Mycoplasma genitalium in Toronto, Ont

Estimates of prevalence and macrolide resistance

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Abstract

Objective To estimate the prevalence of *Mycoplasma genitalium* in Toronto, Ont; detect mutations associated with macrolide and fluoroquinolone resistance; and describe treatment outcomes.

Design Prospective, cross-sectional study.

Setting A sexual health clinic in Toronto.

Participants A consecutive sample of men and women attending the sexual health clinic between September 1, 2013, and December 20, 2013.

Interventions Participants underwent testing for *M genitalium*, along with standard sexually transmitted infection screening. All samples that had positive results for *M genitalium* were tested for mutations associated with resistance to macrolides and fluoroquinolones. *Mycoplasma genitalium* treatment was based on resistance profile and verified with a test of cure.

Main outcome measures Positive results for *M genitalium* and antibiotic resistance.

Results A total of 1193 men and women participated in the study. Overall, 4.5% of the 884 men and 3.2% of the 309 women had positive test results for *M genitalium*. Asymptomatic infection was common (52.0%). Macrolide resistance–mediating mutations were found in 58.0% of the *M genitalium* infections. No treatment failure was observed for azithromycin-treated cases. Treatment failure was suspected for 16.7% of cases treated with moxifloxacin.

Conclusion *Mycoplasma genitalium* is present in Canada, with a prevalence comparable to chlamydia and gonorrhea, and has high macrolide and fluoroquinolone resistance.

EDITOR'S KEY POINTS

- The prevalence of *Mycoplasma genitalium* in this study was 4.2% (95% CI 3.2% to 5.5%), which was comparable to the prevalences of *Chlamydia trachomatis* (5.2%, 95% CI 4.0% to 6.6%) and *Neisseria gonorrhoeae* (4.4%, 95% CI 3.3% to 5.7%).
- Resistance to macrolide (58.0%) and fluoroquinolone (20.0%) treatment was high, which suggests this emerging sexually transmitted infection could rapidly become more prevalent and difficult to treat.
- Moxifloxacin treatment failure was observed and the patients treated with moxifloxacin were already carrying macrolide-resistant strains. Tested treatment options for such multidrug-resistant strains are currently lacking. Doxycycline is likely to be effective in less than 30% of these cases, and for the remaining, new treatment options are urgently needed.

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Le Mycoplasma genitalium à Toronto

Estimations de sa prévalence et de sa résistance aux macrolides

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Résumé

Objectif Estimer la prévalence du *Mycoplasma genitalium* à Toronto, Ontario; détecter la présence des mutations associées à une résistance aux macrolides et aux fluoroquinolones; et décrire les résultats des traitements.

Type d'étude Étude prospective transversale.

Contexte Une clinique de santé sexuelle de Toronto.

Participants Un échantillon consécutif d'hommes et de femmes ayant visité la clinique de santé sexuelle entre le 1er septembre 2013 et le 20 décembre 2013.

Interventions Les participants ont subi un test pour le *M genitalium* en plus du dépistage standard pour les infections à transmission sexuelle. Tous les échantillons trouvés positifs pour le *M genitalium* ont été testés pour des mutations associées à une résistance aux macrolides et aux fluoroquinilones. Le traitement du *M genitalium* était basé sur le profil de résistance et vérifié par un test de guérison.

Principaux paramètres à l'étude Les résultats positifs pour le *M genitalium* et la résistance aux antibiotiques.

Résultats Un total de 1193 hommes et femmes ont participé à l'étude. Dans l'ensemble, 4,5% des 884 hommes et 3,2% des 309 femmes ont eu des résultats positifs pour le *M genitalium*. L'infection était souvent asymptomatique (52,0% des cas). Des mutations responsables de la résistance aux macrolides ont été trouvées dans 58,0% des infections au *M genitalium*. On n'a observé aucun échec pour les cas traités à l'azithromycine. Une possibilité d'échec a été observée dans 16,7% des cas traités à la moxifloxacine.

Conclusion Le *Mycoplasma genitalium* est présent au Canada avec une prévalence comparable à celle de la *Chlamydia* et de la gonorrhée; il est doué d'une résistance élevée aux macrolides et aux fluoroquinolones.

