Mycoplasma genitalium Testing Pattern and Macrolide Resistance: A Danish Nationwide Retrospective Survey

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Background. Mycoplasma genitalium is a common cause of nongonococcal urethritis (NGU) and cervicitis. The aim of the study was to analyze the M. genitalium testing pattern and distribution of positive results according to sex and age in a 5-year period where all diagnostic M. genitalium testing in Denmark was centralized at the Statens Serum Institut. A secondary aim was to estimate the occurrence of macrolide resistance in a 3-year period.

Methods. The study was performed as a nationwide retrospective survey of specimens submitted from general practice, private specialists, and hospitals to Statens Serum Institut for detection of M. genitalium by polymerase chain reaction between 1 January 2006 and 31 December 2010. Macrolide resistance screening was introduced December 2007.

Results. A total of 31,600 specimens from 28,958 patients were tested for M. genitalium, with an increasing trend from 3858 per year in 2006 to 7361 in 2010. The majority (54%) of the patients were tested in general practice. For both sexes, the positive rate increased significantly, from 2.4% to 3.8% for women and from 7.9% to 10.3% for men (P < .0005). Macrolide resistance was detected in 38% (385/1008) of the M. genitalium–positive patients, and the highest rate was found in patients tested at sexually transmitted disease clinics (43%).

Conclusions. Testing for M. genitalium has become important for clinicians treating sexually transmitted infections. In this nationwide survey, macrolide resistance was found in almost 40% of the specimens, raising concern about single-dose azithromycin treatment of NGU, and emphasizing that NGU treatment should be guided by etiologic diagnosis.

Keywords. Mycoplasma genitalium; prevalence; macrolide resistance.

Mycoplasma genitalium is an established cause of sexually transmitted urethritis and cervicitis [1, 2] and may also cause pelvic inflammatory disease [3–6] and infertility in women [7, 8]. In the general population, the prevalence of M. genitalium ranges from 1.1% to 3.3% [9, 10], and among men with symptomatic nonchlamydial, nongonococcal urethritis (NGU), the prevalence ranges from 15% to 35% [2]. Similar to infection with Chlamydia trachomatis, women with M. genitalium are often asymptomatic [11, 12]. Mycoplasma genitalium, like other mycoplasmas, lacks a cell wall, making it inherently resistant to β-lactam antibiotics. In vitro studies suggested that antibiotics of the tetracycline class were active, but treatment trials have shown that doxycycline is inefficient in eradicating the infection [13–15]. Single-dose azithromycin therapy with 1 g is widely used, but is slightly less effective than the extended regimen, as demonstrated in a Scandinavian study, in which single 1-g dose azithromycin cured only 80%–85% of the infections whereas azithromycin at an extended dosage of 500 mg on day 1 followed by 250 mg daily on days 2–5 eradicated the bacterium from 96% of the infected men [14]. Some strains of M. genitalium have developed resistance to azithromycin through mutations in region V of the
23S ribosomal RNA (rRNA) gene, possibly as a result of inappropriate dosage of azithromycin [16]. These findings suggest that the use of single 1-g dose azithromycin treatment of NGU of unknown etiology is problematic [16]. A recent study confirmed that single 1-g dose azithromycin was associated with selection of macrolide-resistant _M. genitalium_ [17], and an Australian study proposed that treatment failure was associated with a higher load of _M. genitalium_ [18]. Currently, moxifloxacin is the most commonly used second-line treatment in patients experiencing azithromycin failure [19, 20], but the use of moxifloxacin is limited by the high cost of the drug, side effects, and the risk of selection of resistant strains [21, 22].

In Denmark, single-dose (1 g) azithromycin or doxycycline 100 mg twice daily for 7 days is routinely given for treatment of NGU, whereas laboratory-confirmed _M. genitalium_ infection is treated with azithromycin 500 mg on day 1, followed by 250 mg daily on days 2–5. Moxifloxacin 400 mg once daily for 7 days is used as a second-line regimen in the case of macrolide resistance. Nucleic acid amplification testing is currently the only diagnostic method available for detection of _M. genitalium_, and no Food and Drug Administration (FDA)–approved assays are presently available [23]. In Denmark, polymerase chain reaction (PCR) for _M. genitalium_ has been centralized at the Statens Serum Institut, where it has been available since 2003. Since 2007, all _M. genitalium_–positive specimens have been tested for macrolide resistance–mediating mutations in region V of the 23S rRNA gene.

