

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-016300
Article Type:	Research
Date Submitted by the Author:	05-Feb-2017
Complete List of Authors:	Gratrix, Jennifer Plitt, Sabrina Turnbull, LeeAnn Smyczek, Petra; University of Alberta, Medicine/Infectious Diseases; Alberta Health Services Brandley, Judith Scarrott, Ron; Alberta Health Services Naidu, Prenilla; Alberta Health Services Parker, Penny; Alberta Health Services Blore, Brenda; Alberta Health Services Bull, Amy; Alberta Health Services Shokoples, Sandy; Alberta Health Services Bertholet, Lindsay; Alberta Health Services Martin, Irene; Public Health Agency of Canada, Bacteriology and Enteric Diseases Chernesky, Max; McMaster Univ./St. Joseph's Healthcare, Read, Ron Singh, AE; University of Alberta, Medicine/Infectious Diseases
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Sexual health
Keywords:	Diagnostic microbiology < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES, BACTERIOLOGY

SCHOLARONE™
Manuscripts

1
2
3 1 **Prevalence and Antibiotic Resistance of *Mycoplasma genitalium* among STI Clinic Attendees**
4
5
6 2 **in Western Canada: a Cross-Sectional Analysis**
7
8
9 3

10
11 4 **Authors and Affiliations:**
12

13 Jennifer Gratrix¹, MSc; Sabrina Plitt², PhD; LeeAnn Turnbull³, BSc, MLT; Petra Smyczek^{1,4}, MD;
14
15 Judith Brandley⁴, BN; Ron Scarrott⁵, RN, BScN; Prenilla Naidu^{3,8}, MD; Penny Parker⁴, BN; Brenda
16
17 Blore⁵; Amy Bull³, MLT; Sandy Shokoples³, MSc, MLT; Lindsay Bertholet¹, MN, RN; Irene Martin⁶,
18
19 BSc; Max Chernesky⁷, PhD; Ron Read⁵, MD, PhD; Ameeta Singh^{4,8}, BMBS, MSc
20
21
22
23
24
25

26 10 ¹ STI Centralized Services, Alberta Health Services, Alberta, Canada; ²Public Health Agency of
27
28 11 Canada, Ottawa, Canada; ³Provincial Laboratory for Public Health, Edmonton, Canada; ⁴
29
30 12 Edmonton STI Clinic, Alberta Health Services, Edmonton, Canada; ⁵Calgary STI Clinic, Alberta
31
32 13 Health Services-, Calgary, Canada; ⁶National Microbiology Laboratory, Winnipeg, Canada;
33
34 14 ⁷McMaster University, Hamilton, Canada; ⁸University of Alberta, Edmonton, Canada.
35
36
37
38
39
40

41 16 **Key Words:** STD Epidemiology, *Mycoplasma genitalium*, STI clinics
42
43
44
45

46 18 **Word Count:** Manuscript: 3000;Abstract:250 ;References: 36;Tables:3
47
48
49
50

51 20 **Corresponding author:** Ameeta E. Singh
52

53 21 3B20 11111-Jasper Ave Edmonton, AB, Canada T5K0L4
54

55 22 Tel: 780 735 5678 ameeta@ualberta.ca
56
57
58
59
60

1
2
3 **Abstract**
4

5 **Objectives:** To determine the prevalence and correlates of *M. genitalium* (MG) infection,
6
7
8 compare test performance of female specimen types, and determine the prevalence of gene
9
10 mutations conferring resistance.
11

12 **Methods:** A cross sectional study was conducted on specimens collected for gonorrhoea (NG)
13
14 and Chlamydia (CT) among Alberta STI Clinic attendees using the *M. genitalium* Transcription
15
16 Mediated Amplification – Research Use Only (RUO) test (Hologic Inc, San Diego, CA). Female
17
18 endocervical and urine specimens were compared. Positive specimens were sequenced for
19
20 23SrRNA, *parC* and *gyrA* genes. Gender-stratified analysis compared test results using Chi-
21
22 square or Fisher’s exact test, Mann-Whitney test, and logistic regression.
23
24
25
26
27

28 **Results:** A total of 2,254 individuals were tested; 53.8% (n=1,212) were male. Male prevalence
29
30 of MG was 5.3%; CT was 5.9% and NG was 1.8%. Correlates of male infection were an NGU
31
32 diagnosis and NG co-infection. MG prevalence for females was 7.2%; CT was 5.8% and NG was
33
34 1.8%. Correlates of female infection were younger age, Indigenous/other ethnicity and CT/NG
35
36 co-infection. There was high concordance (98.1%) of results between urine and cervical swabs.
37
38 Nearly two-thirds of eligible specimens had mutations associated with macrolide resistance and
39
40 12.2% of specimens had a *parC* mutation signifying fluoroquinolone (FQ) resistance.
41
42

43 **Conclusions:** The high prevalence of MG relative to CT and NG supports the incorporation of
44
45 MG testing into routine STI screening and the good concordance of results between urine and
46
47 cervical swabs supports the use of female urine specimens for testing. The high rate of
48
49 resistance to macrolides and FQ raises concerns about treatment options.
50
51
52
53
54
55
56
57
58
59
60

1
2
3 454
5
6 46 Key Words: *Mycoplasma*, Clinical STI Care, Epidemiology (Clinical)7
8 479
10 48 **ARTICLE SUMMARY**11
12
13 49 **Strengths and Limitations of this study**

- 14
-
- 15
-
- 16 50
- 17 • This study is among the largest of global studies examining *Mycoplasma genitalium*
18 prevalence in heterosexual males, men who have sex with men and females attending
19 STI clinics.
20
21 52
 - 22 • We tested for resistance genes to macrolides as well as fluorquinolones.
23
24 53
 - 25 • We compared the performance of female urine specimens with endocervical swabs.
26
27 54
 - 28 • Our study is the first to examine *Mycoplasma genitalium* prevalence by ethnicity.
29
30 55
 - 31 • Although the specimens were collected prospectively, we were only able to collect a
32 limited number of additional variables in addition to standard data collection at the
33 clinics due to time constraints; this may have limited our ability to identify additional
34 correlates of *Mycoplasma genitalium*.
35
36 58
37
38 59
- 39
-
- 40
-
- 41 60

42
43
44 61
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

62 BACKGROUND

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

71
72 Globally, the prevalence of MG using molecular diagnostic tests ranges from 1-4% in men and
73 1-6% in women but is higher in those at risk for STI.⁵ In a recent Eastern Canadian study, male
74 prevalence was 4.5% and prevalence in females was 3.2%.⁶

75
76 In Canada, access to testing for MG is currently largely limited to the referral of suitable
77 specimens to the National Microbiology Laboratory (NML) in Winnipeg. Azithromycin has been
78 recommended for treatment of MG but rising resistance has raised concerns about the use of
79 this drug as the preferred option.⁷ Alternate treatment with moxifloxacin has been proposed
80 but the high cost of this medication, the potential for hepatotoxicity and reports of resistance
81 have also raised concerns.⁸

82

1
2
3 83 Given the anticipated wider availability of test kits to screen for MG in the future, we sought to
4
5 84 determine the prevalence and correlates of MG infection in urogenital specimens from
6
7
8 85 attendees at two Alberta STI clinics, to compare the test performance in different types of
9
10
11 86 urogenital specimens from females, and to determine the prevalence of mutations in genes
12
13
14 87 conferring resistance to macrolides and fluoroquinolones (FQ).
15
16
17
18
19

20 89 **METHODS**

21 90 Specimens collected from January to April, 2016 for NG and CT screening from urogenital sites
22
23 91 among sequential male and female attendees (>17 years old) at two Alberta STI Clinics were
24
25
26 92 tested for MG. Inclusion in the study required that at least two months had elapsed since
27
28 93 being treated for NG or CT to reduce chance visit was related to test of cure from previous
29
30
31 94 infection, and screening could not be part of patient follow-up if named as a sexual contact to a
32
33 95 NG/CT case to remove patients more likely to test positive. All individuals attending the two
34
35
36 96 STI clinics were screened for NG and CT unless they specifically declined: all men were screened
37
38 97 using urine tests while women were either screened with urine tests (asymptomatic, no
39
40
41 98 speculum examination performed) or with an endocervical or vaginal swab (the presence or
42
43 99 complaint of vaginal discharge, odor, or itching and speculum examination performed).
44
45

46 100
47
48 101 Basic demographic and clinical information was collected on the laboratory requisition form
49
50
51 102 and included ethnicity (Caucasian, Indigenous, or Other), presence of symptoms (yes/no; for
52
53 103 females, symptoms were defined as the presence or complaint of vaginal discharge, odor, or
54
55
56 104 itching and for males, urethral discharge or dysuria), diagnosis at the time of visit for those
57
58
59
60

1
2
3 105 undergoing physical examination (NGU, mucopurulent cervicitis (MPC), bacterial vaginosis
4
5
6 106 (BV)/yeast/trichomoniasis (TV) from wet mount) and self-reported HIV status at the time of the
7
8
9 107 patient visit. For male visits only, the gender of the sexual partner was recorded.

10
11 108
12
13 109 The *M. genitalium* Transcription Mediated Amplification – Research Use Only (Hologic Inc, San
14
15
16 110 Diego, CA) test was used to screen endocervical, vaginal and urine specimens. Endocervical
17
18 111 swabs are currently collected in preference to vaginal swabs in our STI clinics. For a female sub-
19
20
21 112 population, test results from endocervical and urine specimens collected on the same
22
23 113 individuals at the same visit were compared and the proportion of concordant results was
24
25
26 114 calculated. The Hologic Aptima Combo 2 assay was used to test for CT and NG. For men, NGU
27
28 115 was diagnosed if upon physical examination the Registered Nurse (RN) found urethral discharge
29
30
31 116 +/- dysuria plus urethral smear with >5 polymorphonuclear leukocytes/ high power field in 5 or
32
33 117 more fields with subsequent negative CT and NG test results. For women, BV, yeast, and TV
34
35
36 118 were diagnosed and treated at the time of visit based on clinical criteria (patient complaint of
37
38 119 abnormal vaginal discharge or RN assessment of abnormal vaginal discharge) for wet mount
39
40
41 120 assessment. The number of wet mounts completed was not collected and therefore we used
42
43 121 the number of individuals who had a pelvic examination as the denominator for prevalence of
44
45
46 122 BV, yeast, and TV. MPC was diagnosed based on the RN assessment of mucopurulent cervical
47
48 123 discharge or cervical friability on pelvic examination and negative tests for CT and NG.

