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Infection and antimicrobial resistance patterns of *Mycoplasma genitalium* among pregnant women

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Contributorship Statement:

All authors were responsible for data entry which was reviewed by the lead author and validated. Irene A. Stafford MD, Kelsey Hummel MD, James J. Dunn PhD, Kenneth L. Muldrew, MD, Alexandra Berra MD, Elizabeth S. Kravitz, BS, Soumya Gogia, BS, Irene Martin BSc, and Erik Munson PhD all contributed to the data collection, data analysis, protocol development and manuscript preparation. Irene A Stafford, MD is the guarantor for the overall content.

Abstract:

Background: *Mycoplasma genitalium* is a sexually transmitted infection. 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 center.

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= .003, .008 and .004 respectively). *M. genitalium* infection was also associated with *T. vaginalis* co-infection and Streptococcus agalactiae (GBS) colonization (p =<0.001 and .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:

Strengths:

- This analysis is one of the largest evaluating prevalence rates of *M. genitalium* in pregnant women presenting for routine care.
- *Mycoplasma genitalium* infection disproportionately affecting young Black pregnant women who are more likely to be co-infected with *Trichomonas vaginalis* and colonized with group B *Streptococcus* (GBS).
- Azithromycin resistance among *M. genitalium* isolates collected from pregnant women was 30.8%

Weaknesses:

- Perinatal outcome data was not recorded.
- Prospective data regarding persistent infection was not collected in this analysis.

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Competing Interest Statement:

The lead author, Irene A Stafford, MD affirms that this manuscript is an honest, accurate and transparent account of the study being reported; that no important aspects of the study have been omitted. The authors report no conflict of interest.

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

Data Sharing:

De-identified data will be available upon written request.

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[21-28].

To date, six studies have assessed the role of *M. genitalium* with pregnancy related complications, including a 2015 meta-analysis (N = 3,128) 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 STI were accounted for (pooled OR 2.3)[22-28]. The meta-analysis by Lis et. al[28] demonstrated the limitations of prior published data mainly related to

varying prevalence rates ranging from 2 – 20 % in women, with scant data concerning rates of infection among pregnant women[4-7,20-29-33]. Characteristics of *M. genitalium* infection, including symptomatology, antibiotic susceptibility patterns and co-infection rates with other STI agents have not been evaluated in pregnant women presenting for care [29-33]. The objective of this study was to determine these characteristics among a cohort of pregnant women in a large tertiary obstetrical care center.

Materials and Methods:

After Institutional Review Board approval from the Baylor College of Medicine, all remnant Aptima Multitest clinician-collected endocervical samples from pregnant women presenting to care between August 30, 2018 and November 30, 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, WI for *M. genitalium* 16S rRNA and *Trichomonas vaginalis* testing by the transcription - mediated amplification technique utilizing Panther System automation (Hologic, Inc., San Diego, CA) 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 *N. gonorrhoeae* and *C. trachamatis* per institutional protocol and guidelines.

M. genitalium positive specimens were shipped to the Public Health Agency of Canada, National Microbiology Laboratory for additional testing. DNA was extracted from the specimens using the MagNA Pure DNA and Viral Nucleic Acid kit (Roche, Laval, Quebec) per manufacturer's instruction. Specimens with detectable *M. genitalium* DNA were subsequently

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analyzed by sequencing the 23S rRNA gene to identify mutations associated with azithromycin resistance and *parC* and *gyrA* genes associated with resistance to moxifloxacin[2,20,21,26,27].

Demographic variables, obstetrical data, pelvic symptoms consistent with cervicitis (pelvic pressure, vaginal discharge, lower abdominal cramping), and STI co-infection [*Neisseria gonorrhoeae*, *Chlamydia trachomatis,* herpes simplex virus, human immunodeficiency virus, *Trichomonas vaginalis*, human papillomavirus (types 16,18)] Bacterial vaginosis and group *B Streptococcus* (GBS) colonization data were extracted from the chart and recorded by the investigators. Patient demographics, clinical characteristics, co-infection with other STI and *M. genitalium* resistance profiles were summarized by means with standard deviations, 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% confidence intervals (CIs) were determined for the resistance profiles. STROBE guidelines were followed for the study design, methods and analysis[34]. All protected health information was removed from discarded samples prior to shipment and all data was entered into a de-identified database using only study numbers to link information at completion of study.

