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Prevalence and Antibiotic Resistance of *Mycoplasma genitalium* among STI Clinic Attendees in Western Canada: a Cross-Sectional Analysis

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Abstract

Objectives: To determine the prevalence and correlates of *M. genitalium* (MG) infection, compare test performance of female specimen types, and determine the prevalence of gene mutations conferring resistance.

Methods: A cross sectional study was conducted on specimens collected for gonorrhea (NG) and Chlamydia (CT) among Alberta STI Clinic attendees using the *M. genitalium* Transcription Mediated Amplification – Research Use Only (RUO) test (Hologic Inc, San Diego, CA). Female endocervical and urine specimens were compared. Positive specimens were sequenced for 23SrRNA, *parC* and *gyrA* genes. Gender-stratified analysis compared test results using Chi-square or Fisher's exact test, Mann-Whitney test, and logistic regression.

Results: A total of 2,254 individuals were tested; 53.8% (n=1,212) were male. Male prevalence of MG was 5.3%; CT was 5.9% and NG was 1.8%. Correlates of male infection were an NGU diagnosis and NG co-infection. MG prevalence for females was 7.2%; CT was 5.8% and NG was 1.8%. Correlates of female infection were younger age, Indigenous/other ethnicity and CT/NG co-infection. There was high concordance (98.1%) of results between urine and cervical swabs. Nearly two-thirds of eligible specimens had mutations associated with macrolide resistance and 12.2% of specimens had a *parC* mutation signifying fluoroquinolone (FQ) resistance.

Conclusions: The high prevalence of MG relative to CT and NG supports the incorporation of MG testing into routine STI screening and the good concordance of results between urine and cervical swabs supports the use of female urine specimens for testing. The high rate of resistance to macrolides and FQ raises concerns about treatment options.

Key Words: *Mycoplasma*, Clinical STI Care, Epidemiology (Clinical)

ARTICLE SUMMARY

Strengths and Limitations of this study

- This study is among the largest of global studies examining *Mycoplasma genitalium* prevalence in heterosexual males, men who have sex with men and females attending STI clinics.
- We tested for resistance genes to macrolides as well as fluorquinolones.
- We compared the performance of female urine specimens with endocervical swabs.
- Our study is the first to examine *Mycoplasma genitalium* prevalence by ethnicity.
- Although the specimens were collected prospectively, we were only able to collect a limited number of additional variables in addition to standard data collection at the clinics due to time constraints; this may have limited our ability to identify additional correlates of *Mycoplasma genitalium*.

BACKGROUND

Mycoplasma genitalium (MG) is an emerging sexually transmissible infection (STI) caused by bacteria belonging to the *Mollicutes* class that lack a cell wall.[1] In males, it has been implicated as an etiologic agent of non-gonococcal urethritis (NGU) and persistent or recurrent urethritis.[1] In females, available evidence suggests that MG infection is significantly associated with an increased risk of cervicitis, pelvic inflammatory disease (PID), preterm birth, spontaneous abortion and risk of infertility is also increased.[2] Studies suggest that PID cases associated with MG may be similar to *Chlamydia trachomatis* (CT) in terms of severity of symptoms and signs.[3] A two fold increased odds of HIV among populations with MG has also been reported.[4]

Globally, the prevalence of MG using molecular diagnostic tests ranges from 1-4% in men and 1-6% in women but is higher in those at risk for STI.[5] In a recent Eastern Canadian study, male prevalence was 4.5% and prevalence in females was 3.2%.[6]

In Canada, access to testing for MG is currently largely limited to the referral of suitable specimens to the National Microbiology Laboratory (NML) in Winnipeg. Azithromycin has been recommended for treatment of MG but rising resistance has raised concerns about the use of this drug as the preferred option.[7] Alternate treatment with moxifloxacin has been proposed but the high cost of this medication, the potential for hepatotoxicity and reports of resistance have also raised concerns.[8]

Given the anticipated wider availability of test kits to screen for MG in the future, we sought to determine the prevalence and correlates of MG infection in urogenital specimens from attendees at two Alberta STI clinics, to compare the test performance in different types of urogenital specimens from females, and to determine the prevalence of mutations in genes conferring resistance to macrolides and fluoroquinolones (FQ).

METHODS

Specimens collected from January to April, 2016 for NG and CT screening from urogenital sites among sequential male and female attendees (>17 years old) at two Alberta STI Clinics were tested for MG. Inclusion in the study required that at least two months had elapsed since being treated for NG or CT to reduce chance visit was related to test of cure from previous infection, and screening could not be part of patient follow-up if named as a sexual contact to a NG/CT case to remove patients more likely to test positive. All individuals attending the two STI clinics were screened for NG and CT unless they specifically declined: all men were screened using urine tests while women were either screened with urine tests (asymptomatic, no speculum examination performed) or with an endocervical or vaginal swab (the presence or complaint of vaginal discharge, odor, or itching and speculum examination performed).

Basic demographic and clinical information was collected on the laboratory requisition form and included ethnicity (Caucasian, Indigenous, or Other), presence of symptoms (yes/no; for females, symptoms were defined as the presence or complaint of vaginal discharge, odor, or itching and for males, urethral discharge or dysuria), diagnosis at the time of visit for those

undergoing physical examination (NGU, mucopurulent cervicitis (MPC), bacterial vaginosis (BV)/yeast/trichomoniasis (TV) from wet mount) and self-reported HIV status at the time of the patient visit. For male visits only, the gender of the sexual partner was recorded.

The *M. genitalium* Transcription Mediated Amplification – Research Use Only *(*Hologic Inc, San Diego, CA) test was used to screen endocervical, vaginal and urine specimens. Endocervical swabs are currently collected in preference to vaginal swabs in our STI clinics. For a female sub-population, test results from endocervical and urine specimens collected on the same individuals at the same visit were compared and the proportion of concordant results was calculated. The Hologic Aptima Combo 2 assay was used to test for CT and NG. For men, NGU was diagnosed if upon physical examination the Registered Nurse (RN) found urethral discharge +/- dysuria plus urethral smear with >5 polymorphonuclear leukocytes/ high power field in 5 or more fields with subsequent negative CT and NG test results. For women, BV, yeast, and TV were diagnosed and treated at the time of visit based on clinical criteria (patient complaint of abnormal vaginal discharge or RN assessment of abnormal vaginal discharge) for wet mount assessment. The number of wet mounts completed was not collected and therefore we used the number of individuals who had a pelvic examination as the denominator for prevalence of BV, yeast, and TV. MPC was diagnosed based on the RN assessment of mucopurulent cervical discharge or cervical friability on pelvic examination and negative tests for CT and NG.