49
50
51 124
52
53 125 All positive specimens for MG were sent to the NML for additional testing. DNA was
54
55
56 126 extracted from the specimens using the QIAamp Viral RNA Mini kit (Qiagen, Toronto,
57
58
59
60

1
2
3 127 Ontario) or the MagNA Pure DNA and Viral Nucleic Acid kit (Roche, Laval, Quebec) as per
4
5
6 128 manufacturer's instructions. Positive specimens were analyzed by sequencing 23SrRNA to
7
8 129 identify mutations associated with macrolide resistance and *parC* and *gyrA* genes
9
10
11 130 associated with resistance to fluoroquinolones.^{9 10}
12
13
14 131
15
16
17 132 Sample size was determined by budgetary costs, impact on clinic staff and an acceptable
18
19 133 margin of error. Using a sample size of 2000, our margin of error was +/- 1% for a 5%
20
21
22 134 prevalence rate with 95% confidence. Gender-stratified analysis was performed to compare
23
24 135 MG test result and MG resistance testing results by demographic and clinical variables using
25
26
27 136 Chi-square or Fisher's exact for discrete variables and Mann-Whitney for continuous variables,
28
29 137 excluding missing data. A two-tailed p-value of <0.05 was defined as statistically significant for
30
31
32 138 univariate analysis. Multivariable logistic regression was performed for both males and females
33
34 139 separately to identify correlates independently associated with a positive MG test result. In
35
36
37 140 addition, the results from endocervical/vaginal swabs were compared to urine specimens for
38
39 141 females and Cohen's Kappa was calculated. A 95% binomial confidence interval (CI) was
40
41
42 142 calculated for each infection prevalence. Data was analyzed using IBM SPSS Statistics version
43
44 143 19.0 (IBM, Armonk, NY, USA). This study was approved by the University of Alberta Health
45
46
47 144 Research Ethics Board.
48
49
50 145

51 146 RESULTS

52
53
54 147 A total of 2,294 individuals were tested. Forty patients were removed due to being <18 years
55
56
57 148 (n=20) and for having more than 1 visit during the study period (n=20). One-half (53.8%;

1
2
3 149 n=1,212) of the study population was men. The male prevalence of MG was 5.3% (95%CI 4.0-
4
5
6 150 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
7
8 151 prevalence was 6.6% with a CT prevalence of 3.4% and NG prevalence of 1.7%. In heterosexual
9
10 152 males, MG prevalence was 4.7% with a CT prevalence of 6.8% and NG prevalence of 2.0%. Of
11
12 153 73 cases of urethritis, 19.2% (n=14) were due to MG. One-third (37.0%; n=27) of NGU cases
13
14 154 were negative for MG, CT, and NG. Univariate correlates significantly associated with a higher
15
16 155 prevalence of MG infection among males included being symptomatic (p=0.001), a diagnosis of
17
18 156 NGU at time of visit (p<0.001), and co-infection with CT or NG (p=0.005 and p<0.001,
19
20 157 respectively) (Table 1). Independent correlates of infection with MG were a diagnosis of NGU
21
22 158 (Adjusted Odds Ratio (AOR)=7.6 95%CI 3.4-17.2) and co-infection with NG (AOR=7.2 95%CI 2.5-
23
24 159 20.4).
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

Category	Female				Male			
	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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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
Gonorrhoea	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
------------	-------------------------	---------------------	-------------------------

Interval

Male

Co-infection with Gonorrhoea	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
Gonorrhoea 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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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.

161

For peer review only

1
2
3 162
4
5
6 163 The overall MG prevalence for females, using any positive test result from endocervical/vaginal
7
8 164 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
9
10 165 1.8% (95%CI 1.0-2.6). Seven cases (1.6%) of MPC were diagnosed among 438 women who had
11
12 166 pelvic examinations. There was high concordance of results between urine and cervical swabs
13
14 167 (98.1%; Table 2; Kappa was 0.85 (95% CI: 0.77-1.0), representing almost perfect agreement.
15
16 168 Three vaginal swabs collected during our study were excluded from the comparison of results
17
18 169 between specimens.
19
20
21
22

23
24 Table 2. Concordance of *M. genitalium* Results from Cervical and Urine Screening among
25
26 Women.
27
28

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

29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47 22+330/359=98.1% concordance between cervical and urine results.
48

49
50 Kappa: 0.85 (95% CI: 0.77-1.0)
51
52

53
54 170
55
56
57
58
59
60

1
2
3 171 Univariate correlates significantly associated with a higher prevalence of MG infection (Table 1)
4
5
6 172 among women were younger median age (<0.001), Indigenous ethnicity (p<0.001), Other
7
8 173 ethnicity (p<0.001), the Edmonton clinic testing location (p=0.003), being pregnant (p=0.04), CT
9
10 174 or NG co-infection (p<0.001, for both), and MPC diagnosis at time of visit (p=0.01). Independent
11
12 175 correlates of infection with MG were younger age (AOR=0.92 95%CI 0.87-0.96), and Indigenous
13
14 176 ethnicity (AOR=4.3 95%CI 2.7-8.1) and Other ethnicity (AOR=2.8 95%CI 1.5-5.3) (vs. Caucasian),
15
16 177 co-infection with CT (AOR=5.1 95%CI 2.6-10.2) and NG (AOR=3.5 95%CI=1.0-11.8).
17
18
19
20
21 178

22
23 179 Macrolide resistance data provided through 23SrRNA sequencing data was available for 73.4%
24
25 180 (n=47) of the 64 positive male specimens. Nearly two-thirds (63.8%; n=30) were found to have
26
27 181 mutations associated with macrolide resistance in either A2058T (n=3), A2058G (n=12), or
28
29 182 A2059G (n=15). There were no variables significantly associated with macrolide resistance,
30
31 183 although MSM was marginally associated with resistance among males (83.3% vs. 51.9%;
32
33 184 p=0.06; Table 3). Resistance to fluoroquinolones was assessed by markers *gyrA* and *parC*.
34
35
36 185 Nearly two-thirds (64.1%; n=41) of positive specimens had *parC* sequences available and 5
37
38 186 (12.2%) specimens had a *parC* mutation (Ser→Ile83, n=4) and (Asp→Tyr87, n=1) signifying
39
40 187 fluoroquinolone resistance. *gyrA* sequencing was performed on 46 specimens and no *gyrA*
41
42 188 mutations were identified.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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	22 (20-26)	26 (22-29)	24 (20-28)	0.04	29 (23-41)	27 (25-40)	28 (24-41)	0.90
(IQR)								
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)	

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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
----------	-----------	-----------	-----------	------	-----------	----------	-----------	------

For peer review only

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
Gonorrhoea	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.

1
2
3 190 Among women, 23SrRNA sequencing data was available for 60.0% (45/75) of positive
4
5
6 191 specimens. Nearly one-half (48.9%; n=22) had a 23SrRNA mutation associated with macrolide
7
8 192 resistance in A2058G (n=11), A2058T (n=5), A2059G (n=6), or A2059C (n=1). In univariate
9
10 193 analysis, younger median age (22y (IQR: 20-26) vs. 26y (IQR: 22-29); p=0.04; Table 3) was the
11
12 194 only variable significantly correlated with macrolide resistance. One-half (50.7%; n=38) of
13
14 195 positive specimens had *parC* sequencing available and only 1 specimen had a mutation
15
16 196 signifying fluoroquinolone resistance (Asp→Tyr87); no *gyrA* mutations were identified.
17
18
19
20
21 197

22 198 **DISCUSSION**

23
24
25 199 Our study underscores the significance of *M. genitalium* as a medically significant pathogen
26
27 200 from urogenital sites. In our male population, the prevalence of MG was 5.3%, within the range
28
29 201 of 3.1%-17.2% reported in males from other STI Clinics.^{6 11 12 13} Although not statistically
30
31 202 significant, the prevalence of MG in our study trended toward being higher in MSM than among
32
33 203 heterosexual males (6.6% vs 4.7%, p=0.18). A recent Dutch study reported a similar prevalence
34
35 204 of MG in males (overall MG prevalence in males 3.1%; 2.5% in MSM, 3.8% in MSW, p=0.13).¹² A
36
37 205 diagnosis of NGU was significantly correlated with MG infection among males in our study
38
39 206 population, in accordance with previous studies reporting a strong association between MG
40
41 207 and NGU independent of Chlamydia infection.¹⁵ In a meta-analysis of studies completed up to
42
43 208 2010, MG was associated with a pooled odds ratio (OR) of 5.5 (95% CI: 4.4-7.0) for NGU.¹
44
45 209
46
47
48
49
50
51
52
53 210 The overall MG prevalence for females was 7.2% (95%CI 5.6-8.8), higher than the range of 3.2-
54
55 211 6% reported in most studies of females STI clinic attendees.^{6 12 14 15} In females, MG has been
56
57
58
59
60