POINTS DE REPÈRE DU RÉDACTEUR

- Dans cette étude, la prévalence du *Mycoplasma genitalium* était de 4,3% (IC à 95% 3,2% à 5,5%), ce qui est comparable aux prévalences de la *Chlamydia Trachomatis* (5,2%, IC à 95% 4,0% à 6,6%) et du *Neisseria gonorrheae* (4,4%, IC à 95% 3,3% à 5,7%).

- La résistance aux macrolides et aux fluoroquinolones était élevée (20%), ce qui suggère que la prévalence de cette nouvelle infection à transmission sexuelle risque d'augmenter et d'être difficile à traiter.

- Chez des patients déjà porteurs d'une souche résistante aux macrolides, la moxifloxacine s'est avérée inefficace. Actuellement, il n'existe pas d'option de traitement ayant fait ses preuves pour ces souches résistantes à plusieurs antibiotiques. La doxycycline est susceptible d'être efficace dans moins de 30% de ces cas, et pour le reste, il faudra de toute urgence de nouvelles options de traitement.

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Mycoplasma genitalium is an emerging sexually transmitted infection (STI). The bacterium has been identified as the causative organism of 10% to 25% of all nongonococcal urethritis (NGU) in men and women,[1] and 2% to 4.5% of asymptomatic NGU cases.[2,3] Clinical presentation is similar to *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.[1,2] *Mycoplasma genitalium* has been detected in the urethra, vagina, and rectum, locations that allow infection to spread via vaginal and anal sex.[4]

The complications of *M genitalium* infection are not fully understood, but infection is suspected to have the same sequelae as infection with *C trachomatis*, including pelvic inflammatory disease, infertility, epididymitis, and increased risk of HIV infection.[1,5] Recent treatment effectiveness studies have demonstrated the emergence of drug resistance in *M genitalium* to antibiotics commonly used to treat NGU.[6-8]

The purpose of our study was to estimate the prevalence of *M genitalium* in Toronto, Ont, assess rates of antimicrobial resistance, and describe treatment outcomes to inform clinical practice.

METHODS

Study design

A prospective and consecutive sample of male, female, and transgender clients seeking sexual health services at the Men/Trans Clinic and Women/Trans Clinic at the Hassle Free Clinic in Toronto between September 1, 2013, and December 20, 2013 was included in the study, regardless of age. Clients could opt out of the study if they did not want to participate or if they did not want to receive *M genitalium* testing. Clients who opted out still received standard STI testing. Our target sample size was 1000 participants.

Biological sampling

Participants provided a first-void urine sample for STI testing. We used urine to test for *M genitalium* to reduce differential bias between male and female participants and to minimize clinic work. The test for *M genitalium* was added as a separate test in the panel. Biological samples were transported to the Public Health Ontario Laboratory with existing daily shipments for laboratory analysis.

Laboratory analysis

A sample of 1800 µL of urine was centrifuged at 20000 *g* for 15 minutes, and the pellet was resuspended in 300 µL of 20% (weight/volume) ion exchange resin (Chelex 100) slurry in Tris-EDTA buffer. The mixture was vortexed for 60 seconds and incubated at 95°C for 10 minutes. After centrifugation at 20000 *g* for 5 minutes, 5 µL of the supernatant was analyzed using real-time polymerase chain reaction (PCR) targeting the gene *MgPa*.[9] Proficiency testing was provided by the Statens Serum Institut in Copenhagen, Denmark, to confirm the test performance within the Public Health Ontario Laboratory. Confirmation of positive samples was performed by real-time PCR using an *M genitalium*–specific 23S target developed at the Public Health Ontario Laboratory.[9] Samples were considered indeterminate if they had a negative confirmatory PCR result.

All specimens with positive test results for *M genitalium* were analyzed for macrolide resistance–mediating mutations in region V on the 23S rRNA gene by PCR assay,[10] and for mutations associated with fluoroquinolone resistance in *parC* and *gyrA*.[6]

Specimen processing occurred 2 to 5 times per week at the Public Health Ontario Laboratory, depending on sample submission volume, with the test results being communicated back to the clinic within approximately 24 hours. All participants who had positive test results for *M genitalium* were contacted for appropriate follow-up and treatment. Sexual contacts of those who had positive results were tested and treated as necessary.

