CITY OF MILWAUKEE
HEALTH DEPARTMENT

Prevalence of *Mycoplasma genitalium* in high risk patients seeking testing at a local Public Health Laboratory

M. Khubbar[1], J. Weiner[1], T. Dasu[1], Z. Amezquita-Montes[1], K. Schieble[1] H. Hermus[1], R. Kryshak[1], J. Dalby[1, 2], S. Bhattacharyya[1]
[1]City of Milwaukee Health Department, [2]School of Medicine and Public Health, University of Wisconsin

Introduction

Mycoplasma genitalium (MG) is a Molicute, fastidious obligate intracellular bacterium and smallest prokaryote capable of self-replication. *M. genitalium*, first identified in 1980s, lives on and in the epithelial cells of the urinary and genital tracts of men and Women. *M. genitalium* is associated with elevated risks of nongonococcal urethritis in men and of cervicitis, pelvic inflammatory disease, preterm birth, infertility, and spontaneous abortion in women. The infection may persist asymptomatic for months or years in individuals. Early diagnosis and effective treatment are, therefore, important in preventing sequelae and ongoing transmission, particularly the transmission of drug-resistant strains to sex partners. Antibiotics targeting cell-wall biosynthesis are ineffective (Penicillins and cephalosporins) as *M. genitalium* lacks cell wall. Current treatment guidelines are inconsistent about the need for presumptive treatment of sexual contacts of *M. genitalium* infected patients, which further complicates as found to be co-infected with commonly known sexually transmitted pathogens.

Due to the generally fastidious requirements for culturing of *Mycoplasma* species in vitro, molecular methods have been the predominant means for detecting *M. genitalium* in clinical specimens. The use of nucleic acid amplification tests (NAATs) employing PCR for detection of *M. genitalium* genomic DNA targets and transcription-mediated amplification (TMA) for detection of *M. genitalium* RNA improves monitoring disease prevalence, co-STI infections, and implement best clinical practices. In this study City of Milwaukee Health Department laboratory (MHDL) determined the prevalence and rate of coinfection of *M. genitalium* (MG) with *C. trachomatis* (CT), *N. gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), and Herpes Simplex Virus (HSV) in local high risk population utilizing only FDA cleared in-vitro diagnostic (IVD) NAAT assay in United States for the detection of ribosomal RNA (23S rRNA) from M. genitalium on Hologic, Inc. Panther system.

Methods

In 2018, Aptima® analyte specific reagents (ASR) validation for MG NAAT involved 219 residual clinical specimens (117 females and 102 males) those previously tested for chlamydia, gonorrhea, trichomonas and/or herpes simplex virus on the Panther® System (Hologic, Inc. Marlborough, MA). A variety of sample types (urine, urethral, vaginal, rectal) and specimen transport tubes (Aptima® Urine, Unisex and Multitest Specimen Transport Tube; MicroTest™ M4 Transport Tube) were validated in this process. Subsequent Aptima® FDA-cleared IVD assay verification in beginning of 2019 involved the use of 81 pre-qualified specimens (45 clinical and 36 Validation Panel samples through Hologic, Inc.).

In this study clinical specimens received between February 2019 till August 2020 by STD clinic laboratory were also analyzed using the Panther system and the FDA-cleared Aptima Combo 2 and Aptima *Trichomonas vaginalis* and *M. genitalium* assays (Hologic), according to the assay package inserts. Swab and urine samples were collected into Aptima swab specimen and urine specimen transport tubes, respectively (Hologic), and either were tested directly or were stored frozen at –70°C and tested at later dates.

Specimens were obtained from symptomatic (cervicitis, vaginitis, or urethritis for females or urethritis for males) and asymptomatic patients seen at sexually transmitted disease clinic. A total of 2,063 subjects were tested between February 2019 till August 2020. The majority of female and male subjects were of younger age (<30 years), black race, and non-Hispanic ethnicity. Prevalence and co-infection of MG with other sexually transmitted organisms (CT, GC, TV, and HSV) was determined.