1
2
3 212 associated with significant morbidity including MPC, PID and infertility, but the association
4
5
6 213 between MG and symptoms is less clear.^{10 16 17} Among female STI clinic attendees in some
7
8
9 214 studies, 40-75% were asymptomatic^{14 15} but a 1994-96 French study reported a very high
10
11 215 prevalence of MG of 38% among symptomatic female STI clinic attendees.¹⁶ The presence of
12
13 216 symptoms was not an independent correlate of MG infection in our study.
14
15
16 217
17
18 218 Independent correlates of female infection with MG in our study were younger age, in contrast
19
20
21 219 to two other studies which reported that the prevalence of MG peaked approximately 5 years
22
23 220 later for both men and women and remained higher in older age groups.^{18 19} Co-infection with
24
25
26 221 CT and NG was common in our patients, confirming the role of MG as a sexually transmitted
27
28 222 pathogen and the probable overlap in behavioural and demographic characteristics for these
29
30
31 223 STIs.
32
33 224
34
35
36 225 Indigenous (First Nations, Inuit, Metis) ethnicity and other non- Caucasian ethnicity were also
37
38 226 significant correlates of MG infection. We were unable to find other studies examining the
39
40
41 227 prevalence of MG by ethnicity but the disproportionately high rates of MG among Indigenous
42
43 228 persons in our study is in keeping with the higher estimated STI prevalence in Canadian
44
45
46 229 Indigenous persons when compared to the overall general population.^{20 21} First Nations persons
47
48 230 represent an estimated 3.8% of the overall Canadian population but chlamydia rates are
49
50
51 231 estimated to be 7 times higher among First Nations adults than the overall population.²⁰ The
52
53 232 reasons for the observed disproportionately high rates of STIs are unclear but Indigenous
54
55
56 233 persons in Canada are also over-represented in adolescent pregnancy and under-represented in

1
2
3 234 sexual health research.²² A recent First Nations Regional Health Survey stressed the importance
4
5
6 235 of colonial history, barriers to health care services and socio-economic disadvantage.²⁰
7
8 236
9
10 237 The optimal specimen type for MG testing remains unresolved with urine specimens considered
11
12 238 acceptable in males and females and in females, vaginal swabs are also considered suitable.²³ In
13
14 239 our study, the near perfect agreement between the test performance in female cervical and
15
16 240 urine swabs is reassuring and supports the use of less invasive urine specimens for testing in
17
18 241 females.
19
20
21
22 242
23
24 243 It is very likely that appropriate treatment of MG infections will result in reduced sexual
25
26 244 transmission as well as prevention of complications.² Alternates to macrolides and FQ, the
27
28 245 antibiotics usually proposed for the treatment of MG, are limited since the lack of a cell wall in
29
30 246 MG precludes the use of penicillins and other beta lactam antibiotics.²⁴ Further complicating
31
32 247 this is that mycoplasmas can develop resistance either by gene mutation or by acquisition of a
33
34 248 resistance gene.²⁴ Since azithromycin has been proposed as the preferred first line agent for the
35
36 249 treatment of MG infections^{25 26}, the high rate of mutations (~2/3 of eligible specimens)
37
38 250 conferring resistance to azithromycin in our study is particularly alarming. Strains of *M.*
39
40 251 *genitalium* began to develop resistance to azithromycin and have continued to do so through
41
42 252 mutations in region V of the 23S ribosomal RNA gene.²⁷ Macrolide resistance rates vary
43
44 253 significantly by geographic region with 58% resistance reported in the only published Canadian
45
46 254 study conducted in Eastern Canada.⁶ This level of resistance is well above the threshold of 5%
47
48 255 resistance above which the World Health Organization typically recommends against the
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 256 routine use of a drug for first line treatment of an STI.²⁸ A recent review reported that the
4
5
6 257 efficacy of azithromycin 1 gm for the treatment of urogenital MG has decreased from 85% prior
7
8
9 258 to 2009 to 60% in early 2015.⁷ This had been postulated to be due to increasing prevalence of
10
11 259 macrolide resistance due to the widespread use of azithromycin for the treatment of CT, NGU
12
13 260 and MPC.^{7 29 30 31} In a recent meta-analysis, persistent MG was associated with a pooled OR of
14
15
16 261 26 (95% CI: 11–57) for persistent urethritis, demonstrating that failure to eradicate MG leads to
17
18 262 persistent or recurrent signs and symptoms of urethritis in the majority of men.⁷ The
19
20
21 263 observation of MG as a significant pathogen in both NGU and MPC has generated much
22
23 264 discussion around whether azithromycin, and especially single dose azithromycin should
24
25
26 265 continue to be recommended as the preferred agent for these STI syndromes.²⁹ Instead it has
27
28 266 been proposed that doxycycline be used as the first line agent because even though it is in only
29
30
31 267 30-40% effective against MG, it does not induce the development of antimicrobial resistance.²⁹
32
33
34 268
35
36 269 Moxifloxacin has been proposed as the drug of choice for treatment failures with
37
38 270 azithromycin^{25 26} but our finding of 12.2% resistance to FQ as assessed by markers *gyrA* and
39
40
41 271 *parC* is also above the 5% threshold set by the WHO.²⁸ Earlier studies reported cure rates of
42
43 272 100% with moxifloxacin.^{30 32 33} However, more recently Tagg et al. reported macrolide
44
45
46 273 resistance-associated mutations in the 23S rRNA gene in 43% of samples and mutations in *parC*
47
48 274 or *gyrA* sequences in 15% of samples.³⁴ Touati et al reported a point mutation in the 23S rRNA
49
50
51 275 gene in 14.2% of samples.³⁵
52
53
54 276
55
56 277 Given the relatively high prevalence of MG in both males and females in ours and other studies,
57
58
59
60

1
2
3 278 the potential for significant morbidity and enhanced HIV transmission, global recommendations
4
5
6 279 for MG screening are currently very diverse in part due to lack of access to good tests for MG.
7
8
9 280 In the absence of an FDA approved test for MG, the U.S. CDC STD Treatment guidelines suggest
10
11 281 that MG be suspected in cases of persistent/recurrent urethritis, cervicitis and PID.²⁵ Canada
12
13 282 has a single Health Canada approved test for MG (Seegene Inc, Seoul, Korea) which is not
14
15
16 283 widely available. The Europeans currently have the broadest recommendations for screening
17
18 284 for MG including persons with STI symptoms and those engaging in high-risk sexual behavior,
19
20
21 285 with a strong recommendation that all positive tests be followed by an assay capable of
22
23 286 detecting macrolide resistance mutations.²⁶
24

25
26 287
27
28 288 Our study has a few limitations. Firstly, our specimens were collected in STI clinic patients in
29
30 289 Western Canada and may not be generalizable to other STI clinics and are likely to be higher
31
32
33 290 than rates reported in non-STI clinic populations. Secondly, although the specimens were
34
35
36 291 collected prospectively, we were only able to collect a limited number of additional variables in
37
38 292 addition to standard data collection at the clinics due to time constraints; this may have limited
39
40
41 293 our ability to identify additional correlates of MG. Thirdly, as tetracycline resistance-associated
42
43 294 mutations have not so far been identified in *M. genitalium*³⁶, we did not test our samples for
44
45
46 295 resistance to doxycycline; this information may have been useful in guiding empiric treatment
47
48 296 regimens for NGU and cervicitis in our region.
49

50
51 297
52
53 298 In summary, our study found a MG prevalence of 6.2% in attendees at two Western Canadian
54
55
56 299 STI Clinics, within the range reported in other studies, but higher than that for chlamydia (in
57
58
59
60

1
2
3 300 females) and gonorrhoea (in both genders). Over one-half of tested isolates were resistant to
4
5
6 301 macrolides. These findings together with the high proportion of asymptomatic carriers who
7
8 302 could facilitate the spread of infection, the potential for significant morbidity and the potential
9
10 303 for enhanced HIV transmission support recommendations for broader screening for MG. The
11
12 304 high prevalence of macrolide resistance also supports the recommendation to follow all
13
14 305 positive tests with an assay that can detect macrolide resistance mutations.²⁶ Judicious use of
15
16 306 antibiotics for the empiric treatment of NGU and MPC is needed to mitigate the further
17
18 307 development of resistance to currently used antibiotics and to optimize treatment of CT, NG
19
20
21 308 and MG. In order to facilitate this, wider access to testing for MG and adaptation of most
22
23 309 existing guidelines will be necessary.
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 310 **ACKNOWLEDGEMENTS**
4

5
6 311 We thank Hologic Inc, Canada for providing the test kits used in this study, the study
7
8 312 participants and staff of the Edmonton and Calgary STI clinics for enrolling patients in the study.
9

10 313

11
12
13 314 **FUNDING**
14

15
16 315 This work was funded in part by an internal grant from Alberta Health Services- STI Centralized
17
18 316 Services to the Alberta Provincial Laboratory for Public Health to complete testing. *M.*
19
20 317 *genitalium* test kits were provided by Hologic Inc, Canada.
21
22

23 318

24
25
26 319 **COMPETING INTERESTS** None
27

28 320

29
30
31 321 **AUTHOR CONTRIBUTIONS** JG, SP, PN, LT, MC, IM, PS, RR, LB and AS developed the study
32
33 322 design, protocol and ethics submission. LB coordinated funding for the study. JG, SP conducted
34
35 323 epidemiologic analyses. JG, SP, AS drafted manuscript. PP, BB, JB, RS coordinated the study in
36
37 324 the clinics. AB, SS, LT and IM coordinated and/or conducted laboratory testing. All authors
38
39 325 contributed to final manuscript review.
40
41