The aim of the study was to analyze the _M. genitalium_ testing pattern and distribution of positive results according to sex, age and healthcare setting in a 5-year period, and to estimate the occurrence of macrolide resistance in specimens in a 3-year period since the introduction of routine screening for resistance.

**METHODS**

**Patients and Specimens**

The study was performed as a retrospective survey of specimens submitted to the Statens Serum Institut, Denmark, for detection of _M. genitalium_ by PCR. During the study period, _M. genitalium_ testing was centralized in Denmark at the Statens Serum Institut. Between 1 January 2006 and 31 December 2010, a total of 31 600 specimens were received from general practitioners, private specialists, and hospitals in Denmark. A number of patients had >1 specimen taken at the same date (eg, from cervix and urethra). In the analysis, only 1 specimen from each patient from a specific date was included; consequently, each case of _M. genitalium_ infection was only represented once. In the case of discrepancy between test results, we included the positive specimen. The classification into general practitioners, private specialists, and hospitals was based on the registration number of the healthcare provider, which is assigned centrally.

Results from specimens submitted from other countries were removed on the basis of the address of the physician requesting the test. Specimens consisted primarily of urogenital swabs and first-void urine samples from patients with symptoms or signs of genital tract infection or from asymptomatic women screened for chlamydia. All specimens were labeled with the central person registry (CPR) number providing information about age and sex of the patient.

**Laboratory Analysis**

DNA was released by boiling with Chelex 100 resin in TE buffer. _Mycoplasma genitalium_ was detected by real-time hydrolysis probe PCR targeting the _mgB_ gene (MG191) [24]. An internal amplification control was included to control for PCR inhibition. All positive results were confirmed in a second PCR targeting the 16S rRNA gene (until 2007) [25] or region V of the 23S rRNA gene (from 2008).

From 1 December 2007, all _M. genitalium_–positive specimens were tested for macrolide resistance–mediating mutations in region V of the 23S rRNA gene by a rapid pyrosequencing assay using a PyroMark Q96 (Qiagen, Hilden, Germany) sequencing platform. In brief, primers Mg 23S-1992Bio (5’-Biotin-CCATCTCCTTGACTGTCGCGCTAT) and Mg 23S-2138R (5’-CCTACCTATTCTCTAGCGTGGTGTT) were used to amplify a fragment of region V of the _M. genitalium_ 23S rRNA gene. The macrolide resistance–mediating mutations were detected by pyrosequencing using the sequencing primer Mg23S-Pyro 5’-TTAAGGCTTCACGGG (details to be described elsewhere). All diagnostic procedures were performed in a laboratory accredited according to ISO 17025, and specimens were generally processed within 24 hours.

**Statistical Analysis**

The statistical comparisons were done using _χ_² test (2-sided _P_ value). Differences with _P_ < .05 (2-sided) were considered statistically significant (GraphPad Software).

Exemption for review by the ethical committee system and for obtaining informed consent was obtained from the Committee on Biomedical Research Ethics for the Capital Region.

**RESULTS**

A total of 31 600 specimens from 28 958 patients were tested for _M. genitalium_ in the 5-year period. We excluded 2642 specimens from patients who had >1 specimen taken at the same date. Of these specimens, 109 (4%) had discrepant test results (ie, inconclusive, negative, positive). All inconclusive results were excluded, leading to a registration of the patient as negative or positive according to the result of the corresponding specimen. In the event of a negative/positive result, the negative specimen was excluded, leading to a registration of the patient...
as positive. Among the specimens from women with discrepancy between test results, the vast majority (69%) were positive in the cervical specimen and negative in the specimen from urethra or urine.

Seventy percent of the specimens were from women, and this proportion remained constant throughout the study period. The yearly number of patients tested increased from 3858 per year in 2006 to 7361 in 2010 (Figure 1).

**Distribution of Specimens Submitted for *M. genitalium* Testing**

The majority (54%) of the patients were tested by general practitioners and a decreasing trend was observed, from 60% of the patients in 2006 to 46% of the patients in 2010. Of the patients tested by general practitioners, 5.7% had results positive for *M. genitalium*, and 40% of the patients were men. Private specialists, primarily gynecologists, tested 35% of the patients. This group accounted for a major increase, from 25% of the patients in 2006 to 44% of the patients tested in 2010. Of the patients tested by private specialists, 2.0% had results positive for *M. genitalium* and only 4% were men. Hospitals, mainly sexually transmitted disease (STD) clinics, tested 11% of the patients. Between 2006 and 2010, the proportion of patients tested by hospital clinics decreased from 15% to 10%; nonetheless, the positive rate increased from 7.8% to 13.0%. Overall, 10.4% of the patients from hospitals had *M. genitalium*-positive results, and 68% were men.