All positive specimens for MG were sent to the NML for additional testing. DNA was extracted from the specimens using the QIAamp Viral RNA Mini kit (Qiagen, Toronto,

Ontario) or the MagNA Pure DNA and Viral Nucleic Acid kit (Roche, Laval, Quebec) as per manufacturer's instructions. Positive specimens were analyzed by sequencing 23SrRNA to identify mutations associated with macrolide resistance and *parC* and *gyrA* genes associated with resistance to fluoroquinolones.[9 10]

Sample size was determined by budgetary costs, impact on clinic staff and an acceptable margin of error. Using a sample size of 2000, our margin of error was +/- 1% for a 5% prevalence rate with 95% confidence. Gender-stratified analysis was performed to compare MG test result and MG resistance testing results by demographic and clinical variables using Chi-square or Fisher's exact for discrete variables and Mann-Whitney for continuous variables, excluding missing data. A two-tailed p-value of <0.05 was defined as statistically significant for univariate analysis. Multivariable logistic regression was performed for both males and females separately to identify correlates independently associated with a positive MG test result. In addition, the results from endocervical/vaginal swabs were compared to urine specimens for females and Cohen's Kappa was calculated. A 95% binomial confidence interval (CI) was calculated for each infection prevalence. Data was analyzed using IBM SPSS Statistics version 19.0 (IBM, Armonk, NY, USA). This study was approved by the University of Alberta Health Research Ethics Board.

RESULTS

A total of 2,294 individuals were tested. Forty patients were removed due to being <18 years (n=20) and for having more than 1 visit during the study period (n=20). One-half (53.8%;

n=1,212) of the study population was men. The male prevalence of MG was 5.3% (95%CI 4.0-6.5); CT was 5.9% (95%CI 4.6-7.3) and NG was 1.8% (95%CI 1.1-2.6). Among MSM, the MG prevalence was 6.6% with a CT prevalence of 3.4% and NG prevalence of 1.7%. In heterosexual males, MG prevalence was 4.7% with a CT prevalence of 6.8% and NG prevalence of 2.0%. Of 73 cases of urethritis, 19.2% (n=14) were due to MG. One-third (37.0%; n=27) of NGU cases were negative for MG, CT, and NG. Univariate correlates significantly associated with a higher prevalence of MG infection among males included being symptomatic (p=0.001), a diagnosis of NGU at time of visit (p<0.001), and co-infection with CT or NG (p=0.005 and p<0.001, respectively) (Table 1). Independent correlates of infection with MG were a diagnosis of NGU (Adjusted Odds Ratio (AOR)=7.6 95%CI 3.4-17.2) and co-infection with NG (AOR=7.2 95%CI 2.5-20.4).

Table 1. Characteristics of *M. genitalium* Cases (Alberta STI Clinics, January to April 2016, N=2,254).

	Female				Male			
Category	Positive (n=75)	Negative (n=967)	Total (n=1,042)	p-value	Positive (n=64)	Negative (n=1,148)	Total (n=1,212)	p-value
Median Age (IQR)	24 (21-28)	28 (24-34)	27 (23-33)	<0.001	28 (25-38)	30 (25-37)	30 (25-37)	0.53
Ethnicity								
Caucasian	33 (44.6)	795 (75.9)	728 (73.5)	<0.001	46 (75.4)	762 (71.0)	808 (71.3)	0.72
Indigenous	23 (31.1)	95 (10.4)	118 (11.9)		3 (4.9)	50 (4.7)	53 (4.7)	
Other	18 (24.3)	126 (13.8)	144 (14.5)		12 (19.7)	261 (24.3)	273 (24.1)	
Sexual Partners								
Heterosexual	-	-		-	37 (61.7)	758 (69.9)	795 (69.5)	0.18
Same Sex	-	-		-	23 (38.3)	326 (30.1)	349 (30.5)	

Testing Location								
Calgary	19 (25.3)	415 (42.9)	434 (41.7)	0.003	27 (42.2)	553 (48.2)	580 (47.9)	0.35
Edmonton	56 (74.7)	552 (57.1)	608 (58.3)		37 (57.8)	595 (51.8)	632 (52.1)	
Symptomatic								
No	44 (59.5)	567 (60.9)	611 (60.8)	0.81	34 (55.7)	817 (74.9)	851 (73.9)	0.001
Yes	30 (40.5)	364 (39.1)	394 (39.2)		27 (44.3)	274 (25.1)	301 (26.1)	
Pregnant								
No	70 (95.9)	903 (99.1)	973 (98.9)	0.04	-	-	-	-
Yes	3 (4.1)	8 (0.9)	11 (1.1)		-	-	-	-
HIV Status								
Negative	58 (84.1)	702 (79.1)	760 (79.4)	0.58	36 (60.0)	644 (61.6)	680 (61.5)	0.64

Positive	0	4 (0.5)	4 (0.4)		2 (3.3)	15 (1.4)	17 (1.5)	
Unknown	11 (15.9)	182 (20.5)	193 (20.2)		22 (36.7)	386 (36.9)	408 (36.9)	
Pelvic Exam								
No	42 (56.0)	562 (58.1)	604 (58.0)	0.81	-	-	-	-
Yes	33 (44.0)	405 (41.9)	438 (42.0)		-	-	-	-
Co-infections								
Chlamydia	18 (24.0)	42 (4.3)	60 (5.8)	<0.001	9 (14.1)	63 (5.5)	72 (5.9)	0.005
Gonorrhea	6 (8.0)	13 (1.3)	19 (1.8)	<0.001	5 (7.8)	17 (1.5)	22 (1.8)	<0.001
MPC[1]/NGU	3 (9.1)	4 (1.0)	7 (1.6)	0.01	9 (15.3)	27 (21.5)	36 (3.2)	<0.001

	Multivariate Analysis			
	Odds Ratio	**95% Confidence Interval**	**Adjusted Odds Ratio**	**95% Confidence**

				Interval
Male				
Co-infection with Gonorrhea	5.6	2.0-15.8	7.2	2.5-20.4
NGU Diagnosis	6.9	3.1-15.6	7.6	3.4-17.2
Female				
Age	0.91	0.87-0.95	0.92	0.87-.96
Indigenous Ethnicity[2]	5.1	2.9-9.1	4.3	2.7-8.1
Other Ethnicity[2]	3.0	1.6-5.5	2.8	1.5-5.3
Chlamydia Co-Infection	7.0	3.8-12.9	5.1	2.6-10.2
Gonorrhea Co-Infection	5.8	2.0-16.6	3.5	1.0-11.8

1. Denominator for MPC is the number of women who underwent a pelvic examination.

2. Referent group is Caucasian ethnicity.

Missing data: ethnicity (females=52, males=78), symptomatic (female=37, males=60), HIV status (female=85, male =107), pregnant=58, sexual partners=68.

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The overall MG prevalence for females, using any positive test result from endocervical/vaginal or urine results, was 7.2% (95%CI 5.6-8.8). CT prevalence was 5.8% (95%CI 4.3-7.2) and NG was 1.8% (95%CI 1.0-2.6). Seven cases (1.6%) of MPC were diagnosed among 438 women who had pelvic examinations. There was high concordance of results between urine and cervical swabs (98.1%; Table 2; Kappa was 0.85 (95% CI: 0.77-1.0), representing almost perfect agreement. Three vaginal swabs collected during our study were excluded from the comparison of results between specimens.

Table 2. Concordance of *M. genitalium* Results from Cervical and Urine Screening among Women.