42 326

43
44
45 327 **DATA SHARING STATEMENT:** No additional data are available.
46
47

48 328
49
50
51
52
53
54
55
56
57
58
59
60

329 REFERENCES

- 1
2
3
4
5
6
7 330 1. **Taylor-Robinson D**, Jensen JS. *Mycoplasma genitalium*: from Chrysalis to multicolored
8
9
10 331 butterfly. *Clin Microbiol Rev* 2011;**24**:498-514.
- 11
12 332 2. **Lis R**, Rowhani-Rahbar A, Manhart LE. *Mycoplasma genitalium* Infection and Female
13
14 333 Reproductive Tract Disease: A Meta-Analysis. *Clin Infect Dis* **2015**;61:418-26.
- 15
16
17 334 3. **Short VL**, Totten PA, Ness RB, *et al*. Clinical presentation of *Mycoplasma genitalium*
18
19 335 infection versus *Neisseria gonorrhoeae* infection among women with pelvic
20
21
22 336 inflammatory disease. *Clin Infect Dis* 2009;**48**:41-7.
- 23
24 337 4. **Napierala Mavedzenge S**, Weiss HA. Association of *Mycoplasma genitalium* and HIV
25
26
27 338 infection: a systematic review and meta-analysis. *AIDS* 2009;**23**:611-620.
- 28
29 339 5. **Manhart LE**, Broad JM, Golden MR. *Mycoplasma genitalium*: should we treat and how?
30
31 340 *Clin Infect Dis* 2011;**53 Suppl 3**:S129-42.
- 32
33
34 341 6. **Gesink D**, Racey CS, Seah C, *et al*. *Mycoplasma genitalium* in Toronto, Canada: Estimates
35
36 342 of Prevalence and Macrolide Resistance. *Can Fam Physician* 2016;**62**:e96-101.
- 37
38
39 343 7. **Lau A**, Bradshaw CS, Lewis D, *et al*. The efficacy of azithromycin for the treatment of
40
41 344 genital *Mycoplasma genitalium*: a systematic review and meta-analysis. *Clin Infect Dis*
42
43 345 2015; **61**:1389–99.
- 44
45
46 346 8. **Bissessor M**, Tabrizi SN, Twin J, *et al*. Macrolide resistance and azithromycin failure in a
47
48 347 *Mycoplasma genitalium*-infected cohort, and response of azithromycin failures to
49
50
51 348 alternative antibiotic regimens. *Clin Infect Dis* 2015; **60**:1228–36.
- 52
53
54
55
56
57
58
59
60

- 1
2
3 349 9. **Jensen JS**. Protocol for the detection of *Mycoplasma genitalium* by PCR from clinical
4
5
6 350 specimens and subsequent detection of macrolide resistance-mediating mutations in
7
8 351 region V of the 23SrRNA gene. *Method Molec Biol* 2012, **903**:129-139.
- 10 352 10. **Shimada Y**, Deguchi T, Nakane K, *et al*. Emergence of clinical strains of *Mycoplasma*
12
13 353 *genitalium* harbouring alterations in *ParC* associated with fluoroquinolone resistance.
14
15
16 354 *Int J Antimicrob Agent* 2010, **36**:255-8.
- 18 355 11. **Getman D**, Jiang A, O'Donnell M, Cohen S. *Mycoplasma genitalium* Prevalence,
19
20
21 356 Coinfection, and Macrolide Antibiotic Resistance Frequency in a Multicenter Clinical
22
23 357 Study Cohort in the United States. *J Clin Microbiol*. 2016;**54**:2278-83.
- 26 358 12. **de Jong AS**, Rahamat-Langendoen JC, van Alphen P, *et al*. Large two-centre study into
27
28 359 the prevalence of *Mycoplasma genitalium* and *Trichomonas vaginalis* in the
29
30
31 360 Netherlands. *Int J STD AIDS* 2016;**27**:856-60.
- 33 361 13. **van der Veer C**, van Rooijen MS, Himschoot M, *et al*. *Trichomonas vaginalis*
34
35
36 362 and *Mycoplasma genitalium*: age-specific prevalence and disease burden in men
37
38 363 attending a sexually transmitted infections clinic in Amsterdam, the Netherlands. *Sex*
39
40
41 364 *Transm Infect* 2016;**92**:83-5.
- 43 365 14. **Falk L**, Fredlund H, Jensen JS. Signs and symptoms of urethritis and cervicitis among
44
45
46 366 women with or without *Mycoplasma genitalium* or *Chlamydia trachomatis* infection. *Sex*
47
48 367 *Transm Infect* 2005; **81**:73–78.
- 51 368 15. **Anagrius C**, Lorange B, Jensen JS. *Mycoplasma genitalium*: prevalence, clinical
52
53
54 369 significance, and transmission. *Sex Transm Infect* 2005; **81**: 458–462.

- 1
2
3 370 16. **Casin I**, Vexiau-Robert D, de la Salmoniere P, *et al*. High prevalence of *Mycoplasma*
4
5
6 371 *genitalium* in the lower genital tract of women attending a sexually transmitted disease
7
8 372 clinic in Paris, France. *Sex Transm Dis* 2002;**29**:353–9.
- 9
10
11 373 17. **Huppert JS**, Mortensen JE, Reed JL, *et al*. *Mycoplasma genitalium* detected by
12
13 374 transcription-mediated amplification is associated with *Chlamydia trachomatis* in
14
15 375 adolescent women. *Sex Transm Dis* 2008;**35**:250-4.
- 16
17
18
19 376 18. **Jensen JS**, Bjørnørnelius E, Dohn B, Lidbrink P. Comparison of first void urine and
20
21 377 urogenital swab specimens for detection of *Mycoplasma genitalium* and *Chlamydia*
22
23 378 *trachomatis* by polymerase chain reaction in patients attending a sexually transmitted
24
25 379 disease clinic. *Sex Transm Dis* 2004;**31**:499–507.
- 26
27
28
29 380 19. **Salado-Rasmussen K**, Jensen JS. *Mycoplasma genitalium* testing pattern and macrolide
30
31 381 resistance: a Danish nationwide retrospective survey. *Clin Infect Dis* 2014; **59**:24–30.
- 32
33
34 382 20. **The First Nations Information Governance Centre**, First Nations Regional Health Survey
35
36 383 (RHS) Phase 2 (2008/10) National Report on Adults, Youth and Children Living in First
37
38 384 Nations Communities. **2012**.
39
40 385 http://fnigc.ca/sites/default/files/First_Nations_Regional_Health_Survey_2008-
41
42 386 [10_National_Report.pdf](http://fnigc.ca/sites/default/files/First_Nations_Regional_Health_Survey_2008-10_National_Report.pdf) (accessed 19 Jan 2017).
- 43
44
45
46 387 21. **Public Health Agency of Canada**. Population-Specific HIV/AIDS Status Report: Aboriginal
47
48 388 Peoples. **2010**. <http://www.catie.ca/sites/default/files/26344.pdf> (accessed 19 Jan
49
50 389 2017).
- 51
52
53
54 390 22. **Devries KM**, Free CJ, Morison L, Saewyc E. Factors associated with pregnancy and STI
55
56 391 among Aboriginal students in British Columbia. *Can J Public Health* 2009;**100**:226-30.
- 57
58
59
60

- 1
2
3 392 23. **Jensen JS**, Cusini M, Gomberg M, Moi H. Background review for the 2016 European
4
5
6 393 guideline on *Mycoplasma genitalium* infections. *J Eur Acad Dermatol Venereol*
7
8 394 2016;**30**:1686-93.
9
10
11 395 24. **Taylor-Robinson D**. Diagnosis and antimicrobial treatment of *Mycoplasma*
12
13 396 *genitalium* infection: sobering thoughts. *Expert Rev Anti Infect Ther* 2014;**12**:715-22.
14
15
16 397 25. **Centers for Disease Control and Prevention**. Sexually Transmitted Diseases Treatment
17
18 398 Guidelines, 2015. <https://www.cdc.gov/std/tg2015/> (accessed 16 Jan 2017).
19
20
21 399 26. **Jensen JS**, Cusini M, Gomberg M. 2016 European Guideline on *Mycoplasma genitalium*
22
23 400 infections.
24
25 401 [http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.p](http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.pdf)
26
27 402 [df](http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.pdf) (accessed 16 Jan 2017).
28
29 403 27. **Jensen JS**, Bradshaw CS, Tabrizi SN, *et al*. Azithromycin treatment failure in *Mycoplasma*
30
31 404 *genitalium*-positive patients with nongonococcal urethritis is associated with induced
32
33 405 macrolide resistance. *Clin Infect Dis* 2008;**47**:1546-53.
34
35
36 406 28. **World Health Organization**. Guidelines for the management of sexually transmitted
37
38 407 infections. 2003. WHO, Geneva, Switzerland.
39
40
41 408 29. **Horner PJ**. Editorial Commentary: *Mycoplasma genitalium* and Declining Treatment
42
43 409 Efficacy of Azithromycin 1 g: What Can We Do? *Clin Infect Dis* 2015;**61**:1400-2.
44
45
46 410 30. **Anagrius C**, Loré B, Jensen JS. Treatment of *Mycoplasma genitalium*: observations from
47
48 411 a Swedish STD Clinic. *PLoS One* 2013; **8**: e61481.
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 412 31. **Ito S**, Shimada Y, Yamaguchi Y, et al. Selection of *Mycoplasma genitalium* strains
4
5
6 413 harbouring macrolide resistance-associated 23S rRNA mutations by treatment with a
7
8 414 single 1 g dose of azithromycin. *Sex Transm Infect* 2011; **87**:412–4.
9
10 415 32. **Bradshaw CS**, Chen MY, Fairley CK. Persistence of *Mycoplasma genitalium* following
11
12 416 azithromycin therapy. *PLoS One* 2008;**3**:e3618.
13
14
15 417 33. **Jernberg E**, Moghaddam A, Moi H. Azithromycin and moxifloxacin for microbiological
16
17 418 cure of *Mycoplasma genitalium* infection: an open study. *Int Journal STD AIDS*
18
19 419 2008;**19**:676–9.
20
21
22 420 34. **Tagg KA**, Jeffreys NJ, Couldwell DL, et al. Fluoroquinolone and macrolide resistance-
23
24 421 associated mutations in *Mycoplasma genitalium*. *J Clin Microbiol* 2013;**51**:2245-9.
25
26
27 422 35. **Touati A**, Peuchant O, Jensen JS, Bébéar C, Pereyre S. Direct detection of macrolide
28
29 423 resistance in *Mycoplasma genitalium* isolates from clinical specimens from France by
30
31 424 use of real-time PCR and melting curve analysis. *J Clin Microbiol* 2014;**52**:1549-55.
32
33
34 425 36. **Couldwell DL**, Lewis DA. *Mycoplasma genitalium* infection: current treatment options,
35
36 426 therapeutic failure, and resistance associated mutations. *Infect Drug Resist* 2015;**8**:147-
37
38 427 61.
39
40
41
42
43
44 428