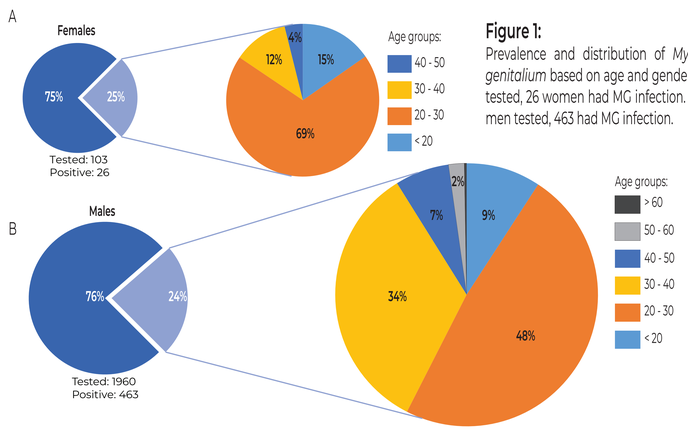

Figure 1:
Prevalence and distribution of *Mycoplasma genitalium* based on age and gender. A. Of 103 tested, 26 women had MG infection. B. Of 1960 men tested, 463 had MG infection.

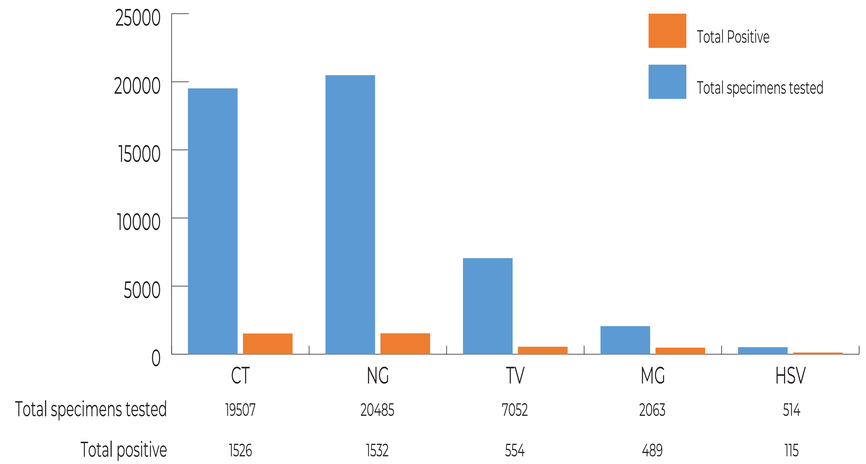

Figure 2:
Prevalence of sexually transmitted organisms among patients tested for a sexually transmitted infection. TV, *Trichomonas vaginalis*; MG, *Mycoplasma genitalium*; CT, *Chlamydia trachomatis*; GC, *Neisseria gonorrhoeae*; HSV (1 &2), Herpes Simplex Virus.

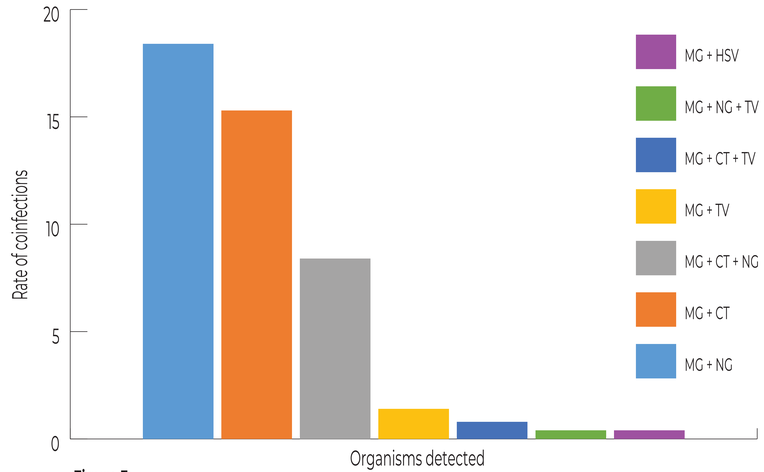

Figure 3:
Rate of coinfection in *Mycoplasma genitalium* positive patients with other sexually transmitted organisms. TV, *Trichomonas vaginalis*; MG, *Mycoplasma genitalium*; CT, *Chlamydia trachomatis*; GC, *Neisseria gonorrhoeae*; HSV, Herpes Simplex Virus 1 & 2.