		Cervix		
		MG Positive	MG Negative	Total
Urine	MG Positive	22	3	25
	MG Negative	4	330	334
	Total	26	333	359

22+330/359=98.1% concordance between cervical and urine results.

Kappa: 0.85 (95% CI: 0.77-1.0)

Univariate correlates significantly associated with a higher prevalence of MG infection (Table 1) among women were younger median age (<0.001), Indigenous ethnicity (p<0.001), Other ethnicity (p<0.001), the Edmonton clinic testing location (p=0.003), being pregnant (p=0.04), CT or NG co-infection (p<0.001, for both), and MPC diagnosis at time of visit (p=0.01). Independent correlates of infection with MG were younger age (AOR=0.92 95%CI 0.87-0.96), and Indigenous ethnicity (AOR=4.3 95%CI 2.7-8.1) and Other ethnicity (AOR=2.8 95%CI 1.5-5.3) (vs. Caucasian), co-infection with CT (AOR=5.1 95%CI 2.6-10.2) and NG (AOR=3.5 95%CI=1.0-11.8).

Macrolide resistance data provided through 23SrRNA sequencing data was available for 73.4% (n=47) of the 64 positive male specimens. Nearly two-thirds (63.8%; n=30) were found to have mutations associated with macrolide resistance in either A2058T (n=3), A2058G (n=12), or A2059G (n=15). There were no variables significantly associated with macrolide resistance, although MSM was marginally associated with resistance among males (83.3% vs. 51.9%; p=0.06; Table 3). Resistance to fluoroquinolones was assessed by markers *gyrA* and *parC*. Nearly two-thirds (64.1%; n=41) of positive specimens had *parC* sequences available and 5 (12.2%) specimens had a *parC* mutation (Ser→Ile83, n=4) and (Asp→Tyr87, n=1) signifying fluoroquinolone resistance. *gyrA* sequencing was performed on 46 specimens and no *gyrA* mutations were identified.

Table 3. Characteristics of Macrolide Resistance in *M. genitalium* Specimens by Gender (Alberta STI Clinics, January to April 2016, N=94).

Category	Macrolide resistance							
	Female				Male			
	Resistance (n=22)	Susceptible (n=23)	Total (n=45)	p-value	Resistance (n=30)	Susceptible (n=17)	Total (n=47)	p-value
Median Age (IQR)	22 (20-26)	26 (22-29)	24 (20-28)	0.04	29 (23-41)	27 (25-40)	28 (24-41)	0.90
Ethnicity								
Caucasian	10 (45.5)	12 (54.5)	22 (50.0)	0.83	22 (75.9)	14 (87.5)	36 (80.0)	0.80
Indigenous	6 (27.3)	5 (22.7)	11 (25.0)		1 (3.4)	0	1 (2.2)	
Other	6 (27.3)	5 (22.7)	11 (25.0)		6 (20.7)	2 (12.5)	8 (17.8)	

Sexual Partners								
Heterosexual	-	-		-	14 (48.3)	13 (81.3)	27 (60.0)	0.06
Same Sex	-	-		-	15 (51.7)	3 (18.8)	18 (40.0)	
Testing Location								
Calgary	5 (22.7)	8 (34.8)	13 (28.9)	0.37	10 (33.3)	9 (52.9)	19 (40.4)	0.19
Edmonton	17 (77.3)	15 (65.2)	32 (71.1)		20 (66.7)	8 (47.1)	28 (59.6)	
Symptomatic								
No	15 (68.2)	14 (63.6)	29 (65.9)	0.75	15 (51.7)	11 (68.8)	26 (57.8)	0.27
Yes	7 (31.8)	8 (36.4)	15 (34.1)		14 (48.3)	5 (31.3)	19 (42.2)	
HIV Status								
Negative	19 (95.0)	16 (80.0)	35 (87.5)	0.34	20 (71.4)	8 (50.0)	28 (63.6)	0.11

Positive	0	0	0		2 (7.1)	0	2 (4.5)	
Unknown	1 (5.0)	4 (20.0)	5 (12.5)		6 (21.4)	8 (50.0)	14 (31.8)	
Co-infections								
Chlamydia	3 (13.6)	7 (30.4)	10 (22.2)	0.28	3 (10.0)	2 (11.8)	5 (10.6)	1.00
Gonorrhea	2 (9.1)	2 (8.7)	4 (8.9)	1.00	2 (6.7)	0	2 (4.3)	0.53
MPC[1]/NGU	0	0	0		6 (20.0)	3 (17.6)	9 (19.1)	1.00

1. Denominator for MPC is the number of women who underwent a pelvic examination.

Missing data: ethnicity (females=1, males=2), symptomatic (female=1, males=2), HIV status (female=5, male =3), sexual partners=2.

Among women, 23SrRNA sequencing data was available for 60.0% (45/75) of positive specimens. Nearly one-half (48.9%; n=22) had a 23SrRNA mutation associated with macrolide resistance in A2058G (n=11), A2058T (n=5), A2059G (n=6), or A2059C (n=1). In univariate analysis, younger median age (22y (IQR: 20-26) vs. 26y (IQR: 22-29); p=0.04; Table 3) was the only variable significantly correlated with macrolide resistance. One-half (50.7%; n=38) of positive specimens had *parC* sequencing available and only 1 specimen had a mutation signifying fluoroquinolone resistance (Asp→Tyr87); no *gyrA* mutations were identified.

DISCUSSION

Our study underscores the significance of *M. genitalium* as a medically significant pathogen from urogenital sites. In our male population, the prevalence of MG was 5.3%, within the range of 3.1%-17.2% reported in males from other STI Clinics.[6 11 12 13] Although not statistically significant, the prevalence of MG in our study trended toward being higher in MSM than among heterosexual males (6.6% vs 4.7%, p=0.18). A recent Dutch study reported a similar prevalence of MG in males (overall MG prevalence in males 3.1%; 2.5% in MSM, 3.8% in MSW, p=0.13).[12] A diagnosis of NGU was significantly correlated with MG infection among males in our study population, in accordance with previous studies reporting a strong association between MG and NGU independent of Chlamydia infection.[1 5] In a meta-analysis of studies completed up to 2010, MG was associated with a pooled odds ratio (OR) of 5.5 (95% CI: 4.4-7.0) for NGU.[1]

The overall MG prevalence for females was 7.2% (95%CI 5.6-8.8), higher than the range of 3.2-6% reported in most studies of females STI clinic attendees.[6 12 14 15] In females, MG has been

associated with significant morbidity including MPC, PID and infertility, but the association between MG and symptoms is less clear.[10 16 17] Among female STI clinic attendees in some studies, 40-75% were asymptomatic[14 15] but a 1994-96 French study reported a very high prevalence of MG of 38% among symptomatic female STI clinic attendees.[16] The presence of symptoms was not an independent correlate of MG infection in our study.

