Prevalence studies of *M. genitalium* and other sexually transmitted pathogens in high risk individuals indicate the need for comprehensive investigation of STIs for accurate diagnosis and effective treatment

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Editor

In this issue of GERMS the paper by Chra P et al.[1] investigates the prevalence of *Mycoplasma genitalium* in the setting of possible other concomitantly occurring sexually transmitted infections (STIs), with demographic, behavioral, epidemiologic, and clinical characteristics of men and women presenting to an urban metropolitan clinic for sexually transmitted diseases (STD) in Athens, Greece. This approach is of interest to clinicians as it employs both traditional microbiology and molecular assay approaches in evaluating STIs as either single, dual- or multi-agent infections.

It is reported that more than one million STIs are acquired every day worldwide with serious ramifications on human sexual and reproductive health.[2] Different STIs can concurrently exist, and may further enhance the risk of transmission of other sexually transmitted pathogens.

Urethritis and cervicitis, the main syndromes caused by STIs, have no standard etiology.[3,4] *Neisseria gonorrhoeae* has been initially linked to these syndromes, and later *Chlamydia trachomatis*.[3,4] *Mycoplasma hominis* was thought to be a cause of non-gonococcal urethritis (NGU), but by the early 1960s it was considered to be less of an etiologic factor.[5] Its prevalence in women with cervicitis varies and has been associated with bacterial vaginosis.[6] *Ureaplasma urealyticum* has also subsequently been associated with NGU in men[3] and cervicitis in women.[7] In addition, while the etiologic roles of *Trichomonas vaginalis* and herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) are clear in NGU[3] and cervicitis,[4] the role of adenoviruses is clear only in urethritis.[3] Association of cervicitis with cytomegalovirus (CMV) has also been reported.[8] *Neisseria meningitidis*, *Haemophilus* spp., *Streptococcus* spp. and *Candida* spp. rarely occur as causative agents of NGU, while the role of Epstein Barr virus in NGU is unclear.[3] Still, the etiological factor in about 30% of NGU cases may not be identified.[9] In this context, the observation that the addition of doxycycline to the treatment regimen of gonococcal urethritis was effective in the treatment of post-gonococcal urethritis (PGU)[10] suggested that *Mycoplasma genitalium* could cause PGU, since this microorganism is often susceptible to tetracyclines.

Investigation of *M. genitalium* has been facilitated by the development of microorganism specific PCR methods. *M. genitalium*, a now recognized sexually transmitted pathogen, has been associated mainly with non-gonococcal non-chlamydial urethritis (NGNCU). It is also associated with balanoposthitis, chronic prostatitis and acute epididymitis in men,[11] with urethritis, cervicitis, endometritis, pelvic inflammatory disease, infertility and adverse pregnancy outcomes in women,[11] and HIV acquisition in both sexes.

M. genitalium prevalence studies seek to assess the importance of this microorganism in the constellation of STIs and determine its incidence, particularly regarding NGU and NGNCU, in an attempt to better define the

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spectrum of STI etiologies. Between 1988 and 2013, *M. genitalium* prevalence in different settings and locations with different population studies and different detection methods has ranged from 0% to 41%.[12] Reports have been published of 6.3% *M. genitalium* prevalence in 2005 among symptomatic males in neighboring Turkey[13] and Sweden,[14] respectively, of 9.4% to 29.2% prevalence among symptomatic individuals in Denmark in 2007,[15] and of 38% *M. genitalium* prevalence among symptomatic women in France in 2002.[16] A study in 2015 from central Greece conducted in individuals referred and investigated specifically for infertility problems, reported absence of *M. genitalium* infection.[17] Pertinent studies published in 2017 report *M. genitalium* prevalence in France corresponding to 3.4% and 5.9% respectively,[18,19] and a 6.6% prevalence among males attending STD clinics in Tel Aviv.[20] In the general population, *M. genitalium* prevalence has been reported as higher than that of *N. gonorrhoeae* but lower than that of *C. trachomatis* in the USA.[21]

At present, the diagnosis of *M. genitalium* infection relies on in-house protocols, since currently no commercially available nucleic acid amplification test (NAAT) assays meet US FDA standards, and the CE marked tests (CE-IVD Assay) on the market suffer from limited validation.[22] Interestingly, a substantial number of studies report prevalence data based on CE-IVD Assay PCR kits and in certain cases these tests are included in national guidelines for the diagnosis of *M. genitalium*. It must be emphasized that molecular assays detect genetic evidence of *M. genitalium*, but cannot distinguish between simple presence of genetic material in latent infection, or contamination, versus active infection or disease; customarily, accurate quantitative cut-offs of (DNA/RNA) copies/mL might be correlated with active infection. Uncertainty regarding definitive diagnoses of *M. genitalium* as an STI can be resolved in many cases by additional standard diagnostic methods such as microscopic findings of inflammation (e.g., increased presence of WBC-pus cells) as well as concomitant symptoms and clinical manifestations, particularly in cases of mono-infection.