Patient and public involvement:

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. Seven samples were inadequate, leaving 719 available for *M. genitalium* testing. Of these, 41 (5.7%)

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were positive. The majority of women in the study group were Hispanic, n= 535 (74.7%) and (72.8%) were multiparous. There were no significant differences in gestational or pre-gestational 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 vs. 28.1 years respectively p = .004) and *M. genitalium* was significantly associated with Black race (p =.003) and Hispanic ethnicity (p = .008). Prevalence rates according to race and ethnicity are shown in 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 to 78 women who tested negative for *M. genitalium* infection (11.8%; p = .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 *Trichomonas vaginalis* (p = <0.001). In addition, the rate of group B *Streptococcus* (GBS) colonization was significantly higher among women infected with *M. genitalium* compared to women who tested negative (58.3% vs. 16.1% respectively p = .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 mutation. Both parC and gyrA gene mutations are associated with moxifloxacin resistance. 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%[20-22]. 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* ($p < .05$ 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[29-33,35].

As described in prior studies, infection with *M. genitalium* was found to be more prevalent among pregnant women compared to N. *gonorrhoeae,* where reported prevalence rates in women remain less than 1%[2-10,20-22.] 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 toward detection of *M. genitalium*[11-9].

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Historically, this organism is extremely challenging to propagate, with few laboratories capable of recovering clinical isolates. 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, 22-28]. Further understanding of this infection as it relates to pregnancy and adverse perinatal outcomes begins with understanding its characteristics as an STI; its association with obstetrical factors, demographics, co-infection patterns and pelvic symptomatology as described in our analysis.

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[29-33,35]. In some countries, strains of multi-drug resistant *M. genitalium* strains exist, limiting therapeutic options[29-33,35]. Although the predicted azithromycin resistance is significantly less in this population compared to 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 the only alternative option 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].

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 is still

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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 labor[11-19, 22-28]. 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. Regardless, the co-infection rate of *Trichomonas 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[1-10,20-22]. An additional interesting result is the significantly higher association of group B streptococcal (GBS) colonization in women infected with *M. genitalium*, a relationship worthy of further investigation. Sample processing was an additional limitation to the study. 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:

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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 center. *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 harboring this STI.

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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 (std)	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)

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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, 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 pre-gestational 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 (std)	22.4 (10.90)	22.2 (10.81)	0.816	22.2 (10.81)
Previous Preterm (< 37 wks)	2/39 (5.1)	63/664 (9.5)	0.568	65/703 (9.3)
Previous PROM (< 37 wks)	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)

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Data presented as N (%) unless otherwise specified
*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.
^^Any of the following symptoms: pelvic pressure, vaginal discharge or lower abdominal cramping
Bolded if significantly different

Table 2: *Mycoplasma genitalium* RNA Detection rates from genital swab collections by Race/Ethnicity

	***Detection of Mycoplasma 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, 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.

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Table 3. Co-Infections with *M. genitalium*

	M. genitalium Positive (N=41) n/N1 (%)	*M. genitalium* Negative (N=678) n/N1 (%)	p-value	Total Population (N=719)
Human papillomavirus 16, 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 I/II	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 *M. genitalium* and Resistance profiles

	Total (N=726)	95% CI
N with sample tested	719	
M. genitalium positive	41 (5.7)	4.0 - 7.4**
23S		
A2058G*	3 (7.3)	1.5 - 19.9
A2058T*	2 (4.9)	0.6 - 16.5
A2059G*	3 (7.3)	1.5 - 19.9
No sequence*	15 (36.6)	22.1 - 53.1
WT*	18 (43.9)	28.5 - 60.3