Independent correlates of female infection with MG in our study were younger age, in contrast to two other studies which reported that the prevalence of MG peaked approximately 5 years later for both men and women and remained higher in older age groups.[18 19] Co-infection with CT and NG was common in our patients, confirming the role of MG as a sexually transmitted pathogen and the probable overlap in behavioural and demographic characteristics for these STIs.

Indigenous (First Nations, Inuit, Metis) ethnicity and other non- Caucasian ethnicity were also significant correlates of MG infection. We were unable to find other studies examining the prevalence of MG by ethnicity but the disproportionately high rates of MG among Indigenous persons in our study is in keeping with the higher estimated STI prevalence in Canadian Indigenous persons when compared to the overall general population.[20 21] First Nations persons represent an estimated 3.8% of the overall Canadian population but chlamydia rates are estimated to be 7 times higher among First Nations adults than the overall population.[20] The reasons for the observed disproportionately high rates of STIs are unclear but Indigenous persons in Canada are also over-represented in adolescent pregnancy and under-represented in

sexual health research.[22] A recent First Nations Regional Health Survey stressed the importance of colonial history, barriers to health care services and socio-economic disadvantage.[20]

The optimal specimen type for MG testing remains unresolved with urine specimens considered acceptable in males and females and in females, vaginal swabs are also considered suitable.[23] In our study, the near perfect agreement between the test performance in female cervical and urine swabs is reassuring and supports the use of less invasive urine specimens for testing in females.

It is very likely that appropriate treatment of MG infections will result in reduced sexual transmission as well as prevention of complications.[2] Alternates to macrolides and FQ, the antibiotics usually proposed for the treatment of MG, are limited since the lack of a cell wall in MG precludes the use of penicillins and other beta lactam antibiotics.[24] Further complicating this is that mycoplasmas can develop resistance either by gene mutation or by acquisition of a resistance gene.[24] Since azithromycin has been proposed as the preferred first line agent for the treatment of MG infections[25 26], the high rate of mutations (~2/3 of eligible specimens) conferring resistance to azithromycin in our study is particularly alarming. Strains of *M. genitalium* began to develop resistance to azithromycin and have continued to do so through mutations in region V of the 23S ribosomal RNA gene.[27] Macrolide resistance rates vary significantly by geographic region with 58% resistance reported in the only published Canadian study conducted in Eastern Canada.[6] This level of resistance is well above the threshold of 5% resistance above which the World Health Organization typically recommends against the

routine use of a drug for first line treatment of an STI.[28] A recent review reported that the efficacy of azithromycin 1 gm for the treatment of urogenital MG has decreased from 85% prior to 2009 to 60% in early 2015.[7] This had been postulated to be due to increasing prevalence of macrolide resistance due to the widespread use of azithromycin for the treatment of CT, NGU and MPC.[7 29 30 31] In a recent meta-analysis, persistent MG was associated with a pooled OR of 26 (95% CI: 11–57) for persistent urethritis, demonstrating that failure to eradicate MG leads to persistent or recurrent signs and symptoms of urethritis in the majority of men.[7] The observation of MG as a significant pathogen in both NGU and MPC has generated much discussion around whether azithromycin, and especially single dose azithromycin should continue to be recommended as the preferred agent for these STI syndromes.[29] Instead it has been proposed that doxycycline be used as the first line agent because even though it is in only 30-40% effective against MG, it does not induce the development of antimicrobial resistance.[29]

Moxifloxacin has been proposed as the drug of choice for treatment failures with azithromycin[25 26] but our finding of 12.2% resistance to FQ as assessed by markers *gyrA* and *parC* is also above the 5% threshold set by the WHO.[28] Earlier studies reported cure rates of 100% with moxifloxacin.[30 32 33] However, more recently Tagg et al. reported macrolide resistance-associated mutations in the 23S rRNA gene in 43% of samples and mutations in *parC* or *gyrA* sequences in 15% of samples.[34] Touati et al reported a point mutation in the 23S rRNA gene in 14.2% of samples.[35]

Given the relatively high prevalence of MG in both males and females in ours and other studies,

the potential for significant morbidity and enhanced HIV transmission, global recommendations for MG screening are currently very diverse in part due to lack of access to good tests for MG. In the absence of an FDA approved test for MG, the U.S. CDC STD Treatment guidelines suggest that MG be suspected in cases of persistent/recurrent urethritis, cervicitis and PID.[25] Canada has a single Health Canada approved test for MG (Seegene Inc, Seoul, Korea) which is not widely available. The Europeans currently have the broadest recommendations for screening for MG including persons with STI symptoms and those engaging in high-risk sexual behavior, with a strong recommendation that all positive tests be followed by an assay capable of detecting macrolide resistance mutations.[26]

Our study has a few limitations. Firstly, our specimens were collected in STI clinic patients in Western Canada and may not be generalizable to other STI clinics and are likely to be higher than rates reported in non-STI clinic populations. Secondly, although the specimens were collected prospectively, we were only able to collect a limited number of additional variables in addition to standard data collection at the clinics due to time constraints; this may have limited our ability to identify additional correlates of MG. Thirdly, as tetracycline resistance-associated mutations have not so far been identified in *M. genitalium*[36], we did not test our samples for resistance to doxycycline; this information may have been useful in guiding empiric treatment regimens for NGU and cervicitis in our region.

In summary, our study found a MG prevalence of 6.2% in attendees at two Western Canadian STI Clinics, within the range reported in other studies, but higher than that for chlamydia (in

females) and gonorrhea (in both genders). Over one-half of tested isolates were resistant to macrolides. These findings together with the high proportion of asymptomatic carriers who could facilitate the spread of infection, the potential for significant morbidity and the potential for enhanced HIV transmission support recommendations for broader screening for MG. The high prevalence of macrolide resistance also supports the recommendation to follow all positive tests with an assay that can detect macrolide resistance mutations.[26] Judicious use of antibiotics for the empiric treatment of NGU and MPC is needed to mitigate the further development of resistance to currently used antibiotics and to optimize treatment of CT, NG and MG. In order to facilitate this, wider access to testing for MG and adaptation of most existing guidelines will be necessary.

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COMPETING INTERESTS None

AUTHOR CONTRIBUTIONS JG, SP, PN, LT, MC, IM, PS, RR, LB and AS developed the study design, protocol and ethics submission. LB coordinated funding for the study. JG, SP conducted epidemiologic analyses. JG, SP, AS drafted manuscript. PP, BB, JB, RS coordinated the study in the clinics. AB, SS, LT and IM coordinated and/or conducted laboratory testing. All authors contributed to final manuscript review.

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Prevalence and Antibiotic Resistance of Mycoplasma genitalium among STI Clinic Attendees in Western Canada: a Cross-Sectional Analysis

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Prevalence and Antibiotic Resistance of *Mycoplasma genitalium* among STI Clinic Attendees in Western Canada: a Cross-Sectional Analysis

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Abstract

Objectives: To determine the prevalence and correlates of *M. genitalium* (MG) infection among males and females, determine the prevalence of gene mutations conferring resistance, and compare test performance of female specimen types.