Results

Clinical validation of *Mycoplasma* Transcription Mediated Assay (TMA) utilizing Analyte Specific Reagents (ASR) provided by Hologic, Inc.

Performed in-house for use as Laboratory Developed Test at the end of 2018. 219 clinical samples that were tested with the Hologic Aptima MG assay at a partner facility on the Panther System were utilized at MHDL for comparison study. There was an overall 100% agreement between expected results and results obtained, and 100% reproducibility with the Aptima *Mycoplasma genitalium* assay. The following specimen types are validated by MHDL: Urine, Urethral swab (clinician collected), Vaginal swab (clinician collected), and Rectal swab (clinician collected). The following specimen transport media are validated by MHDL: Aptima® Urine Specimen Transport Tube, Aptima® Multitest Swab Specimen Transport Tube, Aptima® Unisex Specimen Transport Tube, and MicroTest™ M4 Transport Tube.

Verification of Aptima *Mycoplasma genitalium* assay (IVD), Hologic, Inc.

On January 23, 2019 FDA granted clearance for Hologic's Aptima *Mycoplasma genitalium* IVD assay. There was an overall 97% agreement (N= 81) between expected results and results obtained, and 100% reproducibility with the IVD-labeled Aptima® *M. genitalium* assay.

Prevalence of *M. genitalium* infections

M. genitalium prevalence rates were 24.5% for females (N=103) and 23.6% for males (N= 1960). *M. genitalium* infections were significantly more prevalent in females of ≤30 years of age (69%) and males ≤40 years of age (82%). Rates of detection of *M. genitalium* and other sexually transmitted organisms *C. trachomatis, N. gonorrhoeae, T. vaginalis*, and Herpes Simplex Virus for both female and male patients are presented in Figure 2. *M. genitalium* prevalence rate of 23.75% along with genital Herpes of 22.37% was significantly higher than the prevalence rates for *C. trachomatis* (7.8%), *N. gonorrhoeae* (7.48%), and *T. vaginalis* (7.85%).

Rates of *M. genitalium* single infections and coinfections with *C. trachomatis, N. gonorrhoeae, T. vaginalis*, and Herpes Simplex Virus

Single *M. genitalium* infections represented 55.1% of all *M. genitalium* infections in high-risk population tested at local STD clinic. *M. genitalium* presented in dual infections with *N. gonorrhoeae* in 18.4%, *C. trachomatis* in 15.3%, and both CT & NG in 8.4% of patients. Rate of coinfection with *Trichomonas* and genital Herpes was less than 2%.

Overall 45% of the MG-positive patients presented with at least one laboratory confirmed STI co-infection (chlamydia, gonorrhea, trichomonas, and herpes).

Observations:

- *M. genitalium* is COMMON in females and males in local high-risk population.
- Women are commonly asymptomatic.
- Coinfection with other sexually transmitted organisms is seen particularly with *N. gonorrhoeae* and *C. trachomatis* or both.
- NAAT is more sensitive and specific method as compared to culture that takes up to 6 months.
- Hologic, Inc. Aptima *Mycoplasma genitalium* assay is THE only FDA cleared assay available in United States.
- Availability of accurate and sensitive diagnostic test will not only aid in diagnosis but also physicians will be able to treat their patients appropriately in timely manner.

Conclusion:

Prescription practices by the STI physicians has been optimized based on this data by treating non-specific urethritis (NSU) with doxycycline initially. *M. genitalium*, once identified, is treated with a high-dose Azithromycin regimen following doxycyline and recurring or persistent infections with moxifloxacin. Major achievement in public health practice is adequate treatment for high-risk STI public health clinic population. Our laboratory and STI clinic are exploring impact of macrolide resistance testing on jurisdictional patient cohort to help understand the local rate of macrolide resistance in *Mycoplasma genitalium*.

Future direction:

- To prevent spread and emergence of antibiotic resistant strains wide screening particularly partner testing using NAAT must be considered.
- There is a need for molecular methods to predict the antibiotic resistant to macrolides and fluoroquinolones.

Acknowledgement:

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