**Mycoplasma genitalium**–Positive Specimens

Of the patients tested, 1414 were positive for *M. genitalium* (4.9%). The proportion of *M. genitalium*–positive patients was higher for men (9.0%) than for women (3.1%). For both sexes, a significant increase in the proportion of positive patients was seen from 2006–2008 compared to 2009–2010; the positive rate increased from 2.4% to 3.8% for women and from 7.9% to 10.3% for men, respectively ($P < .0005$; Figure 1). The positive rate was highest among the 25- to 30-year-old men, reaching 14.0% (Figure 2). In women, the 20- to 25-year-olds had the highest positive rate, reaching 5.1% (Figure 3).

**Macrolide Resistance–Mediating Mutations in Region V of the 23S rRNA Gene**

Macrolide resistance detection was attempted for 1121 *M. genitalium*-positive specimens from 1044 patients tested beginning 1 December 2007 and could be completed for 1085 (97%) of the specimens from 1008 (97%) of the patients. Resistance was detected in 385 patients (38.2%). Macrolide resistance was found at a significantly lower rate in patients tested by private specialists (30.3%), whereas resistance was detected in 38.7% and 42.6% of the patients tested by general practitioners and hospitals, respectively ($P = .02$). Throughout the study period, macrolide resistance was found at a constant rate of approximately 40%. The most common mutations were A2058G (61%) and A2059G (35%) (*Escherichia coli* numbering) (Figure 4).

**DISCUSSION**

In Denmark, the vast majority of sexually transmitted infections (STIs) are diagnosed and treated in general practice, which explains the fact that most specimens sent for *M. genitalium*...
testing came from this group. Private specialists, primarily gynecologists, submitted an increasing number of specimens over the years, but with a very low positive rate, probably reflecting that many specimens were collected from asymptomatic women as part of screening for *C. trachomatis*. This explains the lower positive rate for women compared with men.

We found the highest positive rate among patients tested at hospitals, mainly STD clinics, and the majority of these patients

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**Figure 2.** Age distribution of Danish men tested for *Mycoplasma genitalium*. White bars: total number of men tested; black bars: number of men testing positive. The age-specific positivity rate among Danish men is indicated by the dotted line.

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**Figure 3.** Age distribution of Danish women tested for *Mycoplasma genitalium*. White bars: total number of women tested; black bars: number of women testing positive. The age-specific positivity rate among Danish women is indicated by the dotted line.
were from men. This is not surprising, as it has been common practice in STD clinics to restrict *M. genitalium* testing to symptomatic patients with negative *C. trachomatis* and *Neisseria gonorrhoeae* tests, whereas physicians in general practice and private specialist are more likely to test for all 3 pathogens at the initial visit. A significant increase in the positive rate in the period 2009-2010 was observed for both sexes. This increase in the proportion of positive specimens did not completely follow the change in confirmatory assay, but this may explain part of it, as the 23S rRNA gene assay had a slightly higher sensitivity than the previously used confirmatory assay based on the 16S rRNA gene [25]. Furthermore, knowledge about *M. genitalium* as a common cause of genital tract infections increased both in general practice and in STD clinics. Increasing numbers of patients with other STIs, such as *C. trachomatis* [26] and syphilis [27], suggest that high-risk sexual risk behavior is increasingly common, both in the Danish general population and among men who have sex with men, and this might lead to more patients testing positive for STIs.

*Mycoplasma genitalium* had a peak positive rate approximately 5 years later than that of *C. trachomatis* in both men and in women [26]. The prevalence of *C. trachomatis* in the Danish population varies significantly with age and sex [26]. In a population-based survey among 21- to 24-year-old women and men, the *C. trachomatis* prevalence was 8.4% in women and 5.6% in men, and the corresponding *M. genitalium* prevalence was 2.3% in women and 1.1% in men [9]. The lower prevalence of *M. genitalium* may reflect the lower proportion of asymptomatic carriage as suggested in other studies [28]. To some extent, the later peak positive rate for *M. genitalium* may be explained by late diagnosis of persistent infections. Long-term infections with the same strain for >3 years have been reported [29], but the rate of spontaneous clearance of *M. genitalium* infections is not well described. Also, it could be speculated that because *M. genitalium* infection generally carries an approximately 100-fold lower load of organisms than what is found in *C. trachomatis* infection [30], it is less contagious, and because it is less common than *C. trachomatis* infection, more partners and sexual encounters would be needed for infection. This is substantiated by the much higher risk of *M. genitalium* infection in women having ≥3 partners without condoms within the last 12 months as compared with the risk of being *C. trachomatis* positive [30] and with the dramatic increase in *M. genitalium* risk in women having >10 lifetime partners [9].