Treatment of *M genitalium*

Azithromycin (500 mg on day 1, followed by 250 mg per day for 4 days) was the first-line treatment recommended for those with positive results for *M genitalium*. Cases identified as carriers of a strain with a macrolide resistance–mediating mutation were treated with moxifloxacin (400 mg daily for 7 days). Patients were asked to return to the clinic 2 to 4 weeks after treatment to undergo a test of cure (TOC). A repeat positive *M genitalium* PCR test result at the TOC was considered a treatment failure, and moxifloxacin was used to treat these infections even if no azithromycin mutations were detected.

We documented and monitored treatment outcomes to develop best-practice recommendations for uncomplicated (no co-infection, no antibiotic resistance) and complicated (co-infection or antibiotic resistance) *M genitalium* infection. The University of Toronto Research Ethics Board approved this study.

RESULTS

A total of 1193 clinic attendees participated in the study. Participants ranged in age from 19 to 57 years (mean 33 years) and most were men (74.1%).

Prevalence of *M genitalium*

The overall prevalence of *M genitalium* was 4.2% (95% CI 3.2% to 5.5%; **Table 1**). The prevalence of *M genitalium* was higher for men (4.5%, 95% CI 3.3% to 6.1%) than women (3.2%, 95% CI 1.6% to 5.9%); however, it was not significantly

higher (*P*=.33). The prevalence of *C trachomatis* was 5.2% (95% CI 4.0% to 6.6%) and the prevalence of *N gonorrhoeae* was 4.4% (95% CI 3.3% to 5.7%). The *M genitalium* co-infection rate was 12.0% (**Table 1**). Half of the men and 60.0% of the women infected with *M genitalium* reported no symptoms. Most of the asymptomatic infections were identified during routine STI testing for men and routine STI testing and physical examinations for women.

Macrolide and fluoroquinolone resistance

Macrolide resistance–mediating mutations were detected in 29 of the 50 patients (58.0%) with positive results for *M genitalium* (included mutations A2058G and A2059G). Among men, 25 (62.5%) carried macrolide-resistant strains and 13 (52.0%) were symptomatic. Among women, 4 (40.0%) carried macrolide-resistant strains and 3 (75.0%) were symptomatic. Ten individuals (20.0%) harboured strains with the *parC* mutations previously associated with resistance to moxifloxacin[6] (G248T and G259A). No mutations in *gyrA* were identified.

Treatment outcomes

All 50 participants who had positive test results for *M genitalium* were offered treatment, of whom 43 accepted (**Figure 1**). Seven declined treatment of *M genitalium* or indicated they would seek treatment with another doctor.

Following standard practice, all symptomatic NGU cases were treated with doxycycline at the time of presentation (**Figure 1**). Symptomatic NGU patients with positive test results for *M genitalium* were subsequently switched to either azithromycin or moxifloxacin depending on their macrolide-resistance profile. Eleven men were treated with doxycycline owing to symptoms. Three of these men (27.3%) were successfully treated with doxycycline, had negative TOC results for *M genitalium,* and did not require further treatment. The remaining 8 men had positive TOC results for *M genitalium* and were switched to appropriate *M genitalium* treatment (either moxifloxacin or azithromycin). No women were given doxycycline as an initial treatment, and all were treated with azithromycin from the outset.

Azithromycin (total dose of 1.5 g) was administered to 12 men and 6 women (**Figure 1**). Overall, 15 of the 18 patients treated with azithromycin had negative TOC results for *M genitalium* and 3 were lost to follow-up.

