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Retrospective analysis of infection and antimicrobial resistance patterns of *Mycoplasma genitalium* among pregnant women in the southwestern USA

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ABSTRACT

Background *Mycoplasma genitalium* is a sexually transmitted infection (STI) pathogen. There have been no published studies concerning symptomatology, prevalence data, antibiotic resistance profiling or reports of co-infection with other STI in pregnant women.

Objective To describe these characteristics among pregnant women attending prenatal clinics in a large tertiary care centre.

Design Remnant genital samples collected from pregnant women between August 2018 and November 2019 were tested for *M. genitalium* and *Trichomonas vaginalis* by the transcription-mediated amplification technique. Specimens with detectable *M. genitalium* RNA were sequenced for 23S rRNA mutations associated with azithromycin resistance and *parC* and *gyrA* mutations associated with resistance to moxifloxacin. Demographic, obstetric and STI co-infection data were recorded.

Results Of the 719 samples, 41 (5.7 %) were positive for *M. genitalium*. *M. genitalium* infection was associated with black race, Hispanic ethnicity and young age (p=0.003, p=0.008 and p=0.004, respectively). *M. genitalium* infection was also associated with *T. vaginalis* co-infection and *Streptococcus agalactiae* (group B *Streptococcus*) colonisation (p≤0.001 and p=0.002, respectively). Of the 41 positive samples, 26 (63.4%) underwent successful sequencing. Eight (30.8%) had 23S rRNA mutations related to azithromycin resistance. One of 26 (3.8%) positive samples with sequencing results had the gyrA gene mutation and 1 of 18 sequenced samples (5.6%) had the parC gene mutation associated with moxifloxacin resistance.

Conclusions Prevalence rates of *M. genitalium* in pregnant women was 5.7%. *M. genitalium* infection disproportionately affects young black women co-infected with *T. vaginalis*. Pregnant women remain at risk for persistent infection with *M. genitalium* due to decreased azithromycin susceptibility.

Strengths and limitations of this study

- This analysis is one of the largest evaluating prevalence rates of *Mycoplasma genitalium* in pregnant women presenting for routine care.
- *M. genitalium* infection rates were evaluated across race, age and other demographic and obstetrical variables, including co-infections with other sexually transmitted infections.
- Antibiotic resistance patterns were determined among isolates collected from pregnant patients presenting for routine care.
- Perinatal outcome data were not recorded.
- Prospective data regarding persistent infection were not collected in this analysis.

INTRODUCTION

Mycoplasma genitalium is an emerging cause of sexually transmitted disease in women.[1–10] Due to its fastidious nature, culture technique methods have not proven to successfully identify organism in the clinical environment.[1–8] Fortunately, with the recent developments of highly sensitive molecular platforms, *M. genitalium* can expeditiously be detected in urogenital samples with >97% sensitivity.[11–20] As a result, contemporary studies have demonstrated this organism to extend beyond the role as a causative agent for non-gonococcal urethritis among men and has now been implicated in female genital tract pathology, including infectious sequelae similar to *Chlamydia trachomatis*, such as cervicitis, pelvic inflammatory disease and preterm birth.[5 21–32]

To date, six studies have assessed the role of *M. genitalium* with pregnancy-related complications, including a 2015 meta-analysis (N=3128) in which *M. genitalium* was found to be significantly associated with an increased risk of preterm birth prior to 37 weeks (pooled OR=1.89), with an even higher ratio when other sexually transmitted infection (STI) were accounted for (pooled OR=2.3).[21–27]

The meta-analysis by Lis *et al*[27] demonstrated the limitations of prior published data mainly related to varying prevalence rates ranging from 2% to 20% in women, with scant data concerning rates of infection among pregnant women.[4–7 20–32] Characteristics of *M. genitalium* infection, including antibiotic susceptibility patterns and co-infection rates with other STI agents, have not been evaluated in pregnant women presenting for care.[21–32] The objective of this study was to determine these characteristics among a cohort of pregnant women in a large tertiary obstetrical care centre.