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Mutation related to azithromycin resistance^	8/26 (30.8)	14.3 - 51.8
gyrA		
95MET(ATG)->ILE(ATC)*	1 (2.4)	0.06 - 12.9
Inconclusive*	1 (2.4)	0.06 - 12.9
No sequence*	12 (29.3)	16.1 - 45.5
WT*	25 (60.9)	49.4 - 79.9
gyrA mutation ^	1/26 (3.8)	0.09 - 18.4
parC		
83SER(AGT)->ILE(ATT)*	1 (2.4)	0.06 - 12.9
83SER(AGT)->ASN(AAT)*	0	0 - 8.6
Inconclusive*	9 (22.0)	10.6 - 37.6
No sequence*	14 (34.2)	20.1 - 50.6
WT^^	17 (41.5)	26.3 - 57.9
parC mutation^	1/18 (5.6)	0.14 - 27.3
M. genitalium Negative	678 (94.3)	92.6 - 96.0**
M. genitalium positive	1 (5.9)	0.15 - 28.7
M. genitalium Negative	16 (94.1)	71.3 - 99.9

Data presented as N (%)

*Percent of positive for *M. genitalium*

Exact 95% confidence intervals (CI) except for ** which are based on the normal approximation.

^ Denominator is positive samples with conclusive sequencing results

^^ Wild type

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* * * Continuing Review * * *

Continuing Review

1) Is this the submission of translated Spanish consent documents following the initial 3-month approval period? If yes, please upload the IRB-approved documents in the Attachments section. N

If this is the submission of your annual continuing review, please answer the following questions and upload your IRB renewal documents in the Attachments section.

i. Is recruitment active? N

If no, why should study remain active?

ii. Have changes been made since last approval? Y

If yes, indicate specific changes and upload the IRB approval letter in the Attachments section.

There was a change in PI during the study period. Dr. Stafford has now been made a Co-Investigator from the UT Health Science Center in Houston. The study now includes UT Health Science Center, however no specimen collection will occur here.

iii. Total number of Harris Health System participants or patient records reviewed? 1300

iv. Total number of Harris Health System participants enrolled or patient records reviewed since last approval? 1300

v. Please provide a summary of any interim findings and/or publication citations since last approval.

We observed a high percentage of Group B Streptococcus (GBS) positive patients that were also M. genitalium positive. Of the STI studied, this and Trichomonas vaginalis (T. vaginalis) demonstrated the highest statistically significance with M. genitalium infection. Our risk/benefit ratio remains low as there is no increase to patient risk with continued chart reviewing and this information will continue to be de-identified and not linked to the patient's chart. However, the knowledge we can obtain from this study in regards to prevalence and pregnancy in relation to M. genitalium would be high given the number of patients and the fact that this infection has not be heavily studied in this population.

vi. Any serious adverse events reported? N

If yes, please describe

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Please upload all IRB renewal documents in the Attachments section (e.g. approval letter, updated consent documents).

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* * * Personnel Information * * *

Principal Investigator

Harris Health System defines "investigator" as an individual who conducts a research study. If the study is conducted by a team of individuals, the investigator is the responsible leader of the team (21 CFR 312.3[b]). Also referred to as the principal investigator.

Name of Principal Investigator	Degree (MD/PhD)	Title
Kenneth Muldrew	MD, MPH	Medical Director
Email	**Phone**	**Fax**
muldrew@bcm.edu	615-429-6825	
Department	**Mailing Address**	
Pathology/Lab		

Is this personnel credentialed/authorized by Harris Health System to perform the procedure(s) required for this study and been assigned a Harris Health provider number? If you answered no, please contact Monique Okeke at Monique.Okeke@harrishealth.org. Y

Study Coordinator (edit access)

Harris Health System defines a "study coordinator" as an individual who assists the investigator in the conduct of research.

Name of Study Coordinator	Degree (MD/PhD)	Title
Irene Stafford		
Email	**Phone**	**Fax**
petrouia@yahoo.com		
Department	**Mailing Address**	
Obstetrics/Gynecology		

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Is this personnel credentialed/authorized by Harris Health System to perform the procedure(s) required for this study and been assigned a Harris Health contractor number? If you answered no, please contact Monique Okeke at Monique.Okeke@harrishealth.org. Y

Additional Personnel

Name of Additional Personnel	Degree	Title	Department
Elizabeth Kravitz		Medical Student	Obstetrics/Gynecology
Soumya Gogia	BA		Research and Sponsored Programs
Mary Fang		Medical Student	Other
Savannah Bryce	BS	medical student	Obstetrics/Gynecology
Kelsey Hummel			Pathology/Lab

---

* * * Study Affiliate and Location * * *

Study Affiliate and Location

Please select your Harris Health System affiliate institution:
Please attach affiliate IRB approval letter in Attachments section.