Methods: A cross sectional study was conducted on specimens collected for gonorrhea (NG) and Chlamydia (CT) among male and female Alberta STI Clinic attendees using the *M. genitalium* Transcription Mediated Amplification – Research Use Only (RUO) test (Hologic Inc, San Diego, CA). Positive specimens were sequenced for 23SrRNA, *parC* and *gyrA* genes. Gender-stratified analysis compared test results using Chi-square or Fisher's exact test, Mann-Whitney test, and logistic regression. Female endocervical and urine specimens were compared.

Results: A total of 2,254 individuals were tested; 53.8% (n=1,212) were male. Male prevalence of MG was 5.3%; CT was 5.9% and NG was 1.8%. Correlates of male infection were an NGU diagnosis and NG co-infection. MG prevalence for females was 7.2%; CT was 5.8% and NG was 1.8%. Correlates of female infection were younger age, Indigenous/other ethnicity and CT/NG co-infection. Nearly two-thirds of eligible specimens had mutations associated with macrolide resistance and 12.2% of specimens had a *parC* mutation signifying possible moxifloxacin resistance. There was high concordance (98.1%) of results between urine and endocervical swabs.

Conclusions: The high prevalence of MG relative to CT and NG supports the incorporation of MG testing into routine STI screening. The high rate of resistance to macrolides and moxifloxacin raises concerns about treatment options. The good concordance of results between urine and endocervical swabs supports the use of female urine specimens for testing.

Key Words: *Mycoplasma*, Clinical STI Care, Epidemiology (Clinical)

Strengths and Limitations of this study

- The main strength of this study is that it is among the largest of global studies examining *Mycoplasma genitalium* prevalence in heterosexual males, men who have sex with men and females attending STI clinics.
- Since we were only able to collect a small number of additional variables over standard clinic protocol due to time constraints, this may have limited our ability to identify additional correlates of *Mycoplasma genitalium* infection.

BACKGROUND

Mycoplasma genitalium (MG) is an emerging sexually transmissible infection (STI) caused by bacteria belonging to the *Mollicutes* class that lack a cell wall.[1] In males, it has been implicated as an etiologic agent of non-gonococcal urethritis (NGU) and persistent or recurrent urethritis.[1] In females, available evidence suggests that MG infection is significantly associated with an increased risk of cervicitis, pelvic inflammatory disease (PID), preterm birth, spontaneous abortion and risk of infertility is also increased.[2] Studies suggest that PID cases associated with MG may be similar to *Chlamydia trachomatis* (CT) in terms of severity of symptoms and signs.[3] A two fold increased odds of HIV among populations with MG has also been reported.[4]

Globally, the prevalence of MG using molecular diagnostic tests ranges from 1-4% in men and 1-6% in women but is higher in those at risk for STI.[5] In a recent Eastern Canadian study, male prevalence was 4.5% and prevalence in females was 3.2%.[6]

In Canada, access to testing for MG is currently largely limited to the referral of suitable specimens to the National Microbiology Laboratory (NML) in Winnipeg. Azithromycin has been recommended for treatment of MG but rising resistance has raised concerns about the use of this drug as the preferred option.[7] Alternate treatment with moxifloxacin has been proposed but the high cost of this medication, the potential for hepatotoxicity and reports of resistance have also raised concerns.[8]

Given the anticipated wider availability of test kits to screen for MG in the future, we sought to determine the prevalence and correlates of MG infection in urogenital specimens from attendees at two Alberta STI clinics, to compare the test performance in different types of urogenital specimens from females, and to determine the prevalence of mutations in genes conferring resistance to macrolides and moxifloxacin.

METHODS

Specimens collected from January to April 2016 for NG and CT screening from urogenital sites among sequential male and female attendees (>17 years old) at two Alberta STI Clinics were tested for MG. Inclusion in the study required that at least two months had elapsed since being treated for NG or CT to reduce chance visit was related to test of cure from previous infection, and screening could not be part of patient follow-up if named as a sexual contact to a NG/CT case to remove patients more likely to test positive. All individuals attending the two STI clinics were screened for NG and CT unless they specifically declined: all men were screened using urine tests while women were either screened with urine tests (mostly asymptomatic) or with an endocervical or vaginal swab (mostly symptomatic).

Basic demographic and clinical information was collected on the laboratory requisition form and included ethnicity (Caucasian, Indigenous, or Other), presence of symptoms (yes/no; for females, symptoms were defined as the presence or complaint of vaginal discharge, odor, or itching and for males, urethral discharge or dysuria), diagnosis at the time of visit for those undergoing physical examination (NGU, mucopurulent cervicitis (MPC)) and self-reported HIV

status at the time of the patient visit. For male visits only, the gender of the sexual partner was recorded. For men, NGU was diagnosed if upon physical examination the Registered Nurse (RN) found urethral discharge +/- dysuria plus urethral smear with >5 polymorphonuclear leukocytes/ high power field in 5 or more fields with subsequent negative CT and NG test results. For women, MPC was diagnosed based on the RN assessment of mucopurulent cervical discharge or cervical friability on vaginal speculum examination.

The *M. genitalium* Transcription Mediated Amplification – Research Use Only *(*Hologic Inc, San Diego, CA) test was used to screen endocervical, vaginal, and urine specimens. Endocervical swabs are currently collected in preference to vaginal swabs in our STI clinics. For a female sub-population, test results from endocervical and urine specimens collected on the same individuals at the same visit were compared and the proportion of concordant results was calculated. The Hologic Aptima Combo 2 assay was used to test for CT and NG.

All positive specimens for MG were sent to the NML for additional testing. DNA was extracted from the specimens using the QIAamp Viral RNA Mini kit (Qiagen, Toronto, Ontario) or the MagNA Pure DNA and Viral Nucleic Acid kit (Roche, Laval, Quebec) as per manufacturer's instructions. Positive specimens were analyzed by sequencing 23SrRNA to identify mutations associated with macrolide resistance and *parC* and *gyrA* genes associated with potential resistance to moxifloxacin.[9 10]

Sample size was determined by budgetary costs, impact on clinic staff and an acceptable margin of error. Using a sample size of 2000, our margin of error was +/- 1% for a 5% prevalence rate with 95% confidence. Gender-stratified analysis was performed to compare MG test result and MG resistance testing results by demographic and clinical variables using Chi-square or Fisher's exact for discrete variables and Mann-Whitney for continuous variables, excluding missing data. A two-tailed p-value of <0.05 was defined as statistically significant for univariate analysis. Multivariable logistic regression was performed for both males and females separately to determine adjusted estimates of odds ratios (AOR) and 95% CI for correlates independently associated with a positive MG test result. All variables with a statistical significance of p< 0.10 in univariate analysis were considered in the regression models. Variables were removed from the model if they were deemed to be non-significant or did not contribute significantly to the overall model. In addition, the results from endocervical swabs were compared to urine specimens for females and Cohen's Kappa was calculated. A 95% binomial confidence interval (CI) was calculated for each infection prevalence. Data was analyzed using IBM SPSS Statistics version 19.0 (IBM, Armonk, NY, USA). This study was approved by the University of Alberta Health Research Ethics Board.