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-016300.R1
Article Type:	Research
Date Submitted by the Author:	11-Apr-2017
Complete List of Authors:	Gratrix, Jennifer; Alberta Health Services, STI Centralized Services Plitt, Sabrina; Public Health Agency of Canada Turnbull, LeeAnn; Provincial Laboratory for Public Health Smyczek, Petra; Alberta Health Services, Edmonton STI Clinic; Alberta Health Services, Centralized STI Services Brandley, Judith; Alberta Health Services, Edmonton STI Clinic Scarrott, Ron; Alberta Health Services, Calgary STI Clinic Naidu, Prenilla; Provincial Laboratory for Public Health Parker, Penny; Alberta Health Services, Edmonton STI Clinic Blore, Brenda; Alberta Health Services, Calgary STI Clinic Bull, Amy; Provincial Laboratory for Public Health Shokoples, Sandy; Provincial Laboratory for Public Health Bertholet, Lindsay; Alberta Health Services, Centralized STI Services Martin, Irene; Public Health Agency of Canada, Bacteriology and Enteric Diseases Chernesky, Max; McMaster Univ./St. Joseph's Healthcare, Read, Ron; Alberta Health Services, Calgary STI Clinic Singh, AE; University of Alberta, Medicine/Infectious Diseases; Alberta Health Services, Edmonton STI Clinic
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Sexual health
Keywords:	Diagnostic microbiology < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES, mycoplasma, clinical sti care

SCHOLARONE™
Manuscripts

1
2
3 1 **Prevalence and Antibiotic Resistance of *Mycoplasma genitalium* among STI Clinic Attendees**
4
5
6 2 **in Western Canada: a Cross-Sectional Analysis**
7
8
9 3

10
11 4 **Authors and Affiliations:**

12
13 5 Jennifer Gratrix¹, MSc; Sabrina Plitt², PhD; LeeAnn Turnbull³, BSc, MLT; Petra Smyczek^{1,4}, MD;
14
15 6 Judith Brandley⁴, BN; Ron Scarrott⁵, RN, BScN; Prenilla Naidu^{3,8}, MD; Penny Parker⁴, BN; Brenda
16
17 7 Blore⁵; Amy Bull³, MLT; Sandy Shokoples³, MSc, MLT; Lindsay Bertholet¹, MN, RN; Irene Martin⁶,
18
19 8 BSc; Max Chernesky⁷, PhD; Ron Read⁵, MD, PhD; Ameeta Singh^{4,8}, BMBS, MSc
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

10 ¹ STI Centralized Services, Alberta Health Services, Alberta, Canada; ²Public Health Agency of
11 Canada, Ottawa, Canada; ³Provincial Laboratory for Public Health, Edmonton, Canada; ⁴
12 Edmonton STI Clinic, Alberta Health Services, Edmonton, Canada; ⁵Calgary STI Clinic, Alberta
13 Health Services-, Calgary, Canada; ⁶National Microbiology Laboratory, Winnipeg, Canada;
14 ⁷McMaster University, Hamilton, Canada; ⁸University of Alberta, Edmonton, Canada.

16 **Key Words:** STD Epidemiology, *Mycoplasma genitalium*, STI clinics

18 **Word Count:** Manuscript: 3023;Abstract:256 ;References: 39;Tables:3

20 **Corresponding author:** Ameeta E. Singh

21 3B20 11111-Jasper Ave Edmonton, AB, Canada T5K0L4

22 Tel: 780 735 5678

ameeta@ualberta.ca

1
2
3 **Abstract**
4

5 **Objectives:** To determine the prevalence and correlates of *M. genitalium* (MG) infection among
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
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 gonorrhoea (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.

1
2
3 45 Key Words: *Mycoplasma*, Clinical STI Care, Epidemiology (Clinical)
4
5

6 46 **Strengths and Limitations of this study**
7

- 8 47 • The main strength of this study is that it is among the largest of global studies examining
9
10 48 *Mycoplasma genitalium* prevalence in heterosexual males, men who have sex with men
11
12 and females attending STI clinics.
13
14 49
15
16 50 • Since we were only able to collect a small number of additional variables over standard
17
18 51 clinic protocol due to time constraints, this may have limited our ability to identify
19
20 52 additional correlates of *Mycoplasma genitalium* infection.
21
22
23
24 53
25
26 54
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

55 BACKGROUND

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

64
65 Globally, the prevalence of MG using molecular diagnostic tests ranges from 1-4% in men and
66 1-6% in women but is higher in those at risk for STI.⁵ In a recent Eastern Canadian study, male
67 prevalence was 4.5% and prevalence in females was 3.2%.⁶

68
69 In Canada, access to testing for MG is currently largely limited to the referral of suitable
70 specimens to the National Microbiology Laboratory (NML) in Winnipeg. Azithromycin has been
71 recommended for treatment of MG but rising resistance has raised concerns about the use of
72 this drug as the preferred option.⁷ Alternate treatment with moxifloxacin has been proposed
73 but the high cost of this medication, the potential for hepatotoxicity and reports of resistance
74 have also raised concerns.⁸

75

1
2
3 76 Given the anticipated wider availability of test kits to screen for MG in the future, we sought to
4
5
6 77 determine the prevalence and correlates of MG infection in urogenital specimens from
7
8
9 78 attendees at two Alberta STI clinics, to compare the test performance in different types of
10
11 79 urogenital specimens from females, and to determine the prevalence of mutations in genes
12
13 80 conferring resistance to macrolides and moxifloxacin.
14
15
16 81

18 82 **METHODS**

20
21 83 Specimens collected from January to April 2016 for NG and CT screening from urogenital sites
22
23 84 among sequential male and female attendees (>17 years old) at two Alberta STI Clinics were
24
25
26 85 tested for MG. Inclusion in the study required that at least two months had elapsed since
27
28 86 being treated for NG or CT to reduce chance visit was related to test of cure from previous
29
30
31 87 infection, and screening could not be part of patient follow-up if named as a sexual contact to a
32
33 88 NG/CT case to remove patients more likely to test positive. All individuals attending the two
34
35
36 89 STI clinics were screened for NG and CT unless they specifically declined: all men were screened
37
38 90 using urine tests while women were either screened with urine tests (mostly asymptomatic) or
39
40
41 91 with an endocervical or vaginal swab (mostly symptomatic).
42
43
44 92

46 93 Basic demographic and clinical information was collected on the laboratory requisition form
47
48 94 and included ethnicity (Caucasian, Indigenous, or Other), presence of symptoms (yes/no; for
49
50 95 females, symptoms were defined as the presence or complaint of vaginal discharge, odor, or
51
52 96 itching and for males, urethral discharge or dysuria), diagnosis at the time of visit for those
53
54
55 97 undergoing physical examination (NGU, mucopurulent cervicitis (MPC)) and self-reported HIV
56
57
58
59
60

1
2
3 98 status at the time of the patient visit. For male visits only, the gender of the sexual partner was
4
5
6 99 recorded. For men, NGU was diagnosed if upon physical examination the Registered Nurse (RN)
7
8
9 100 found urethral discharge +/- dysuria plus urethral smear with >5 polymorphonuclear
10
11 101 leukocytes/ high power field in 5 or more fields with subsequent negative CT and NG test
12
13 102 results. For women, MPC was diagnosed based on the RN assessment of mucopurulent cervical
14
15
16 103 discharge or cervical friability on vaginal speculum examination.
17

18
19 104

20
21 105 The *M. genitalium* Transcription Mediated Amplification – Research Use Only (Hologic Inc, San
22
23 106 Diego, CA) test was used to screen endocervical, vaginal, and urine specimens. Endocervical
24
25
26 107 swabs are currently collected in preference to vaginal swabs in our STI clinics. For a female sub-
27
28 108 population, test results from endocervical and urine specimens collected on the same
29
30
31 109 individuals at the same visit were compared and the proportion of concordant results was
32
33 110 calculated. The Hologic Aptima Combo 2 assay was used to test for CT and NG.
34
35

36 111

37
38 112 All positive specimens for MG were sent to the NML for additional testing. DNA was
39
40
41 113 extracted from the specimens using the QIAamp Viral RNA Mini kit (Qiagen, Toronto,
42
43 114 Ontario) or the MagNA Pure DNA and Viral Nucleic Acid kit (Roche, Laval, Quebec) as per
44
45
46 115 manufacturer's instructions. Positive specimens were analyzed by sequencing 23SrRNA to
47
48 116 identify mutations associated with macrolide resistance and *parC* and *gyrA* genes
49
50
51 117 associated with potential resistance to moxifloxacin.^{9 10}
52

53
54 118
55
56
57
58
59
60

1
2
3 119 Sample size was determined by budgetary costs, impact on clinic staff and an acceptable
4
5
6 120 margin of error. Using a sample size of 2000, our margin of error was +/- 1% for a 5%
7
8
9 121 prevalence rate with 95% confidence. Gender-stratified analysis was performed to compare
10
11 122 MG test result and MG resistance testing results by demographic and clinical variables using
12
13 123 Chi-square or Fisher's exact for discrete variables and Mann-Whitney for continuous variables,
14
15
16 124 excluding missing data. A two-tailed p-value of <0.05 was defined as statistically significant for
17
18
19 125 univariate analysis. Multivariable logistic regression was performed for both males and females
20
21 126 separately to determine adjusted estimates of odds ratios (AOR) and 95% CI for correlates
22
23 127 independently associated with a positive MG test result. All variables with a statistical
24
25
26 128 significance of $p < 0.10$ in univariate analysis were considered in the regression models.
27
28
29 129 Variables were removed from the model if they were deemed to be non-significant or did not
30
31 130 contribute significantly to the overall model. In addition, the results from endocervical swabs
32
33
34 131 were compared to urine specimens for females and Cohen's Kappa was calculated. A 95%
35
36 132 binomial confidence interval (CI) was calculated for each infection prevalence. Data was
37
38
39 133 analyzed using IBM SPSS Statistics version 19.0 (IBM, Armonk, NY, USA). This study was
40
41 134 approved by the University of Alberta Health Research Ethics Board.
42
43
44 135