In the Chra P et al. paper, *M. genitalium* was detected by two different PCRs (conventional PCR targeting the V1/V3 hypervariable regions of the 16S rRNA gene and MGB TaqMan Real-Time PCR) with different gene targets, implementing stringent criteria to ensure that positive results were true positive. Appropriate statistical techniques were utilized to depict values and variables according to sample size. The overall prevalence of *M. genitalium* was found to be 5.7%, with a 6.4% and 4.9% prevalence among males and females respectively. Its prevalence among symptomatic patients, symptomatic males and symptomatic females was 5.6%, 5.7% and 5.4%, respectively.

A particular strength of this prospective study includes the concomitant investigation of an array of alternative STD pathogens namely *Neisseria gonorrhoeae*, *Ureaplasma* spp., *Mycoplasma hominis*, *Trichomonas vaginalis*, *Candida* spp., bacterial vaginosis using Amsel's criteria, and *Chlamydia trachomatis* detected by PCR (Cobas Amplicor PCR platform). This approach enabled the authors to record *M. genitalium* as mono-infection, as well as dual, triple or quadruple co-infections utilizing both PCR and the standard methods employed for diagnostic microbiology, such as direct microscopic findings, which demonstrate evidence of inflammation in the urogenital tract, enabling correlation of this evidence with clinical manifestations in symptomatic individuals.

Among the six *M. genitalium* infected males, five (5/6) had microscopic findings, symptoms and signs of urethritis, three of whom (3/5) were *M. genitalium* mono-infected. These findings provide and support evidence that *M. genitalium* independently contributes to pathogenicity.

Microscopic findings from samples taken and cultures performed for the isolation of *N. gonorrhoeae* and *Candida* spp. can assist in appropriate antibiotic selection, especially for *N. gonorrhoeae* isolations, where the microscopic findings could also be utilized as a prognostic risk factor. This diagnostic strategy of not

necessarily employing molecular assays for the detection of *N. gonorrhoeae* is based on bibliography which suggests that nucleic acid methods are not appreciably better than the results obtained with a proficient specimen transport and culture system.[23]

In this study, the presence of *M. genitalium* infection was associated with the presence of Mycoplasmataceae family members (*M. hominis* and *Ureaplasma* spp.) while 40% of *M. genitalium* detections pertained to co-infection with only *Ureaplasma* spp. This suggests a possibility of common risk factors or a susceptibility link, and demands further studies to elucidate whether a first acquired and probably often untreated STI may determine or affect a subsequent occurring sequence of infections. Thus, it needs to be determined whether certain sequential infections behave as an innocent bystander or as in a "quorum sensing like" manner, with particular infectious agents taking center stage when present in co-infections. In that respect, *Ureaplasma* spp. should be characterized at the species and serovar level with (multiplex) real time PCR methods which are serovar specific and determine *U. parvum*-biovar 1 corresponding to serovars 1, 3, 6, 14, and *U. urealyticum*-biovar 2 corresponding to serovars 2, 4, 5, 7-13. It should be noted that no dual co-infection of *M. genitalium* with *Neisseria gonorrhoeae* or *Chlamydia trachomatis* was observed in this study.

Comprehensive studies appropriately detecting and recording all STI pathogens in individuals at high risk for STDs could accurately and fully correlate STIs with direct microscopy findings and clinical manifestations, providing essential information to assess differential pathogenicity.

An association between antibiotic use and *M. genitalium* infection in this study may indicate prior inappropriate choice of antibiotics, insufficient dosage or treatment duration, but also unavoidably points to increasing concern for emerging resistance to presumably appropriate antibiotics such as doxycycline, azithromycin, moxifloxacin and pristinamycin or pristinamycin combined with doxycycline.[24] This inevitably supports the re-examination and resistance testing at specified post-treatment periods, until confirmation of infection resolution.

Overall in this study, younger age individuals showed greater vulnerability, the majority of *M. genitalium* infected persons were smokers, while multiple sexual partners and condom use did not have a differential effect on isolation frequency. Further studies should be conducted to consolidate all facets of clinical, demographic and social parameters pertaining to STIs and *M. genitalium* in particular.

In conclusion, it is worth considering how far we have come in addressing the diagnostic process for STIs: although a great number of STIs are mono-infections, STIs need to be investigated as a constellation of possible concomitant infections that may accumulate as sequential infections over time, and may be related to sexual practices and sexual networks. *M. genitalium* is an important STI to seek out, especially in NGNCU and PGU cases. It is self-evident that in cases where no satisfactory diagnosis is reached, an expanded range of microbial and viral agents (and here it is proposed that HPV should be included) should be sought by utilizing both molecular and standard microbiological techniques.

Conflicts of interest: GP participated in data acquisition, literature search and laboratory work in the manuscript by Chra P et al.