Design

After institutional review board's approval from the Baylor College of Medicine, all remnant Aptima Multitest clinician-collected endocervical samples from pregnant women presenting to care between 30 August 2018 and 30 November 2019 were placed in the Aptima swab specimen transport tube, stored for up to 30 days and shipped monthly by overnight mail to Marquette University, Milwaukee, Winconsin, USA, for *M. genitalium* 16S rRNA and *Trichomonas vaginalis* testing by the transcription-mediated amplification technique using Panther System automation (Hologic, San Diego, California, USA) as previously described.[11–20] Only one sample collected at intake to care was used for each patient presenting obstetrical care and received testing with the Aptima swab for *Neisseria gonorrhoeae* and *C. trachomatis* per institutional protocol and guidelines.

M. genitalium positive specimens were shipped to the National Microbiology Laboratory, Public Health Agency of Canada, for additional testing. DNA was extracted from the specimens using the MagNA Pure DNA and Viral Nucleic Acid kit (Roche, Laval) per manufacturer's instruction. Specimens with detectable *M. genitalium* DNA were subsequently analysed by sequencing the 23S rRNA gene to identify mutations associated with azithromycin resistance and *parC* and *gyrA* genes associated with resistance to moxifloxacin.[20 28–32]

Demographic variables, obstetrical data, pelvic symptoms consistent with cervicitis (pelvic pressure, vaginal discharge and lower abdominal cramping) and STI co-infection (*N. gonorrhoeae, C. trachomatis,* herpes simplex virus, HIV, *T. vaginalis* and human papillomavirus (types 16 and 18)) were collected. Bacterial vaginosis and group B *Streptococcus* (GBS) colonisation data were extracted from the chart and recorded by the investigators. Patient characteristics, co-infection with other STI and *M. genitalium* resistance profiles were summarised by means with SD, or frequencies with percentages. Fisher's exact test or the Wilcoxon rank sum test was used to determine differences between women positive and negative for *M. genitalium* in demographic, clinical characteristics and co-infections with other STIs. Exact 95% CIs were determined for the resistance profiles. Strengthening the Reporting of Observational Studies in Epidemiology guidelines were followed for the study design, methods and analysis.[33] All protected health information was removed from discarded samples prior to shipment and all data were entered into a de-identified database using only study numbers to link information at completion of study. Patient consent was not obtained, as this project was a retrospective chart review study involving otherwise discarded samples.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research. We used de-identified database involving otherwise discarded samples and chart review. There was no patient involved for this study.

RESULTS

During the study period, 726 remnant samples were collected from all pregnant women from the obstetric clinics at Baylor College of Medicine that underwent routine STI testing at intake to care. Seven samples were inadequate, leaving 719 samples available for *M. genitalium* testing. Of these, 41 (5.7%) were positive. The majority of women in the study group were Hispanic, n=535 (74.7%), and n=72.8% were multiparous. There were no significant differences in gestational or pregestational diabetes, hypertensive disorders in pregnancy and illicit substance use between infected and non-infected women. The demographic and obstetric variables of the study group according to *M. genitalium* infection status are demonstrated in table 1. The mean age of women infected with *M. genitalium* was younger than non-infected women (24.9 years vs 28.1 years, respectively; p=0.004) and *M. genitalium* was significantly associated with black race (p=0.003) and Hispanic ethnicity (p=0.008) (table 2). At the time of sample collection, 12.1% (85/701) reported pelvic complaints (pelvic pain, vaginal discharge or lower abdominal cramping). Seven women with positive results for infection with *M. genitalium* were symptomatic (18%) compared with 78 women who tested negative for *M. genitalium* infection (11.8%; p=0.307).

Table 3 demonstrates the association between *M. genitalium* and co-infection with other STI. *M. genitalium* infection was significantly associated with women co-infected with *T. vaginalis* (p≤0.001). In addition, the rate of GBS colonisation was significantly higher among women infected with *M. genitalium* compared with women who tested negative (58.3% vs 16.1%, respectively; p=0.002).

Of the samples with detectable *M. genitalium* RNA, 26 (63.4 %) were of sufficient quantity to undergo conclusive sequencing analysis for azithromycin resistance. Of these, 8/26 (30.7%) were found to have 23S rRNA mutations (A2059G) associated with azithromycin resistance. Of the 18 samples that were of sufficient quantity to undergo sequencing analysis for the *parC* gene mutation, one (5.6%) was found to have the *parC* (Ser→Asn83) gene mutation. Of the 26 samples that were of sufficient quantity to undergo sequencing analysis for the *gyrA* gene mutation, one (3.8%) was found to have that gene