45 46 136 **RESULTS**

47
48 137 A total of 2,294 individuals were tested. Forty patients were removed due to being <18 years
49
50
51 138 (n=20) and for having more than 1 visit during the study period (n=20). The overall MG
52
53
54 139 prevalence was 6.2% (95%CI 5.2-7.2). One-half (53.8%; n=1,212) of the study population was
55
56 140 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
57
58
59
60

1
2
3 141 NG was 1.8% (95%CI 1.1-2.6). Among MSM, the MG prevalence was 6.6% with a CT prevalence
4
5
6 142 of 3.4% and NG prevalence of 1.7%. In heterosexual males, MG prevalence was 4.7% with a CT
7
8
9 143 prevalence of 6.8% and NG prevalence of 2.0%. Of 73 cases of urethritis, 19.2% (n=14) were
10
11 144 due to MG. One-third (37.0%; n=27) of NGU cases were negative for MG, CT, and NG.
12
13 145 Univariate correlates significantly associated with a higher prevalence of MG infection among
14
15
16 146 males included being symptomatic (p=0.001), a diagnosis of NGU (p<0.001), and co-infection
17
18
19 147 with CT or NG (p=0.005 and p<0.001, respectively) (Table 1). Independent correlates of
20
21 148 infection with MG were a diagnosis of NGU (Adjusted Odds Ratio (AOR)=7.6 95%CI 3.4-17.2) and
22
23 149 co-infection with NG (AOR=7.2 95%CI 2.5-20.4).
24
25
26 150
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

Category	Female				Male				Grand Total
	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	24 (21-	28 (24-	27 (23-	<0.001	28 (25-	30 (25-	30 (25-	0.53	29
(IQR)	28)	34)	33)		38)	37)	37)		(24-
									35)
Ethnicity									
Caucasian	33	795	728	<0.001	46	762	808	0.72	1,536
	(44.6)	(75.9)	(73.5)		(75.4)	(71.0)	(71.3)		(72.3)
Indigenous	23	95	118		3 (4.9)	50 (4.7)	53 (4.7)		171
	(31.1)	(10.4)	(11.9)						(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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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)

	11	182	193		22	386	408		601
Unknown	(15.9)	(20.5)	(20.2)		(36.7)	(36.9)	(36.9)		(29.1)

Co-infections

Chlamydia	18	42 (4.3)	60 (5.8)	<0.001	9 (14.1)	63 (5.5)	72 (5.9)	0.005	132
	(24.0)								(5.9)

Gonorrhoea	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	36 (3.2)	<0.001	-
						(21.5)			

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

Multivariate Analysis				
	Odds Ratio	95% Confidence Interval	Adjusted Odds Ratio	95% Confidence Interval
Male¹				
Co-infection with Gonorrhoea	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
Female²				
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

For peer review only

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
Gonorrhoea 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.

1
2
3 152
4
5
6 153 The overall MG prevalence for females, using any positive test result from endocervical/vaginal
7
8 154 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
9
10 155 1.8% (95%CI 1.0-2.6). Seven cases (0.7%) of MPC were diagnosed.

11
12
13 156 Univariate correlates significantly associated with a higher prevalence of MG infection (Table 1)
14
15
16 157 among women were younger age ($p<0.001$), Indigenous ethnicity ($p<0.001$), Other ethnicity
17
18 158 ($p<0.001$), the Edmonton clinic testing location ($p=0.003$), being pregnant ($p=0.04$), CT or NG
19
20 159 co-infection ($p<0.001$, for both), and MPC diagnosis ($p=0.01$). Independent correlates of
21
22 160 infection with MG were younger age (AOR=0.92 95%CI 0.87-0.96), Indigenous ethnicity
23
24 161 (AOR=4.3 95%CI 2.7-8.1) and Other ethnicity (AOR=2.8 95%CI 1.5-5.3) (vs. Caucasian), co-
25
26 162 infection with CT (AOR=5.1 95%CI 2.6-10.2) and NG (AOR=3.5 95%CI=1.0-11.8).

27
28
29 163
30
31
32
33 164 Macrolide resistance data provided through 23SrRNA sequencing data was available for two-
34
35 165 thirds (66.2%; $n=92$) of the 139 positive MG specimens. No significant differences were found
36
37 166 between specimens that were and were not sequenced for age, gender, symptoms, same sex
38
39 167 partners (for male cases only), NG, or CT results. There was a significant difference in ethnicity,
40
41 168 with fewer specimens from Indigenous cases being typed (46.2%) than from non-Indigenous
42
43 169 cases (70.6%; $p=0.02$). However, when stratified by gender, the significance was lost (women:
44
45 170 $p=0.17$, men= 0.17). Over one-half (56.5%; $n=52$) of specimens were found to have mutations
46
47 171 associated with macrolide resistance. Of the 73.4% ($n=47$) positive male specimens sequenced,
48
49 172 nearly two-thirds (63.8%; $n=30$) were found to have mutations in either A2058T ($n=3$), A2058G
50
51 173 ($n=12$), or A2059G ($n=15$). There were no variables significantly associated with macrolide
52
53
54
55
56
57
58
59
60

1
2
3 174 resistance, although MSM was marginally associated with resistance among males (83.3% vs.
4
5
6 175 51.9%; p=0.06; Table 2). Resistance to moxifloxacin was assessed by markers *gyrA* and *parC*.
7
8 176 Nearly two-thirds (64.1%; n=41) of positive male specimens had *parC* sequences available and 5
9
10 177 (12.2%) specimens had a *parC* mutation (Ser→Ile83, n=4) and (Asp→Tyr87, n=1) signifying
11
12 178 possible moxifloxacin resistance. *gyrA* sequencing was performed on 46 specimens and no *gyrA*
13
14 179 mutations were identified.
15
16
17
18
19 180
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

Category	Macrolide resistance								Grand Total
	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	22 (20-26)	26 (22-29)	24 (20-	0.04	29 (23-41)	27 (25-40)	28 (24-	0.90	26
(IQR)			28)				41)		(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)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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)
Gonorrhoea	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.

181 Among women, 23SrRNA sequencing data was available for 60.0% (45/75) of positive
 182 specimens. Nearly one-half (48.9%; n=22) had a 23SrRNA mutation associated with macrolide
 183 resistance in A2058G (n=11), A2058T (n=5), A2059G (n=6), or A2059C (n=1). In univariate
 184 analysis, younger median age (22y (IQR: 20-26) vs. 26y (IQR: 22-29); p=0.04; Table 3) was the
 185 only variable significantly correlated with macrolide resistance. One-half (50.7%; n=38) of
 186 positive specimens had *parC* sequencing available and only 1 specimen had a mutation
 187 signifying potential moxifloxacin resistance (Asp→Tyr87); no *gyrA* mutations were identified.
 188
 189 Among the subpopulation of women who had both endocervical swabs and urine collected,
 190 there was high concordance of results (98.1%; Table 3; Kappa was 0.85 (95% CI: 0.75-0.96),
 191 representing excellent agreement. Only three vaginal swabs were collected during the study
 192 period, therefore concordance with urine specimens was not calculated. This subpopulation of
 193 women was more likely to have symptoms (61.1%) than those with urines only (27.5%;
 194 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)

195

196

197 DISCUSSION

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

205

206 The overall MG prevalence for females was 7.2% (95%CI 5.6-8.8), higher than the range of 3.2-
207 6% reported in most studies of females STI clinic attendees.^{6 12 14 15} In females, MG has been
208 associated with significant morbidity including MPC, PID and infertility, but the association
209 between MG and symptoms is less clear.^{10 16 17} Among female STI clinic attendees in some
210 studies, 40-75% were asymptomatic^{14 15} but a 1994-96 French study reported a very high
211 prevalence of MG of 38% among symptomatic female STI clinic attendees.¹⁶ The presence of
212 symptoms was not an independent correlate of MG infection in our study.

1
2
3 213
4
5
6 214 Independent correlates of female infection with MG in our study were younger age, in contrast
7
8
9 215 to two other studies which reported that the prevalence of MG peaked approximately 5 years
10
11 216 later for both men and women and remained higher in older age groups.^{18 19} Co-infection with
12
13 217 CT and NG was common in our patients, confirming the role of MG as a sexually transmitted
14
15
16 218 pathogen and the probable overlap in behavioural and demographic characteristics for these
17
18
19 219 STIs.