RESULTS

A total of 2,294 individuals were tested. Forty patients were removed due to being <18 years (n=20) and for having more than 1 visit during the study period (n=20). The overall MG prevalence was 6.2% (95%CI 5.2-7.2). One-half (53.8%; n=1,212) of the study population was men. The male prevalence of MG was 5.3% (95%CI 4.0-6.5); CT was 5.9% (95%CI 4.6-7.3) and

NG was 1.8% (95%CI 1.1-2.6). Among MSM, the MG prevalence was 6.6% with a CT prevalence of 3.4% and NG prevalence of 1.7%. In heterosexual males, MG prevalence was 4.7% with a CT prevalence of 6.8% and NG prevalence of 2.0%. Of 73 cases of urethritis, 19.2% (n=14) were due to MG. One-third (37.0%; n=27) of NGU cases were negative for MG, CT, and NG. Univariate correlates significantly associated with a higher prevalence of MG infection among males included being symptomatic (p=0.001), a diagnosis of NGU (p<0.001), and co-infection with CT or NG (p=0.005 and p<0.001, respectively) (Table 1). Independent correlates of infection with MG were a diagnosis of NGU (Adjusted Odds Ratio (AOR=7.6 95%CI 3.4-17.2) and co-infection with NG (AOR=7.2 95%CI 2.5-20.4).

Table 1. Characteristics of *M. genitalium* Cases (Alberta STI Clinics, January to April 2016, N=2,254).

	Female				Male				Grand Total
Category	Positive (n=75)	Negative (n=967)	Total (n=1,042)	p-value	Positive (n=64)	Negative (n=1,148)	Total (n=1,212)	p-value	
Median Age (IQR)	24 (21-28)	28 (24-34)	27 (23-33)	<0.001	28 (25-38)	30 (25-37)	30 (25-37)	0.53	29 (24-35)
Ethnicity									
Caucasian	33 (44.6)	795 (75.9)	728 (73.5)	<0.001	46 (75.4)	762 (71.0)	808 (71.3)	0.72	1,536 (72.3)
Indigenous	23 (31.1)	95 (10.4)	118 (11.9)		3 (4.9)	50 (4.7)	53 (4.7)		171 (8.1)
Other	18	126	144		12	261	273		417

	(24.3)	(13.8)	(14.5)		(19.7)	(24.3)	(24.1)		(19.6)
Sexual Partners									
Heterosexual	-	-		-	37	758	795	0.18	-
					(61.7)	(69.9)	(69.5)		
Same Sex	-	-		-	23	326	349		-
					(38.3)	(30.1)	(30.5)		
Testing Location									
Calgary	19	415	434	0.003	27	553	580	0.35	1,014
	(25.3)	(42.9)	(41.7)		(42.2)	(48.2)	(47.9)		(45.0)
Edmonton	56	552	608		37	595	632		1,240
	(74.7)	(57.1)	(58.3)		(57.8)	(51.8)	(52.1)		(55.0)
Symptomatic									

No	44	567	611	0.81	34	817	851	0.001	1,462
	(59.5)	(60.9)	(60.8)		(55.7)	(74.9)	(73.9)		(67.8)
Yes	30	364	394		27	274	301		695
	(40.5)	(39.1)	(39.2)		(44.3)	(25.1)	(26.1)		(32.2)
Pregnant									
No	70	903	973	0.04	-	-	-	-	-
	(95.9)	(99.1)	(98.9)						
Yes	3 (4.1)	8 (0.9)	11 (1.1)		-	-	-	-	-
HIV Status									
Negative	58	702	760	0.58	36	644	680	0.64	1,440
	(84.1)	(79.1)	(79.4)		(60.0)	(61.6)	(61.5)		(69.8)
Positive	0	4 (0.5)	4 (0.4)		2 (3.3)	15 (1.4)	17 (1.5)		21
									(1.0)

	Unknown	11 (15.9)	182 (20.5)	193 (20.2)		22 (36.7)	386 (36.9)	408 (36.9)		601 (29.1)
	Co-infections									
	Chlamydia	18 (24.0)	42 (4.3)	60 (5.8)	<0.001	9 (14.1)	63 (5.5)	72 (5.9)	0.005	132 (5.9)
	Gonorrhea	6 (8.0)	13 (1.3)	19 (1.8)	<0.001	5 (7.8)	17 (1.5)	22 (1.8)	<0.001	41 (1.8)
MPC/NGU		3 (4.0)	4 (0.4)	7 (0.7)	0.01	9 (15.3)	27 (21.5)	36 (3.2)	<0.001	-

	Multivariate Analysis			
	Odds Ratio	95% Confidence Interval	Adjusted Odds Ratio	95% Confidence Interval
Male[1]				
Co-infection with Gonorrhea	5.6	2.0-15.8	7.2	2.5-20.4
NGU Diagnosis	6.9	3.1-15.6	7.6	3.4-17.2
Female[2]				
Age (increase in one year)	0.91	0.87-0.95	0.92	0.87-.96
Indigenous Ethnicity[3]	5.1	2.9-9.1	4.3	2.7-8.1

Other Ethnicity[3]	3.0	1.6-5.5	2.8	1.5-5.3
Chlamydia Co-Infection	7.0	3.8-12.9	5.1	2.6-10.2
Gonorrhea Co-Infection	5.8	2.0-16.6	3.5	1.0-11.8

1. Symptoms and CT co-infection were not retained in the final model due to lack of significance.

2. Testing location, pregnancy and MPC diagnosis were not retained in the final model due to lack of significance.

3. Referent group is Caucasian ethnicity.

Missing data: ethnicity (females=52, males=78), symptomatic (female=37, males=60), HIV status (female=85, male =107), pregnant=58, sexual partners=68.

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The overall MG prevalence for females, using any positive test result from endocervical/vaginal or urine results, was 7.2% (95%CI 5.6-8.8). CT prevalence was 5.8% (95%CI 4.3-7.2) and NG was 1.8% (95%CI 1.0-2.6). Seven cases (0.7%) of MPC were diagnosed.

Univariate correlates significantly associated with a higher prevalence of MG infection (Table 1) among women were younger age (p<0.001), Indigenous ethnicity (p<0.001), Other ethnicity (p<0.001), the Edmonton clinic testing location (p=0.003), being pregnant (p=0.04), CT or NG co-infection (p<0.001, for both), and MPC diagnosis (p=0.01). Independent correlates of infection with MG were younger age (AOR=0.92 95%CI 0.87-0.96), Indigenous ethnicity (AOR=4.3 95%CI 2.7-8.1) and Other ethnicity (AOR=2.8 95%CI 1.5-5.3) (vs. Caucasian), co-infection with CT (AOR=5.1 95%CI 2.6-10.2) and NG (AOR=3.5 95%CI=1.0-11.8).