Table 1 Demographics and baseline obstetrical characteristics

	M. genitalium positive (N=41)	*M. genitalium* negative (N=678)	P value§	Total population (N=719)
Age, mean (SD) (in years)	24.9 (4.89)	28.1 (6.93)	**0.004**	27.9 (6.87)
<20	5 (12.2)	80 (11.9)	0.021	85 (11.9)
20–34	35 (85.4)	462 (68.4))		497 (69.4)
35 or more	1 (2.4)	133 (19.7)		134 (18.7)
Race/ethnicity	41	675	**0.003**† **0.004**‡	716
White/Hispanic	23 (56.1)	522 (77.3)		545 (76.1)
White/non-Hispanic	4 (9.8)	26 (3.9)		30 (4.2)
Black/Hispanic	0	2 (0.3)		2 (0.3)
Black/non-Hispanic	14 (34.2)	99 (14.7)		113 (15.8)
Other (Asian, Native Hawaiian/Pacific Islander and American Indian/Alaskan Native)	0	21 (3.1)		21 (2.9)
Unknown	0	5 (0.7)		5 (0.7)
Nulliparous	18 (42.9)	183 (27.2)	0.031	201 (28.2)
Hypertensive disorders of pregnancy	5/39 (12.8)	54/658 (8.2)	0.3661	59/697 (8.5)
Diabetes mellitus (GDM or pregestational DM)	1/39 (2.6)	67/658 (10.2)	0.1637	68/697 (9.8)
Illicit drug use during pregnancy	0/38 (0)	25/654 (3.8)	0.390	25/692 (3.6)
Tobacco use during pregnancy	2/38 (5.3)	14/655 (2.1)	0.2171	16/693 (2.3)
Alcohol use during pregnancy	3/38 (7.9)	11/652 (1.7)	0.0368	14/690 (2.0)
GA at specimen collection, mean (SD)	22.4 (10.90)	22.2 (10.81)	0.816	22.2 (10.81)
Previous preterm (<37 weeks)	2/39 (5.1)	63/664 (9.5)	0.568	65/703 (9.3)
Previous PROM (<37 weeks)	0/39 (0)	15/651 (2.3)	1.00	15/690 (2.2)
Cervicitis symptoms*	7/39 (18.0)	78/662 (11.8)	0.307	85/701 (12.1)
Cerclage in index pregnancy	0/39 (0)	6/664 (0.9)	1.00	6/703 (0.9)
Twin pregnancy	0/39 (0)	9/670 (1.3)	1.00	9/709 (1.3)

Data presented as N (%) unless otherwise specified.
Bolded if significantly different.
*Any of the following symptoms: pelvic pressure, vaginal discharge or lower abdominal cramping.
†P value compares black vs non-black.
‡P value compares Hispanic (including Mexican and unknown) vs non-Hispanic.
§P value from Fisher's exact test or Wilcoxon rank sum test.
GDM, Gestational Diabetes Mellitus; *M. genitalium*, *Mycoplasma genitalium*.

Table 2 *Mycoplasma genitalium* RNA detection rates from genital swab collections by race/ethnicity

	Detection of *M. genitalium* RNA (n/N1 (% of subjects))
Race/ethnicity	
White/Hispanic	23/545 (4.2)
White/non-Hispanic	4/30 (13.3)
Black/Hispanic	0/2 (0)
Black/non-Hispanic	14/113 (12.4)
Other (Asian, Native Hawaiian/Pacific Islander and American Indian/Alaskan Native)	0/21 (0)
Race, p value	0.003
Black	14/115 (12.2)
Non-black	27/601 (4.5)
Ethnicity, p value	0.008
Hispanic	23/535 (4.3)
Non-Hispanic	18/179 (10.1)

P value from Fisher's exact test.
N1=number of women tested for the infection with a non-missing value

mutation. Both *parC* and *gyrA* gene mutations are associated with moxifloxacin resistance. Sequencing results of all samples are demonstrated in table 4.