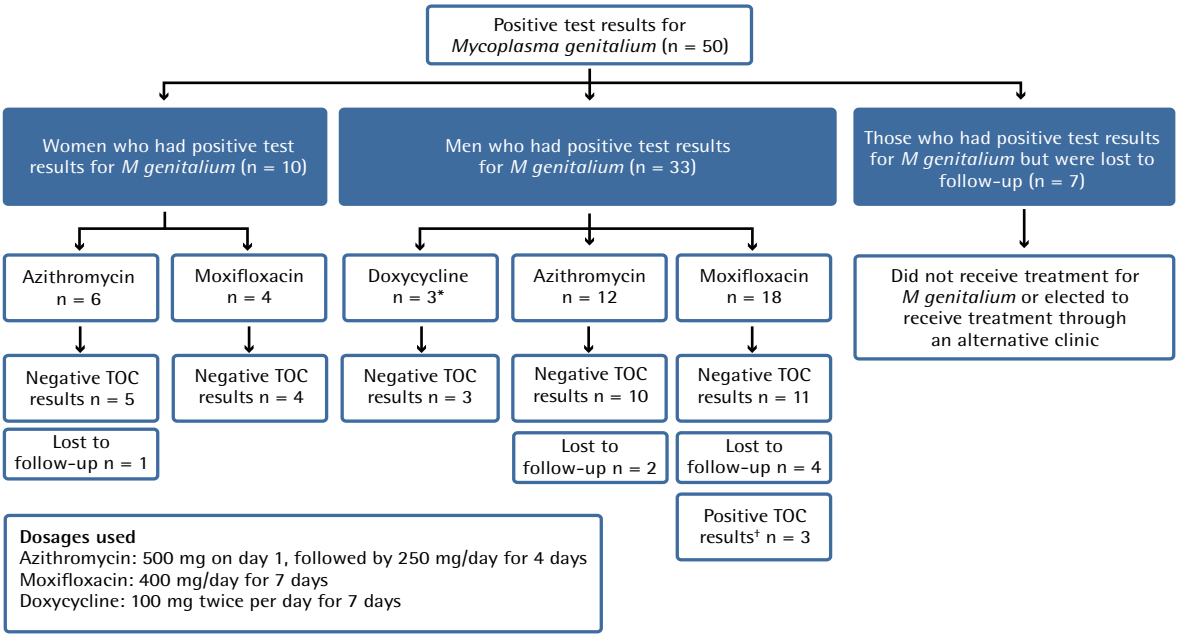
Moxifloxacin was administered to 18 men and 4 women (**Figure 1**). Four were lost to follow-up and 18 underwent TOCs. Of these, 11 (61.1%) had negative TOC results for *M genitalium* and 3 (16.7%) had positive TOC results for *M genitalium*, after the recommended 2- to 4-week wait period and self-reported abstinence from sexual intercourse, although these results would be more certain if they had waited 4 to 6 weeks after treatment. Of the 3 patients with positive TOC results, 2 continued to have positive test results with no evidence of clearing the infection.

Three co-infected patients were successfully treated with moxifloxacin and 1 co-infected patient was

Table 1. Estimated *Mycoplasma genitalium* prevalence, including key characteristics and co-infections

CHARACTERISTIC	N	PREVALENCE, % (95% CI)
All participants (n = 1193)		
Positive test results for *Mycoplasma genitalium*	50	4.2 (3.2 to 5.5)
Positive test results for *Chlamydia trachomatis*	62	5.2 (4.0 to 6.6)
Positive test results for *Neisseria gonorrhoeae*	52	4.4 (3.3 to 5.7)
Men/Trans Clinic (n = 884)		
Indeterminate	2	0.2 (0.0 to 0.8)
Positive test results for *M genitalium*	40	4.5 (3.3 to 6.1)
• Symptomatic	20	50.0 (33.8 to 66.2)
• Macrolide resistance	25	62.4 (45.8 to 77.3)
Co-infections		
• *M genitalium*, *C trachomatis*, and *N gonorrhoeae*	1	0.1 (0.0 to 0.6)
• *M genitalium* and *N gonorrhoeae*	4	0.5 (0.1 to 1.2)
• *C trachomatis* and *N gonorrhoeae*	11	1.2 (0.6 to 2.2)
Women/Trans Clinic (n = 309)		
Indeterminate	3	1.0 (0.2 to 2.8)
Positive test results for *M genitalium*	10	3.2 (1.6 to 5.9)
• Symptomatic	4	40.0 (12.2 to 73.8)
• Macrolide resistance	4	40.0 (12.2 to 73.8)
Co-infections		
• *M genitalium* and *C trachomatis*	1	0.3 (0.0 to 1.8)