Macrolide resistance was found at a constant rate of approximately 40% throughout the study period, indicating that screening for mutations in region V of the 23S rRNA gene should be part of the diagnostic procedure to guide clinicians to the correct treatment. Comparable rates of macrolide resistance and similar distributions of the most common A2058G and A2059G mutations have been reported recently from both the United Kingdom and Australia [31, 32], where the prevalence of macrolide resistance–associated mutations was 41% and 43%, respectively. From antimicrobial susceptibility testing and clinical experience, no difference in the cure rate
for azithromycin according to the type of mutation would be expected, as both lead to very high-level azithromycin resistance [16]. We found a significantly lower rate of macrolide resistance in patients tested at private specialists, perhaps reflecting that many of these specimens were from asymptomatic women not previously treated for STIs. By contrast, the highest rate of macrolide resistance was found in patients tested at hospitals, mainly STD clinics. Although not documented in this report, we speculate that patients attending STD clinics may have a more risky sexual behavior than the general population and consequently have a higher risk of having had a previous NGU treated with the widely used single 1-g dose of azithromycin. This may have induced selection of azithromycin-resistant M. genitalium strains not detected initially. Widespread macrolide resistance is being increasingly reported; in a recent randomized controlled trial, Manhart et al found that the cure rate after 1 g of azithromycin was unexpectedly low and not significantly different than that of doxycycline [15], probably reflecting preexisting macrolide resistance in the population and induced resistance. In Greenland, where the M. genitalium prevalence is high, a study found that 100% of M. genitalium–positive cases carried macrolide resistance, although the number of samples investigated was relatively low [33].

The prevalence of M. genitalium in the general Danish population is low [9] and does not seem to justify screening of asymptomatic persons. However, several reports suggest selective testing of high-risk populations based on sexual history, where the number of partners (both recently and lifetime) seem to be strong predictors of risk [9, 10, 18]. Our results suggest that the routine testing of patients with symptoms of genital tract infection or patients requesting screening for STDs due to risk behavior should include routine testing for M. genitalium to avoid syndromic management of genital tract infections. However, in settings where access to M. genitalium testing is limited or where testing is limited by high costs, selective testing could be reserved for C. trachomatis–negative patients with genital tract infections and persisting symptoms.

Some limitations to our study must be addressed. First, only a minority of Danish patients are tested for M. genitalium, even in the presence of symptoms. Most physicians test primarily for C. trachomatis, and for comparison, 339 704 C. trachomatis tests were performed in Denmark in 2012 with an overall positive rate of 7.8% [26]. These tests are almost exclusively nucleic acid amplification tests, and are performed at regional public microbiology laboratories. Thus, the population reported here may be selected and the prevalence overestimated. Second, a number of patients had duplicate specimens taken at the same event. We decided to exclude duplicate specimens so that each event of M. genitalium infection was only represented with 1 specimen. Furthermore, we included the positive specimen in the case of discrepancy, although this could potentially overestimate the prevalence compared with settings where only 1 specimen per patient is tested. Third, information about treatment regimen (ie, prior azithromycin use), was lacking due to the retrospective design of the study. However, the study is strengthened by the very large sample size and the centralized testing of M. genitalium in Denmark, including specimens submitted from general practice. Furthermore, the unique CPR number assigned to all individuals in Denmark at birth or upon immigration provided information about sex and age of all patients.

In conclusion, diagnostic testing for M. genitalium has become an important supplement for clinicians treating STIs. Men between 20 and 30 years of age had positive rates >10%, whereas the positive rate for women was much lower, probably reflecting screening of asymptomatic women. Macrolide resistance was found in almost 40% of the positive specimens, emphasizing the need for detection of resistance mutations to guide therapy. We suggest that testing guidelines for STIs are modified to include routine testing for M. genitalium and detection of macrolide resistance–mediating mutations to ensure that treatment of genital tract infections is guided by etiologic diagnosis.

Notes

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Potential conflicts of interest. Both authors: No reported conflicts.

Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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