X Baylor College of Medicine
X UTHealth - Houston
MD Anderson Cancer Center
Texas Woman's University
Prairie View A&M University
University of Houston
University of Houston - Clearlake
University of Texas Medical Branch - Galveston
X Harris Health System
Other

STUDY LOCATION (we strongly recommend that you discuss this study with applicable Unit/Health Center representatives) (Check all that apply)

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X Ben Taub General Hospital
Unit/Specific Clinic(s) Pathology

Lyndon B. Johnson General Hospital
Unit/Specific Clinic(s)

Quentin Mease Hospital
Unit/Specific Clinic(s)

The Ambulatory Surgery Center (ACS) at LBJ
Thomas Street Health Center
Acres Home Health Center
Aldine Health Center
Baytown Health Center
Casa De Amigos Health Center
Gulfgate Health Center
MLK Health Center
Northwest Health Center
Vallbona Health Center
Settegast Health Center
E.A. Squatty Lyons Health Center
Strawberry Health Center
School Based Clinics
Unit/Specific Clinic(s)

Homeless Clinics
Unit/Specific Clinic(s)

El Franco Lee Health Center
Dental Center
Riverside Dialysis Center
Smith Clinic
Unit/Specific Clinic(s)

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* * * Funding * * *

NONE--This project does not have any external funding. If you want to add funding for the study, please uncheck "NONE."

Funding

Add external funding source(s) below: Sponsor, Federal, or Other. Select "None" above if there is no external funding for the study.

Commercial

Sponsor Name
Hologic Corp, Malborough, MA: Please specify

---

* * * District Resources and Methodology * * *

Harris Health System Resources and Methodology

Study Title

H-44123: A Point Prevalence Study of Mycoplasma Genitalium among Pregnant Women in Houston, TX

Study Information

Please attach affiliate IRB application/summary in Attachments section.

Affiliate IRB Protocol Number: H-44123

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Sample Size: (Harris Health participants ONLY) 1300

1. Harris Health System Resources

Will any Harris Health resources or services be utilized for research purposes? Y

Indicate which of the following services will be used for research-specific procedures only. Select all that apply.

Investigational Drug Services

X Pathology/Laboratory Services

a. Data Report Search

X b. Block/Slide/Sample Retrieval

c. Stain/Test or Procedure

d. Other

Nursing Service

Radiology Service

Patient data provided by Information Technology (IT). Email research@harrishealth.org to request an IT Research Report Request.

Nuclear Medicine Service

Health Information Management (Chart Review)

Other (specify):

2. Methodology

Please check here if the protocol does not involve patient care or clinical interventions (e.g. medical record review, employee survey research).

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a) **Specify which protocol procedures and/or tests are considered routine clinical care and will be billed to the patient or insurance.**

Nothing will be billed to the patient on insurance. We will be testing residual, otherwise discarded urogenital samples from pregnant women that have been stored..

b) **Specify which protocol procedures and/or tests are being done solely for research purposes using Harris Health resources. Items included here will be included in the Harris Health financial agreement and reimbursed by the study sponsor. If a coverage analysis has been completed, please upload a copy in the Attachments section.**

We will be using previously analyzed and stored urogenital samples (Hologic NG/CT NAAT) collected from pregnant women at Ben Taub between July 1, 2020 and June 30, 2021. These samples will be de-identified, assigned a study identification number, and shipped to Dr. Erik Munson with the Clinical Laboratory Science department in the College of Health Sciences, Marquette University, Milwaukee, WI for M. genitalium testing using the Hologic transcription-mediated amplification (TMA) Panther system.

No costs will be accrued at Ben Taub hospital. We will be collecting stored samples with the help of pathologists Drs. Dunn-Urbonas and Muldrew. The PI will de-identify, label, and ship samples to Marquette University. Hologic corp will be providing all reagents and supplies to Dr. Erik Munson to test the samples using the TMA Panther system.

The PI will be accessing patient's medical records to record limited demographic data including age, race, ethnicity, parity, and STI coinfection.

