseman JB. Determination of infectious load of *Mycoplasma genitalium* in clinical samples of human vaginal cells. J Clin Microbiol 2004;42:746-52.
31. Jensen JS, Björnelius E, Dohn B, Lidbrink P. Comparison of first void urogenital swab specimens for detection of *Mycoplasma genitalium* and *Chlamydia trachomatis* by polymerase chain reaction in patients attending a sexually transmitted disease clinic. Sex Transm Dis 2004;31:499-507.
32. Ito S, Shimada Y, Yamaguchi Y, Yasuda M, Yokoi S. Selection of *Mycoplasma genitalium* strains harbouring macrolide resistance-associated 23S RNA mutations by treatment with a single dose of azithromycin. Sex Transm Infect 2011;87412-4.
33. Lucier TS, Heitzman K, Liu SK, Hu PC. Transition mutations in the 23S RNA of erythromycin-resistant isolates of *Mycoplasma pneumoniae*. Antimicrob Agents Chemother 1995;39:2770-3.