References

1. Chra P, Papaparaskevas J, Papadogeorgaki E, Panos G, Leontsinidis M, Arsenis G, Tsakris A. Prevalence of *Mycoplasma genitalium* and other sexually-transmitted pathogens among high-risk individuals in Greece. GERMS 2018;8:12-20. [Crossref]
2. World Health Organization. Sexually transmitted infections (STI), Fact sheet. 2016. Accessed on: 23 February 2018. Available at: http://www.who.int/mediacentre/factsheets/fs110/en/
3. Martin D, Bowie W. Urethritis in males. In Holmes KK, et al (Eds). Sexually transmitted diseases (3rd Ed.) 1999:833-44.
4. Lusk MJ, Konecny P. Cervicitis: a review. Curr Opin Infect Dis 2008;21:49-55. [Crossref]

5. Csonka GW, Williams RE, Corse J. T strain *Mycoplasma* in nongonococcal urethritis. Ann N Y Acad Sci 1967;143:794-8. [Crossref]
6. Shah SS. *Mycoplasma hominis*. In: Long S, Pickering L, Prober C (Eds). Principles and Practice of Pediatric Infectious Diseases (Fourth Edition). Saunders; 2012.
7. Paavonen J, Critchlow CW, DeRouen T, et al. Etiology of cervical inflammation. Am J Obstet Gynecol 1986;154:556-64. [Crossref]
8. McGalie CE, McBride HA, McCluggage WG. Cytomegalovirus infection of the cervix: morphological observations in five cases of a possibly under-recognised condition. J Clin Pathol 2004;57:691-4. [Crossref]
9. Moi H, Blee K, Horner PJ. Management of non-gonococcal urethritis. BMC Infect Dis 2015;15:294. [Crossref]
10. Mclean KA, Evans BA, Lim JM, Azadin BS. Postgonococcal urethritis: a double-blind study of doxycycline vs placebo. Genitourin Med 1990;66:20-3. [Crossref]
11. Taylor-Robinson D, Jensen JS. *Mycoplasma genitalium*: from Chrysalis to multicolored butterfly. Clin Microbiol Rev 2011;24:498-514. [Crossref]
12. Daley GM, Russell DB, Tabrizi SN, McBride J. *Mycoplasma genitalium*: a review. Int J STD AIDS 2014;25:475-87. [Crossref]
13. Dolapci I, Tekeli A, Ozsan M, Yaman O, Ergin S, Elhan A. Detecting of *Mycoplasma genitalium* in male patients with urethritis symptoms in Turkey by polymerase chain reaction. Saudi Med J 2005;26:64-8.
14. Anagrius C, Loré B, Jensen JS. *Mycoplasma genitalium*: prevalence, clinical significance, and transmission. Sex Transm Infect 2005;81:458-62. [Crossref]
15. Andersen B, Sokolowski I, Østergaard L, Kjølseth Møller J, Olesen F, Jensen JS. *Mycoplasma genitalium*: prevalence and behavioural risk factors in the general population. Sex Transm Infect 2007;83:237-41. [Crossref]
16. Casin I, Vexiau-Robert D, De La Salmonière P, Eche A, Grandry B, Janier M. High prevalence of *Mycoplasma genitalium* in the lower genitourinary tract of women attending a sexually transmitted disease clinic in Paris, France. Sex Transm Dis 2002;29:353-9. [Crossref]
17. Ikonomidis A. Venetis C, Georgantzis D, et al. Prevalence of *Chlamydia trachomatis*, *Ureaplasma* spp., *Mycoplasma genitalium* and *Mycoplasma hominis* among outpatients in central Greece: absence of tetracycline resistance gene *tet*(M) over a 4-year period study. New Microbes New Infect 2015;9:8-10. [Crossref]
18. Pereyre S, Laurier Nadalié C, Bébéar C, investigator group. *Mycoplasma genitalium* and *Trichomonas vaginalis* in France: a point prevalence study in people screened for sexually transmitted diseases. Clin Microbiol Infect 2017;23:122.e1-7. [Crossref]
19. Le Roy C, Pereyre S, Hénin N, Bébéar C. French prospective clinical evaluation of the Aptima *Mycoplasma genitalium* CE-IVD assay and macrolide resistance detection using three distinct assays. J Clin Microbiol 2017;55:3194-200. [Crossref]
20. Gottesman T, Yossepowitch O, Samra Z, Rosenberg S, Dan M. Prevalence of *Mycoplasma genitalium* in men with urethritis and in high risk asymptomatic males in Tel Aviv: a prospective study. Int J STD AIDS 2017;28:127-32. [Crossref]
21. Manhart LE, Holmes KK, Hughes JP, Houston LS, Totten PA. *Mycoplasma genitalium* among young adults in the United States: an emerging sexually transmitted infection. Am J Public Health 2007;97:1118-1125. [Crossref]
22. Jensen JS, Cusini M, Gomberg M, Moi H. 2016 European guideline on *Mycoplasma genitalium* infections. J Eur Acad Dermatol Venereol 2016;30:1650-6. [Crossref]
23. Ng L, Martin IE. The laboratory diagnosis of *Neisseria gonorrhoeae*. Can J Infect Dis Med Microbiol 2005;16:15-25. [Crossref]
24. Read TRH, Jensen JS, Fairley CK, et al. Use of pristinamycin for macrolide-resistant *Mycoplasma genitalium* infection. Emerg Infect Dis 2018;24:328-35. [Crossref]

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