---

* * * Recruiting and Advertising * * *

3. Recruitment and Advertising

Are you requesting access to Harris Health facilities for recruitment purposes ONLY (e.g. posting of flyers, screening medical records)? Patients will be required to undergo all research interventions, including the process of informed consent, at an off-site, non-Harris Health location. N

Are you requesting approval to post recruitment flyers in a Harris Health System facility? If N

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yes, a copy of the flyer must be uploaded in the Attachments section and will be stamped with the Harris Health approval.

In the Attachments section, please attach a copy of all subject recruitment materials that will be used to recruit Harris Health System patients.

---

* * * Informed Consent * * *

4. Informed Consent

If your protocol involves a physical intervention that may incur research-related injuries, the injury disclaimer below MUST be included in the English and Spanish consent documents. Your application will be returned if the disclaimer is not present.

"In the event of injury resulting from this research, (your institution) and/or the Harris Health System (name of Harris Health facility or facilities) are not able to offer financial compensation nor to absorb the costs of medical treatment. However, necessary facilities, emergency treatment and professional services will be available to you, just as they are to the general community."

a) Will WRITTEN informed consent be obtained from participants in this study? N

b) Will patients who only speak Spanish be included in this study? Please note, it is Harris Health policy that this population be included unless there is a scientific rationale to exclude them.
If no, please provide a scientific rationale for excluding this population.

Are foreign language consent forms, other than Spanish, being used for this study? (e.g. Arabic, Chinese, Vietnamese)?

In the Attachments section, please upload all IRB-approved informed consent documents.

For studies enrolling Spanish-speaking only participants, please ensure a translated full Spanish consent

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document is uploaded in the Attachments section. If the Spanish consent document is pending approval by the affiliate IRB, Harris Health will grant a 3-month approval , at which time submission of the translated document is required for continued approval.

*** Attachments ***

5. Attachments

Please attach the below items, if applicable.

- Affiliate IRB approval letter
- Affiliate IRB protocol summary/application
- Affiliate IRB-approved consent forms (all languages)
- Information Technology Research Report Request
- Subject recruitment materials used to recruit Harris Health System patients

Type	Attachment Name	Attached Date	Submitted Date
Affiliate IRB application	H-44123 IRB application	09/24/2018	09/24/2018
Affiliate IRB approval letter	Amendment Letter - IRB approval	09/24/2018	09/24/2018
Affiliate IRB approval letter	Human Approval Letter	09/24/2018	09/24/2018
Harris Health Financial Agreement	H-44123 Irene Stafford Financial Agreement 9-27-18	09/27/2018	09/27/2018
Affiliate IRB approval letter	Human Approval Letter_asp	09/11/2019	02/10/2020
Affiliate IRB approval letter	Consent Waiver Memorandum	05/23/2020	05/29/2020
Affiliate IRB approval letter	Human Amendment Information	05/23/2020	05/29/2020
Affiliate IRB approval letter	Amendment Letter	05/23/2020	05/29/2020
Affiliate IRB approval letter	Human Approval Letter__2020	05/23/2020	05/29/2020

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Affiliate IRB approval letter	Human Protocol Report__2020	05/29/2020	05/29/2020
Affiliate IRB approval letter	MGen Study Renewal 7_7_20-6_16_21_baylor approval letter	07/10/2020	07/10/2020
Affiliate IRB application	MGen Study Renewal 7_7_20-6_16_21_baylor approved protocol	07/10/2020	07/10/2020

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* * * Assurance * * *

Assurance

The Principal Investigator of this study provides the following assurances:

The eProtocol application submitted for this study is complete and accurate.

The PI acknowledges responsibility for the conduct of this project as described in the Harris Health System Administrative Review application.

The PI has evaluated the protocol and determined that s/he has sufficient resources to conduct the study as submitted and necessary to protect subjects who enroll in the study.

All co- or sub-investigators, study coordinators, and other research personnel to whom the PI delegates study-related responsibilities will receive thorough training in human subjects protections as well as in the specific details of study procedures.

The PI will not begin the study until s/he has received notification of final Harris Health System Administrative approval.

The PI acknowledges his/her responsibility for the accuracy of all documents submitted to the Harris Health System Office of Research on his/her behalf.

The PI will comply with all Harris Health System Office of Research requests regarding the status of the study.

The PI will seek and obtain Harris Health System Administrative approval for all study modifications.