Figure 1. Flowchart of the treatment outcomes of patients with positive test results for *Mycoplasma genitalium* in Toronto, Ont, in 2013



TOC—test of cure.
*A total of 11 patients initially received doxycycline based on symptoms, and were subsequently switched to either azithromycin or moxifloxacin, with the exception of 3 who had negative TOC results after treatment with doxycycline.
†There were 3 potential treatment failures in those taking moxifloxacin (2 had resolved symptoms).

successfully treated with doxycycline. Two co-infected patients were lost to follow-up.

DISCUSSION

Mycoplasma genitalium is present in Toronto. The prevalence of *M genitalium* was comparable to the prevalence of *C trachomatis* and *N gonorrhoeae*, although our prevalence is likely underestimated, as the relative sensitivity of *M genitalium* detection is only 61% for urine samples.[4] Resistance to macrolide (58.0%) and fluoroquinolone (20.0%) treatment was high and comparable to the resistance observed in other studies.[7]

Macrolide and fluoroquinolone resistance poses an important clinical challenge to treating both *M genitalium* and NGU. Azithromycin (1 g or extended dose [500 mg on day 1, followed by 250 mg per day for 4 days]) has been the preferred first-line treatment of *M genitalium,* and moxifloxacin has been recommended as a second-line treatment when treatment failures are observed.[11] Doxycycline is not a viable first-line option, given rates of clinical cure as low as 17.1%.[8]

Currently, the *Canadian Guidelines on Sexually Transmitted Infections* recommend treating NGU with 1 week of doxycycline (100 mg twice a day) or a single dose of azithromycin (1 g).[12] This treatment schedule might not be sufficient to eradicate *M genitalium* NGU, leading to treatment failure, persistent NGU, and potential development of macrolide resistance.

Mycoplasma genitalium infection is a common cause of persistent NGU, especially if doxycycline or azithromycin treatment failure is observed. In this situation, moxifloxacin should be considered, in particular if azithromycin was used as the initial treatment. Given the high level of macrolide resistance in this population, second-line treatment with azithromycin after doxycycline might not be an optimal solution unless macrolide susceptibility has been documented.

Moxifloxacin treatment failure was observed in our study and the patients treated with moxifloxacin were already carrying macrolide-resistant strains. Tested treatment options for such multidrug-resistant strains are currently lacking. Doxycycline is likely to be effective in less than 30% of these cases, and for the remaining, new treatment options are urgently needed. Fluoroquinolone resistance and failure after moxifloxacin treatment has been reported recently[7] and seems to be increasing. The rise of *M genitalium* resistance to commonly used therapies in the treatment of NGU emphasizes the need to specifically identify *M genitalium* and treat it appropriately to avoid perpetuating resistance.

Conclusion

Our study provides evidence that *M genitalium* is present in Canada. The prevalence of *M genitalium* will be lower in the general population than it is for the high-risk population in this study. At the same time, prevalence comparable to *C trachomatis* and *N gonorrhoeae* and high resistance to first-line treatment recommendations suggest this emerging STI could rapidly become more prevalent and difficult to treat. These results underscore the need to better care for and educate patients in transmission prevention. Future studies should collect information on epidemiologic risk factors and co-infection status to assist with this endeavour.

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Contributors

Dr Gesink contributed to the conception of the work; all authors contributed to the design of the work. **Dr Allen**, **Mr Mitterni**, **Dr Juzkiw**, **Ms Jamieson**, **Ms Racey**, **Ms Seah**, **Dr Zittermann**, **Mr Singh**, and **Dr Jensen** contributed to the acquisition of the data for the work. **Dr Allen** and **Ms Racey** contributed to the analysis of the data; all authors contributed to the interpretation of the results; and all authors were involved in drafting, critically reviewing, revising, and approving the manuscript. All authors are accountable for all aspects of the work, its accuracy, and its integrity.

Competing interests

None declared

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