20
21 220
22
23 221 Indigenous (First Nations, Inuit, Metis) ethnicity and other non- Caucasian ethnicity were also
24
25
26 222 significant correlates of MG infection. Other studies have reported higher rates of MG in non-
27
28 223 Caucasian populations.^{20 21} Our finding of disproportionately high rates of MG among
29
30
31 224 Indigenous persons is in keeping with the higher estimated STI prevalence in Canadian
32
33
34 225 Indigenous persons when compared to the overall general population.^{22 23} First Nations persons
35
36 226 represent an estimated 3.8% of the overall Canadian population but chlamydia rates are
37
38
39 227 estimated to be 7 times higher among First Nations adults than the overall population.²² The
40
41 228 reasons for the observed disproportionately high rates of STIs are unclear but Indigenous
42
43
44 229 persons in Canada are also over-represented in adolescent pregnancy and under-represented in
45
46 230 sexual health research.²⁴ A recent First Nations Regional Health Survey stressed the importance
47
48
49 231 of colonial history, barriers to health care services and socio-economic disadvantage.²²

50
51 232
52
53 233 It is very likely that appropriate treatment of MG infections will result in reduced sexual
54
55
56 234 transmission as well as prevention of complications.² Alternates to macrolides and

1
2
3 235 moxifloxacin, the antibiotics usually proposed for the treatment of MG, are limited since the
4
5
6 236 lack of a cell wall in MG precludes the use of penicillins and other beta lactam antibiotics.²⁵
7
8 237 Further complicating this is that mycoplasmas can develop resistance either by gene mutation
9
10 238 or by acquisition of a resistance gene.²⁶ Since azithromycin has been proposed as the preferred
11
12 239 first line agent for the treatment of MG infections^{27 28}, the high rate of mutations (~2/3 of
13
14 240 eligible specimens) conferring resistance to azithromycin in our study is particularly alarming.
15
16 241 Strains of *M. genitalium* began to develop resistance to azithromycin and have continued to do
17
18 242 so through mutations in region V of the 23S ribosomal RNA gene.²⁹ Macrolide resistance rates
19
20 243 vary significantly by geographic region with 58% resistance reported in the only published
21
22 244 Canadian study conducted in Eastern Canada.⁶ This level of resistance is well above the
23
24 245 threshold of 5% resistance above which the World Health Organization typically recommends
25
26 246 against the routine use of a drug for first line treatment of an STI.³⁰ A recent review reported
27
28 247 that the efficacy of azithromycin 1 gm for the treatment of urogenital MG has decreased from
29
30 248 85% prior to 2009 to 60% in early 2015.⁷ This had been postulated to be due to increasing
31
32 249 prevalence of macrolide resistance due to the widespread use of azithromycin for the
33
34 250 treatment of CT, NGU and MPC.^{7 31 32 33} In a recent meta-analysis, persistent MG was associated
35
36 251 with a pooled OR of 26 (95% CI: 11–57) for persistent urethritis, demonstrating that failure to
37
38 252 eradicate MG leads to persistent or recurrent signs and symptoms of urethritis in the majority
39
40 253 of men.⁷ The observation of MG as a significant pathogen in both NGU and MPC has generated
41
42 254 much discussion around whether azithromycin, and especially single dose azithromycin should
43
44 255 continue to be recommended as the preferred agent for these STI syndromes.³¹ Instead it has
45
46 256 been proposed that doxycycline be used as the first line agent because even though it is in only
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 257 30-40% effective against MG, it does not induce the development of antimicrobial resistance.³¹
4
5
6 258
7
8 259 Moxifloxacin has been proposed as the drug of choice for treatment failures with
9
10 260 azithromycin^{27 28} but our finding of potentially 12.2% resistance to moxifloxacin as assessed by
11
12 261 markers *gyrA* and *parC* is also above the 5% threshold set by the WHO.³⁰ Earlier studies
13
14 262 reported cure rates of 100% with moxifloxacin.^{32 34 35} However, more recently Tagg et al.
15
16 263 reported macrolide resistance-associated mutations in the 23S rRNA gene in 43% of samples
17
18 264 and mutations in *parC* or *gyrA* sequences in 15% of samples.³⁶ Touati et al reported a point
19
20 265 mutation in the 23S rRNA gene in 14.2% of samples.³⁷
21
22
23
24
25
26 266
27
28 267 Despite the relatively high prevalence of MG in both males and females in ours and other
29
30 268 studies, the potential for significant morbidity and enhanced HIV transmission, global
31
32 269 recommendations for MG screening are currently very diverse in part due to lack of access to
33
34 270 good tests for MG. In the absence of an FDA approved test for MG, the U.S. CDC STD Treatment
35
36 271 guidelines suggest that MG be suspected in cases of persistent/recurrent urethritis, cervicitis
37
38 272 and PID.²⁷ Canada has a single Health Canada approved test for MG (Seegene Inc, Seoul, Korea)
39
40 273 which is not widely available. The Europeans currently have the broadest recommendations for
41
42 274 screening for MG including persons with STI symptoms and those engaging in high-risk sexual
43
44 275 behavior, with a strong recommendation that all positive tests be followed by an assay capable
45
46 276 of detecting macrolide resistance mutations.²⁸
47
48
49
50
51
52
53
54 277
55
56
57
58
59
60

1
2
3 278 The optimal specimen type for MG testing remains unresolved with urine specimens considered
4
5
6 279 acceptable in males and females and in females, vaginal swabs are also considered suitable.²⁵ In
7
8 280 our study, the excellent agreement between the test performance in female endocervical
9
10 281 swabs and urine is reassuring and supports the use of less invasive urine specimens for testing
11
12 282 in females. It should be noted, however, that the comparison of test positivity in female urine
13
14 283 and female endocervical swabs is likely biased since the subpopulation included in this
15
16 284 calculation were more likely to be symptomatic than those not included. Organism burden may
17
18 285 play a role in whether a woman is symptomatic or asymptomatic, and organism burden is also
19
20 286 likely associated with test positivity.³⁸
21
22
23
24
25
26
27

28 288 Our study has a few limitations. Firstly, our specimens were collected in STI clinic patients in
29
30 289 Western Canada and may not be generalizable to other STI clinics and are likely to be higher
31
32 290 than rates reported in non-STI clinic populations. Secondly, although the specimens were
33
34 291 collected prospectively, we were only able to collect a limited number of additional variables in
35
36 292 addition to standard data collection at the clinics due to time constraints; this may have limited
37
38 293 our ability to identify additional correlates of MG. Thirdly, as tetracycline resistance-associated
39
40 294 mutations have not so far been identified in *M. genitalium*³⁹, we did not test our samples for
41
42 295 resistance to doxycycline; this information may have been useful in guiding empiric treatment
43
44 296 regimens for NGU and cervicitis in our region.
45
46
47
48
49
50

51 297
52
53 298 In summary, our study found a MG prevalence of 6.2% in attendees at two Western Canadian
54
55 299 STI Clinics, within the range reported in other studies, but higher than that for chlamydia (in
56
57
58
59
60

1
2
3 300 females) and gonorrhoea (in both genders). Over one-half of tested isolates were resistant to
4
5
6 301 macrolides. These findings together with the high proportion of asymptomatic carriers who
7
8 302 could facilitate the spread of infection, the potential for significant morbidity and the potential
9
10 303 for enhanced HIV transmission support recommendations for broader screening for MG. The
11
12 304 high prevalence of macrolide resistance also supports the recommendation to follow all
13
14 305 positive tests with an assay that can detect macrolide resistance mutations.²⁸ Judicious use of
15
16 306 antibiotics for the empiric treatment of NGU and MPC is needed to mitigate the further
17
18 307 development of resistance to currently used antibiotics and to optimize treatment of CT, NG
19
20
21 308 and MG. In order to facilitate this, wider access to testing for MG and adaptation of most
22
23 309 existing guidelines will be necessary.
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 310 **ACKNOWLEDGEMENTS**
4

5
6 311 We thank Hologic Inc, Canada for providing the test kits used in this study, the study
7
8 312 participants and staff of the Edmonton and Calgary STI clinics for enrolling patients in the study.
9

10 313

11
12
13 314 **FUNDING**
14

15
16 315 This work was funded in part by an internal grant from Alberta Health Services- STI Centralized
17
18 316 Services to the Alberta Provincial Laboratory for Public Health to complete testing. *M.*
19
20 317 *genitalium* test kits were provided by Hologic Inc, Canada.
21
22

23 318

24
25
26 319 **COMPETING INTERESTS** None
27

28 320

29
30
31 321 **AUTHOR CONTRIBUTIONS** JG, SP, PN, LT, MC, IM, PS, RR, LB and AS developed the study
32
33 322 design, protocol and ethics submission. LB coordinated funding for the study. JG, SP conducted
34
35 323 epidemiologic analyses. JG, SP, AS drafted manuscript. PP, BB, JB, RS coordinated the study in
36
37 324 the clinics. AB, SS, LT and IM coordinated and/or conducted laboratory testing. All authors
38
39 325 contributed to final manuscript review.
40
41
42

43 326

44
45
46 327 **DATA SHARING STATEMENT:** No additional data are available.
47

48 328

49
50
51 329 **DISCLAIMER**
52

53
54 330 The opinions expressed in this manuscript are those of the authors and should not be construed
55
56 331 to be those of any affiliated organization or entity.
57
58
59
60

332 REFERENCES

- 333 1. **Taylor-Robinson D**, Jensen JS. *Mycoplasma genitalium*: from Chrysalis to multicolored
334 butterfly. *Clin Microbiol Rev* 2011;**24**:498-514.
- 335 2. **Lis R**, Rowhani-Rahbar A, Manhart LE. *Mycoplasma genitalium* Infection and Female
336 Reproductive Tract Disease: A Meta-Analysis. *Clin Infect Dis* **2015**;61:418-26.
- 337 3. **Short VL**, Totten PA, Ness RB, *et al*. Clinical presentation of *Mycoplasma genitalium*
338 infection versus *Neisseria gonorrhoeae* infection among women with pelvic
339 inflammatory disease. *Clin Infect Dis* 2009;**48**:41-7.
- 340 4. **Napierala Mavedzenge S**, Weiss HA. Association of *Mycoplasma genitalium* and HIV
341 infection: a systematic review and meta-analysis. *AIDS* 2009;**23**:611-620.
- 342 5. **Manhart LE**, Broad JM, Golden MR. *Mycoplasma genitalium*: should we treat and how?
343 *Clin Infect Dis* 2011;**53 Suppl 3**:S129-42.
- 344 6. **Gesink D**, Racey CS, Seah C, *et al*. *Mycoplasma genitalium* in Toronto, Canada: Estimates
345 of Prevalence and Macrolide Resistance. *Can Fam Physician* 2016;**62**:e96-101.
- 346 7. **Lau A**, Bradshaw CS, Lewis D, *et al*. The efficacy of azithromycin for the treatment of
347 genital *Mycoplasma genitalium*: a systematic review and meta-analysis. *Clin Infect Dis*
348 2015; **61**:1389–99.
- 349 8. **Bissessor M**, Tabrizi SN, Twin J, *et al*. Macrolide resistance and azithromycin failure in a
350 *Mycoplasma genitalium*-infected cohort, and response of azithromycin failures to
351 alternative antibiotic regimens. *Clin Infect Dis* 2015; **60**:1228–36.