DISCUSSION

Prevalence rates of *M. genitalium* in this large cohort of pregnant women approximate rates reported in non-pregnant women at 5.7%.[4–7 20–32] Infection with *M. genitalium* was more prevalent among women at risk for other STI, including black race, young age and co-infection with *T. vaginalis* (p<0.05 for all). Although macrolide resistance patterns from isolates collected form non-pregnant patients approach 50%, azithromycin resistance was detected in 30% of isolates collected from the cohort and 5.6% demonstrated moxifloxacin resistance.[28–32 34–40]

As described in prior studies, infection with *M. genitalium* was found to be more prevalent among pregnant women compared with *N. gonorrhoeae,* where reported prevalence rates in women remain less than 1%.[2–10 20–32 37 40] The adverse health impacts of the more common STI, including *N. gonorrhoeae,* syphilis, *C. trachomatis* and herpes simplex virus, on pregnant women are well understood.[2–10] These have been studied for decades and standard screening and treatment protocols are practiced nationwide with the support of evidence-based guidelines and recommendations for clinical management.[10] A comparable body of evidence is not available for *M. genitalium*, largely because this organism is relatively understudied as a cause of female genital tract infectious morbidity.[6 7 9] A contributing factor to this paradox is that researchers have been unable to apply many of the same culture-based mechanisms and point-of-care testing often used for the diagnosis of other STI towards detection of *M. genitalium.*[11–20]

With the advent of molecular-based technologies used in research protocols evaluating associations of *M. genitalium* with adverse reproductive outcomes, this organism has been associated with premature birth, premature rupture of membranes, spontaneous abortion, cervicitis and infertility, implicating this organism as a pathogen in pregnant as well as non-pregnant women.[11–19 21–32] Further understanding of this infection as it relates to pregnancy and adverse perinatal outcomes begins with understanding its characteristics as an STI, and its association with obstetrical factors, demographics, co-infection patterns and pelvic symptomatology as described in our analysis.

Table 3 Co-infections with *Mycoplasma genitalium*

	M. genitalium positive (N=41) n/N1 (%)	*M. genitalium* negative (N=678) n/N1 (%)	P value	Total population (N=719)
Human papillomavirus, types 16 and 18	4/14 (28.6)	43/281 (15.3)	0.251	47/295 (15.9)
Bacterial vaginosis	5/18 (27.8)	98/340 (28.8)	1.000	103/255 (28.8)
Trichomonas vaginalis	7/40 (17.5)	18/677 (2.7)	**<0.001**	25/717 (3.5)
Chlamydia trachomatis	6/39 (15.4)	54/670 (8.1)	0.131	60/709 (8.5)
Neisseria gonorrhoeae	0/39 (0)	7/670 (1.0)	1.000	7/709 (1.0)
Hepatitis B	0/39 (0)	2/637 (0.3)	1.000	2/676 (0.3)
Hepatitis C	0/17 (0)	1/281 (0.4)	1.000	1/298 (0.3)
Syphilis	1/37 (2.7)	8/639 (1.3)	0.399	9/676 (1.3)
Herpes simplex virus 1/2	3/6 (50.0)	23/104 (22.1)	0.143	26/110 (23.6)
Group B *Streptococcus*	7/12 (58.3)	40/248 (16.1)	0.002	47/260 (18.1)

N1=number of women tested for the infection with a non-missing value. P value from Fisher's exact test.

Table 4 Prevalence of *Mycoplasma genitalium* and resistance profiles

	Total (N=726)	95% CI
N with sample tested	719	
M. genitalium positive	41 (5.7)	4.0 to 7.4§
23S		
A2058G*	3 (7.3)	1.5 to 19.9
A2058T*	2 (4.9)	0.6 to 16.5
A2059G*	3 (7.3)	1.5 to 19.9
No sequence*	15 (36.6)	22.1 to 53.1
WT*	18 (43.9)	28.5 to 60.3
Mutation related to azithromycin resistance†	8/26 (30.8)	14.3 to 51.8
GyrA		
95MET(ATG)→ILE(ATC)*	1 (2.4)	0.06 to 12.9
Inconclusive*	1 (2.4)	0.06 to 12.9
No sequence*	12 (29.3)	16.1 to 45.5
WT*	25 (60.9)	49.4 to 79.9
GyrA mutation †	1/26 (3.8)	0.09 to 18.4
ParC		
83SER(AGT)→ILE(ATT)*	1 (2.4)	0.06 to 12.9
83SER(AGT)→ASN(AAT)*	0	0 to 8.6
Inconclusive*	9 (22.0)	10.6 to 37.6
No sequence*	14 (34.2)	20.1 to 50.6
WT‡	17 (41.5)	26.3 to 57.9
ParC mutation†	1/18 (5.6)	0.14 to 27.3
M. genitalium negative	678 (94.3)	92.6 to 96.0§
M. genitalium positive	1 (5.9)	0.15 to 28.7
M. genitalium negative	16 (94.1)	71.3 to 99.9

Data presented as N (%).
*Per cent of positive for *M. genitalium*.
†Denominator is positive samples with conclusive sequencing results.
‡Wild type.
§Exact 95% CIs except for which are based on the normal approximation.