Macrolide resistance data provided through 23SrRNA sequencing data was available for two-thirds (66.2%; n=92) of the 139 positive MG specimens. No significant differences were found between specimens that were and were not sequenced for age, gender, symptoms, same sex partners (for male cases only), NG, or CT results. There was a significant difference in ethnicity, with fewer specimens from Indigenous cases being typed (46.2%) than from non-Indigenous cases (70.6%; p=0.02). However, when stratified by gender, the significance was lost (women: p=0.17, men=0.17). Over one-half (56.5%; n=52) of specimens were found to have mutations associated with macrolide resistance. Of the 73.4% (n=47) positive male specimens sequenced, nearly two-thirds (63.8%; n=30) were found to have mutations in either A2058T (n=3), A2058G (n=12), or A2059G (n=15). There were no variables significantly associated with macrolide

resistance, although MSM was marginally associated with resistance among males (83.3% vs. 51.9%; p=0.06; Table 2). Resistance to moxifloxacin was assessed by markers *gyrA* and *parC*. Nearly two-thirds (64.1%; n=41) of positive male specimens had *parC* sequences available and 5 (12.2%) specimens had a *parC* mutation (Ser→Ile83, n=4) and (Asp→Tyr87, n=1) signifying possible moxifloxacin resistance. *gyrA* sequencing was performed on 46 specimens and no *gyrA* mutations were identified.

Table 2. Characteristics of Macrolide Resistance in *M. genitalium* Specimens by Gender (Alberta STI Clinics, January to April 2016, N=92).

Category	Macrolide resistance								
	Female				Male				Grand Total
	Resistance (n=22)	Susceptible (n=23)	Total (n=45)	p-value	Resistance (n=30)	Susceptible (n=17)	Total (n=47)	p-value	
Median Age (IQR)	22 (20-26)	26 (22-29)	24 (20-28)	0.04	29 (23-41)	27 (25-40)	28 (24-41)	0.90	26 (22-31)
Ethnicity									
Caucasian	10 (45.5)	12 (54.5)	22 (50.0)	0.83	22 (75.9)	14 (87.5)	36 (80.0)	0.80	58 (65.2)
Indigenous	6 (27.3)	5 (22.7)	11 (25.0)		1 (3.4)	0	1 (2.2)		12

								(13.5)
Other	6 (27.3)	5 (22.7)	11 (25.0)		6 (20.7)	2 (12.5)	8 (17.8)	19 (21.3)
Sexual Partners								
Heterosexual	-	-	-		14 (48.3)	13 (81.3)	27 (60.0)	0.06 -
Same Sex	-	-	-		15 (51.7)	3 (18.8)	18 (40.0)	-
Testing Location								
Calgary	5 (22.7)	8 (34.8)	13 (28.9)	0.37	10 (33.3)	9 (52.9)	19 (40.4)	0.19 32 (34.8)
Edmonton	17 (77.3)	15 (65.2)	32 (71.1)		20 (66.7)	8 (47.1)	28 (59.6)	60 (65.2)

Symptomatic									
No	15 (68.2)	14 (63.6)	29 (65.9)	0.75	15 (51.7)	11 (68.8)	26 (57.8)	0.27	55 (61.8)
Yes	7 (31.8)	8 (36.4)	15 (34.1)		14 (48.3)	5 (31.3)	19 (42.2)		34 (38.2)
HIV Status									
Negative	19 (95.0)	16 (80.0)	35 (87.5)	0.34	20 (71.4)	8 (50.0)	28 (63.6)	0.11	63 (75.0)
Positive	0	0	0		2 (7.1)	0	2 (4.5)		2 (2.4)
Unknown	1 (5.0)	4 (20.0)	5 (12.5)		6 (21.4)	8 (50.0)	14 (31.8)		19 (22.6)
Co-infections									

Chlamydia	3 (13.6)	7 (30.4)	10 (22.2)	0.28	3 (10.0)	2 (11.8)	5 (10.6)	1.00	15 (16.3)
Gonorrhea	2 (9.1)	2 (8.7)	4 (8.9)	1.00	2 (6.7)	0	2 (4.3)	0.53	6 (6.5)
MPC/NGU	0	0	0		6 (20.0)	3 (17.6)	9 (19.1)	1.00	-

Missing data: ethnicity (females=1, males=2), symptomatic (female=1, males=2), HIV status (female=5, male =3), sexual partners=2.

Among women, 23SrRNA sequencing data was available for 60.0% (45/75) of positive specimens. Nearly one-half (48.9%; n=22) had a 23SrRNA mutation associated with macrolide resistance in A2058G (n=11), A2058T (n=5), A2059G (n=6), or A2059C (n=1). In univariate analysis, younger median age (22y (IQR: 20-26) vs. 26y (IQR: 22-29); p=0.04; Table 3) was the only variable significantly correlated with macrolide resistance. One-half (50.7%; n=38) of positive specimens had *parC* sequencing available and only 1 specimen had a mutation signifying potential moxifloxacin resistance (Asp→Tyr87); no *gyrA* mutations were identified.

Among the subpopulation of women who had both endocervical swabs and urine collected, there was high concordance of results (98.1%; Table 3; Kappa was 0.85 (95% CI: 0.75-0.96), representing excellent agreement. Only three vaginal swabs were collected during the study period, therefore concordance with urine specimens was not calculated. This subpopulation of women was more likely to have symptoms (61.1%) than those with urines only (27.5%; p<0.001).

Table 3. Concordance of *M. genitalium* Results from Cervical and Urine Screening among Women.

		Cervix		
		MG Positive	MG Negative	Total
Urine	MG Positive	22	3	25
	MG Negative	4	333	337

Total	26	336	362

22+333/362=98.1% concordance between cervical and urine results.

Kappa: 0.85 (95% CI: 0.75-0.96)

DISCUSSION

Our study underscores the significance of *M. genitalium* as a medically significant pathogen from urogenital sites. In our male population, the prevalence of MG was 5.3%, within the range of 3.1%-17.2% reported in males from other STI Clinics.[6 11 12 13] A diagnosis of NGU was significantly correlated with MG infection among males in our study population, in accordance with previous studies reporting a strong association between MG and NGU independent of Chlamydia infection.[1, 5] In a meta-analysis of studies completed up to 2010, MG was associated with a pooled odds ratio (OR) of 5.5 (95% CI: 4.4-7.0) for NGU.[1]

The overall MG prevalence for females was 7.2% (95%CI 5.6-8.8), higher than the range of 3.2-6% reported in most studies of females STI clinic attendees.[6 12 14 15] In females, MG has been associated with significant morbidity including MPC, PID and infertility, but the association between MG and symptoms is less clear.[10 16 17] Among female STI clinic attendees in some studies, 40-75% were asymptomatic[14 15] but a 1994-96 French study reported a very high prevalence of MG of 38% among symptomatic female STI clinic attendees.[16] The presence of symptoms was not an independent correlate of MG infection in our study.

Independent correlates of female infection with MG in our study were younger age, in contrast to two other studies which reported that the prevalence of MG peaked approximately 5 years later for both men and women and remained higher in older age groups.[18 19] Co-infection with CT and NG was common in our patients, confirming the role of MG as a sexually transmitted pathogen and the probable overlap in behavioural and demographic characteristics for these STIs.