- 1
2
3 352 9. **Jensen JS**. Protocol for the detection of *Mycoplasma genitalium* by PCR from clinical
4
5
6 353 specimens and subsequent detection of macrolide resistance-mediating mutations in
7
8 354 region V of the 23SrRNA gene. *Method Molec Biol* 2012, **903**:129-139.
9
10 355 10. **Shimada Y**, Deguchi T, Nakane K, *et al*. Emergence of clinical strains of *Mycoplasma*
11
12 356 *genitalium* harbouring alterations in *ParC* associated with fluoroquinolone resistance.
13
14
15 357 *Int J Antimicrob Agent* 2010, **36**:255-8.
16
17 358 11. **Getman D**, Jiang A, O'Donnell M, Cohen S. *Mycoplasma genitalium* Prevalence,
18
19 359 Coinfection, and Macrolide Antibiotic Resistance Frequency in a Multicenter Clinical
20
21 360 Study Cohort in the United States. *J Clin Microbiol*. 2016;**54**:2278-83.
22
23
24 361 12. **de Jong AS**, Rahamat-Langendoen JC, van Alphen P, *et al*. Large two-centre study into
25
26 362 the prevalence of *Mycoplasma genitalium* and *Trichomonas vaginalis* in the
27
28 363 Netherlands. *Int J STD AIDS* 2016;**27**:856-60.
29
30
31 364 13. **van der Veer C**, van Rooijen MS, Himschoot M, *et al*. *Trichomonas vaginalis*
32
33 365 and *Mycoplasma genitalium*: age-specific prevalence and disease burden in men
34
35 366 attending a sexually transmitted infections clinic in Amsterdam, the Netherlands. *Sex*
36
37 367 *Transm Infect* 2016;**92**:83-5.
38
39 368 14. **Falk L**, Fredlund H, Jensen JS. Signs and symptoms of urethritis and cervicitis among
40
41 369 women with or without *Mycoplasma genitalium* or *Chlamydia trachomatis* infection. *Sex*
42
43 370 *Transm Infect* 2005; **81**:73–78.
44
45
46 371 15. **Anagrius C**, Lorange B, Jensen JS. *Mycoplasma genitalium*: prevalence, clinical significance,
47
48 372 and transmission. *Sex Transm Infect* 2005; **81**: 458–462.
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 373 16. **Casin I**, Vexiau-Robert D, de la Salmoniere P, *et al*. High prevalence of *Mycoplasma*
4
5
6 374 *genitalium* in the lower genital tract of women attending a sexually transmitted disease
7
8 375 clinic in Paris, France. *Sex Transm Dis* 2002;**29**:353–9.
- 10
11 376 17. **Huppert JS**, Mortensen JE, Reed JL, *et al*. *Mycoplasma genitalium* detected by
12
13 377 transcription-mediated amplification is associated with *Chlamydia trachomatis* in
14
15 378 adolescent women. *Sex Transm Dis* 2008;**35**:250-4.
- 18
19 379 18. **Jensen JS**, Bjørnørnelius E, Dohn B, Lidbrink P. Comparison of first void urine and
20
21 380 urogenital swab specimens for detection of *Mycoplasma genitalium* and *Chlamydia*
22
23 381 *trachomatis* by polymerase chain reaction in patients attending a sexually transmitted
24
25 382 disease clinic. *Sex Transm Dis* 2004;**31**:499–507.
- 28
29 383 19. **Salado-Rasmussen K**, Jensen JS. *Mycoplasma genitalium* testing pattern and macrolide
30
31 384 resistance: a Danish nationwide retrospective survey. *Clin Infect Dis* 2014; **59**:24–30.
- 33
34 385 20. **Sonnenberg P**, Ison CA, Clifton S, *et al*. Epidemiology of *Mycoplasma genitalium* in
35
36 386 British men and women aged 16–44 years: evidence from the third National Survey of
37
38 387 Sexual Attitudes and Lifestyles (Natsal-3). *Int J Epidemiol* 2015;**44**:1982-94.
- 41
42 388 21. **Hancock EB**, Manhart LE, Nelson SJ, *et al*. Comprehensive assessment of
43
44 389 sociodemographic and behavioral risk factors for *Mycoplasma genitalium* infection in
45
46 390 women. *Sex Transm Dis* 2010;**37**:777-83.
- 48
49 391 22. **The First Nations Information Governance Centre**, First Nations Regional Health Survey
50
51 392 (RHS) Phase 2 (2008/10) National Report on Adults, Youth and Children Living in First
52
53 393 Nations Communities. **2012**.

- 1
2
3 394 http://fnigc.ca/sites/default/files/First_Nations_Regional_Health_Survey_2008-
4
5
6 395 [10_National_Report.pdf](http://fnigc.ca/sites/default/files/First_Nations_Regional_Health_Survey_2008-10_National_Report.pdf) (accessed 19 Jan 2017).
7
8 396 **23. Public Health Agency of Canada.** Population-Specific HIV/AIDS Status Report: Aboriginal
9
10
11 397 Peoples. **2010.** <http://www.catie.ca/sites/default/files/26344.pdf> (accessed 19 Jan
12
13 398 2017).
14
15
16 399 **24. Devries KM,** Free CJ, Morison L, Saewyc E. Factors associated with pregnancy and STI
17
18 400 among Aboriginal students in British Columbia. *Can J Public Health* 2009;**100**:226-30.
19
20
21 401 **25. Jensen JS,** Cusini M, Gomberg M, Moi H. Background review for the 2016 European
22
23 402 guideline on *Mycoplasma genitalium* infections. *J Eur Acad Dermatol Venereol*
24
25 403 2016;**30**:1686-93.
26
27
28
29 404 **26. Taylor-Robinson D.** Diagnosis and antimicrobial treatment of *Mycoplasma*
30
31 405 *genitalium* infection: sobering thoughts. *Expert Rev Anti Infect Ther* 2014;**12**:715-22.
32
33
34 406 **27. Centers for Disease Control and Prevention.** Sexually Transmitted Diseases Treatment
35
36 407 Guidelines, 2015. <https://www.cdc.gov/std/tg2015/> (accessed 16 Jan 2017).
37
38
39 408 **28. Jensen JS,** Cusini M, Gomberg M. 2016 European Guideline on *Mycoplasma genitalium*
40
41 409 infections.
42
43
44 410 [http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.p](http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.pdf)
45
46 411 [df](http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.pdf) (accessed 16 Jan 2017).
47
48
49 412 **29. Jensen JS,** Bradshaw CS, Tabrizi SN, *et al.* Azithromycin treatment failure in *Mycoplasma*
50
51 413 *genitalium*-positive patients with nongonococcal urethritis is associated with induced
52
53 414 macrolide resistance. *Clin Infect Dis* 2008;**47**:1546-53.
54
55
56
57
58
59
60

- 1
2
3 415 30. **World Health Organization.** Guidelines for the management of sexually transmitted
4
5
6 416 infections. 2003. WHO, Geneva, Switzerland.
7
8
9 417 31. **Horner PJ.** Editorial Commentary: *Mycoplasma genitalium* and Declining Treatment
10
11 418 Efficacy of Azithromycin 1 g: What Can We Do? *Clin Infect Dis* 2015;**61**:1400-2.
12
13
14 419 32. **Anagnius C, Loré B, Jensen JS.** Treatment of *Mycoplasma genitalium*: observations from
15
16 420 a Swedish STD Clinic. *PLoS One* 2013; **8**: e61481.
17
18
19 421 33. **Ito S, Shimada Y, Yamaguchi Y, et al.** Selection of *Mycoplasma genitalium* strains
20
21 422 harbouring macrolide resistance-associated 23S rRNA mutations by treatment with a
22
23 423 single 1 g dose of azithromycin. *Sex Transm Infect* 2011; **87**:412–4.
24
25
26 424 34. **Bradshaw CS, Chen MY, Fairley CK.** Persistence of *Mycoplasma genitalium* following
27
28 425 azithromycin therapy. *PLoS One* 2008;**3**:e3618.
29
30
31 426 35. **Jernberg E, Moghaddam A, Moi H.** Azithromycin and moxifloxacin for microbiological
32
33 427 cure of *Mycoplasma genitalium* infection: an open study. *Int Journal STD AIDS*
34
35 428 2008;**19**:676–9.
36
37
38 429 36. **Tagg KA, Jeffreys NJ, Couldwell DL, et al.** Fluoroquinolone and macrolide resistance-
39
40 430 associated mutations in *Mycoplasma genitalium*. *J Clin Microbiol* 2013;**51**:2245-9.
41
42
43 431 37. **Touati A, Peuchant O, Jensen JS, Bébéar C, Pereyre S.** Direct detection of macrolide
44
45 432 resistance in *Mycoplasma genitalium* isolates from clinical specimens from France by
46
47 433 use of real-time PCR and melting curve analysis. *J Clin Microbiol* 2014;**52**:1549-55.
48
49
50 434 38. **Michel CE, Sonnex C, Carne CA, et al.** *Chlamydia trachomatis* load at matched anatomic
51
52 435 sites: implications for screening strategies. *J Clin Microbiol* 2007;**45**:1395-402.
53
54
55
56
57
58
59
60

- 1
2
3 436 39. **Couldwell DL**, Lewis DA. *Mycoplasma genitalium* infection: current treatment options,
4
5
6 437 therapeutic failure, and resistance associated mutations. *Infect Drug Resist* 2015;**8**:147-
7
8 438 61.
9

10 439
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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.