A unique finding of this study relates to antimicrobial susceptibility profiles of *M. genitalium* isolated from this pregnant cohort. Although detection rates of macrolide resistance determinants approach 30% in our population, published rates of macrolide resistance approach 50% in isolates collected from men.[21–32 34 38] In some countries, strains of multidrug-resistant *M. genitalium* strains exist, limiting therapeutic options.[21–32 34 38] Although the predicted azithromycin resistance is significantly less in this population compared with prior published reports involving men and women, pregnant women remain at significant risk for persistent antenatal infection due to decreased azithromycin susceptibility. The number of cases (n=2) identified with predicted moxifloxacin resistance in this study was low, but it is of concern as extended dose moxifloxacin is currently one of the few alternative options for treatment of macrolide-resistant *M. genitalium* strains, an option not available to pregnant women due to potential fetal teratogenicity and the assigned pregnancy classification.[10 35–38]

Pristinamycin, an antimicrobial agent synthesised from macrolide and depsipeptide components, has demonstrated promising results as a second-line treatment option with a 75% cure rate of *M. genitalium* in preliminary studies.[38] Although not significantly different from moxifloxacin in treatment efficacy among non-pregnant people, pristinamycin remains a potential option during pregnancy and in other situations where fluoroquinolones have failed or are contraindicated.[38]

Data on which to determine whether prenatal treatment of *M. genitalium* can reduce the incidence of pelvic complaints, preterm birth or any other adverse perinatal outcome are still lacking. Future research is warranted to examine relationships between *Mycoplasmas* and pregnancy, given that some of these organisms may be mechanistically related in their ability to induce inflammatory

cytokines, potentially leading to preterm labour.[11–19 21–27] This gap in knowledge is a significant impediment for implicating this organism as a notifiable cause of reproductive tract disease, and for evidence-based improvement of the current prenatal STI screening and treatment guidelines.

The limitations of our study include the lack of perinatal outcome correlates and a low representation of other STI. The number required to determine meaningful perinatal outcome data, that is, preterm birth, after adjusting for prior preterm birth, using a conservative OR of 1.3 per Lis *et al*, would require over 17 000 patients to determine a 30% difference in this outcome, even when using higher published prevalence rates among women of 15% and a macrolide resistance rate of 25%.[21–32 39] The information provided in this manuscript can inform research scientists for future prospective studies, including a large, randomised-controlled treatment trial to prevent preterm birth related to *M. genitalium* infection.

Of note, the co-infection rate of *T. vaginalis* with *M. genitalium* was significant, as was the association of this infection with demographic risk factors common among women with other STI, such as young age and black race.[1–10 20 21 39 40] An additional interesting result is the significantly higher association of GBS colonisation in women infected with *M. genitalium*, a relationship worthy of further investigation. Sample processing was an additional limitation to the study, as samples were shipped across multiple sites, subjecting the samples to preprocessing degradation. Only 68% of samples contained sufficient material for sequencing for conclusive antibiotic resistance profiling. As these samples were remnant samples that had undergone testing for *N. gonorrhoeae* and *C. trachomatis* prior to *M. genitalium* testing, the potential for a reduction in sample quantity was not unexpected, contributing to lower yields. Further prospective studies involving sample collection for *M. genitalium* testing either alone or simultaneously with other STI detected by the Panther transcription-mediated amplification method would result in higher concentrations of genetic material for sequencing analysis.

CONCLUSION

Our analysis demonstrates that the prevalence of *M. genitalium* is 5.7% among a large cohort of pregnant women attending prenatal care in an urban academic centre. *M. genitalium* shares features of other STI, including common demographic risk factors, such as black race and young age. Of the samples with detectable *M. genitalium* RNA that underwent sequencing, 30% were found to have mutations for resistance to azithromycin. If future studies demonstrate a relationship between *M. genitalium* and adverse perinatal outcomes, alternative therapeutic regimens based on antibiotic susceptibility profiles will need to be determined for the pregnant patient harbouring this STI.

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