Indigenous (First Nations, Inuit, Metis) ethnicity and other non- Caucasian ethnicity were also significant correlates of MG infection. Other studies have reported higher rates of MG in non-Caucasian populations.[20 21] Our finding of disproportionately high rates of MG among Indigenous persons is in keeping with the higher estimated STI prevalence in Canadian Indigenous persons when compared to the overall general population.[22 23] First Nations persons represent an estimated 3.8% of the overall Canadian population but chlamydia rates are estimated to be 7 times higher among First Nations adults than the overall population.[22] The reasons for the observed disproportionately high rates of STIs are unclear but Indigenous persons in Canada are also over-represented in adolescent pregnancy and under-represented in sexual health research.[24] A recent First Nations Regional Health Survey stressed the importance of colonial history, barriers to health care services and socio-economic disadvantage.[22]

It is very likely that appropriate treatment of MG infections will result in reduced sexual transmission as well as prevention of complications.[2] Alternates to macrolides and

moxifloxacin, the antibiotics usually proposed for the treatment of MG, are limited since the lack of a cell wall in MG precludes the use of penicillins and other beta lactam antibiotics.[25] Further complicating this is that mycoplasmas can develop resistance either by gene mutation or by acquisition of a resistance gene.[26] Since azithromycin has been proposed as the preferred first line agent for the treatment of MG infections[27 28], the high rate of mutations (~2/3 of eligible specimens) conferring resistance to azithromycin in our study is particularly alarming. Strains of *M. genitalium* began to develop resistance to azithromycin and have continued to do so through mutations in region V of the 23S ribosomal RNA gene.[29] Macrolide resistance rates vary significantly by geographic region with 58% resistance reported in the only published Canadian study conducted in Eastern Canada.[6] This level of resistance is well above the threshold of 5% resistance above which the World Health Organization typically recommends against the routine use of a drug for first line treatment of an STI.[30] A recent review reported that the efficacy of azithromycin 1 gm for the treatment of urogenital MG has decreased from 85% prior to 2009 to 60% in early 2015.[7] This had been postulated to be due to increasing prevalence of macrolide resistance due to the widespread use of azithromycin for the treatment of CT, NGU and MPC.[7 31 32 33] In a recent meta-analysis, persistent MG was associated with a pooled OR of 26 (95% CI: 11–57) for persistent urethritis, demonstrating that failure to eradicate MG leads to persistent or recurrent signs and symptoms of urethritis in the majority of men.[7] The observation of MG as a significant pathogen in both NGU and MPC has generated much discussion around whether azithromycin, and especially single dose azithromycin should continue to be recommended as the preferred agent for these STI syndromes.[31] Instead it has been proposed that doxycycline be used as the first line agent because even though it is in only

30-40% effective against MG, it does not induce the development of antimicrobial resistance.[31]

Moxifloxacin has been proposed as the drug of choice for treatment failures with azithromycin[27 28] but our finding of potentially 12.2% resistance to moxifloxacin as assessed by markers *gyrA* and *parC* is also above the 5% threshold set by the WHO.[30] Earlier studies reported cure rates of 100% with moxifloxacin.[32 34 35] However, more recently Tagg et al. reported macrolide resistance-associated mutations in the 23S rRNA gene in 43% of samples and mutations in *parC* or *gyrA* sequences in 15% of samples.[36] Touati et al reported a point mutation in the 23S rRNA gene in 14.2% of samples.[37]

Despite the relatively high prevalence of MG in both males and females in ours and other studies, the potential for significant morbidity and enhanced HIV transmission, global recommendations for MG screening are currently very diverse in part due to lack of access to good tests for MG. In the absence of an FDA approved test for MG, the U.S. CDC STD Treatment guidelines suggest that MG be suspected in cases of persistent/recurrent urethritis, cervicitis and PID.[27] Canada has a single Health Canada approved test for MG (Seegene Inc, Seoul, Korea) which is not widely available. The Europeans currently have the broadest recommendations for screening for MG including persons with STI symptoms and those engaging in high-risk sexual behavior, with a strong recommendation that all positive tests be followed by an assay capable of detecting macrolide resistance mutations.[28]

The optimal specimen type for MG testing remains unresolved with urine specimens considered acceptable in males and females and in females, vaginal swabs are also considered suitable.[25] In our study, the excellent agreement between the test performance in female endocervical swabs and urine is reassuring and supports the use of less invasive urine specimens for testing in females. It should be noted, however, that the comparison of test positivity in female urine and female endocervical swabs is likely biased since the subpopulation included in this calculation were more likely to be symptomatic than those not included. Organism burden may play a role in whether a woman is symptomatic or asymptomatic, and organism burden is also likely associated with test positivity.[38]

Our study has a few limitations. Firstly, our specimens were collected in STI clinic patients in Western Canada and may not be generalizable to other STI clinics and are likely to be higher than rates reported in non-STI clinic populations. Secondly, although the specimens were collected prospectively, we were only able to collect a limited number of additional variables in addition to standard data collection at the clinics due to time constraints; this may have limited our ability to identify additional correlates of MG. Thirdly, as tetracycline resistance-associated mutations have not so far been identified in *M. genitalium*[39], we did not test our samples for resistance to doxycycline; this information may have been useful in guiding empiric treatment regimens for NGU and cervicitis in our region.

In summary, our study found a MG prevalence of 6.2% in attendees at two Western Canadian STI Clinics, within the range reported in other studies, but higher than that for chlamydia (in

females) and gonorrhea (in both genders). Over one-half of tested isolates were resistant to macrolides. These findings together with the high proportion of asymptomatic carriers who could facilitate the spread of infection, the potential for significant morbidity and the potential for enhanced HIV transmission support recommendations for broader screening for MG. The high prevalence of macrolide resistance also supports the recommendation to follow all positive tests with an assay that can detect macrolide resistance mutations.[28] Judicious use of antibiotics for the empiric treatment of NGU and MPC is needed to mitigate the further development of resistance to currently used antibiotics and to optimize treatment of CT, NG and MG. In order to facilitate this, wider access to testing for MG and adaptation of most existing guidelines will be necessary.

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COMPETING INTERESTS None

AUTHOR CONTRIBUTIONS JG, SP, PN, LT, MC, IM, PS, RR, LB and AS developed the study design, protocol and ethics submission. LB coordinated funding for the study. JG, SP conducted epidemiologic analyses. JG, SP, AS drafted manuscript. PP, BB, JB, RS coordinated the study in the clinics. AB, SS, LT and IM coordinated and/or conducted laboratory testing. All authors contributed to final manuscript review.

DATA SHARING STATEMENT: No additional data are available.

DISCLAIMER

The opinions expressed in this manuscript are those of the authors and should not be construed to be those of any affiliated organization or entity.

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-9
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9,18, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	17
Outcome data	15*	Report numbers of outcome events or summary measures	9,18-19
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	

		(*b*) Report category boundaries when continuous variables were categorized	9,18-19
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	25-29
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	30
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	25-29
Generalisability	21	Discuss the generalisability (external validity) of the study results	30
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	32

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.