The PI will promptly report any unexpected or otherwise significant adverse events or unanticipated problems or incidents that may occur in the course of this study.

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The PI will notify the Harris Health System Office of Research when his/her research has been completed or terminated.

X The Principal Investigator has read and agrees to abide by the above obligations.

Please click on the 'Check for Completeness' button in the left navigation to check if your application is complete.

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* * * Event History * * *

Event History

Date	Status	View Attachments	Letters
09/24/2018	NEW FORM CREATED		
09/24/2018	NEW FORM SUBMITTED	Y	
09/24/2018	NEW FORM PANEL ASSIGNED		
09/24/2018	NEW FORM REVIEWER(S) ASSIGNED		
09/27/2018	NEW FORM SUBMITTED (CYCLE 1)	Y	
09/28/2018	NEW FORM APPROVED	Y	Y
09/05/2019	PROTOCOL EXPIRED		
09/11/2019	CONTINUING REVIEW 1 FORM CREATED		
09/11/2019	CONTINUING REVIEW 1 FORM SUBMITTED	Y	
09/11/2019	CONTINUING REVIEW 1 FORM PANEL REASSIGNED		
09/17/2019	CONTINUING REVIEW 1 FORM APPROVED	Y	Y
02/10/2020	AMENDMENT 1 FORM CREATED		

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02/10/2020	AMENDMENT 1 FORM SUBMITTED	Y	
02/10/2020	AMENDMENT 1 FORM APPROVED	Y	Y
02/18/2020	AMENDMENT 2 FORM CREATED		
04/03/2020	AMENDMENT 2 FORM SUBMITTED	Y	
04/03/2020	AMENDMENT 2 FORM APPROVED	Y	Y
04/23/2020	CONTINUING REVIEW 2 FORM CREATED		
05/29/2020	CONTINUING REVIEW 2 FORM SUBMITTED	Y	
06/09/2020	CONTINUING REVIEW 2 FORM RETURNED		
07/10/2020	CONTINUING REVIEW 2 FORM RESUBMITTED	Y	
07/14/2020	CONTINUING REVIEW 2 FORM APPROVED	Y	Y

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
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	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	4-6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6
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	4-6
Bias	9	Describe any efforts to address potential sources of bias	4-6
Study size	10	Explain how the study size was arrived at	4-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4-6
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
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	6-7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6-7
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	6-7

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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 (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-7
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	7-10
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	7-10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
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	N/a

*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 http://www.strobe-statement.org.

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A retrospective analysis of infection and antimicrobial resistance patterns of *Mycoplasma genitalium* among pregnant women in the southwestern United States

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Contributorship Statement:

All authors were responsible for data entry which was reviewed by the lead author and validated. Irene A. Stafford MD, Kelsey Hummel MD, James J. Dunn PhD, Kenneth L. Muldrew, MD, Alexandra Berra MD, Elizabeth S. Kravitz, BS, Soumya Gogia, BS, Irene Martin BSc, and Erik Munson PhD all contributed to the data collection, data analysis, protocol development and manuscript preparation. Irene A Stafford, MD is the guarantor for the overall content.

Abstract:

Background: *Mycoplasma genitalium* is a sexually transmitted infection 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 center.

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= .003, .008 and .004 respectively). *M. genitalium* infection was also associated with *T. vaginalis* co-infection and Streptococcus agalactiae (GBS) colonization (p =<0.001 and .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:

Strengths:

- This analysis is one of the largest evaluating prevalence rates of *M. genitalium* in pregnant women presenting for routine care.
- *Mycoplasma 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.

Weaknesses:

- Perinatal outcome data was not recorded.
- Prospective data regarding persistent infection was not collected in this analysis.

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Competing Interest Statement:

The lead author, Irene A Stafford, MD affirms that this manuscript is an honest, accurate and transparent account of the study being reported; that no important aspects of the study have been omitted. The authors report no conflict of interest.

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

Data Sharing:

Extra data can be accessed via the Dryad data repository at http://datadryad.org/ with the doi: 10.5061/dryad.qrfj6q5fq

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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[21-33].

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To date, six studies have assessed the role of *M. genitalium* with pregnancy related complications, including a 2015 meta-analysis (N = 3,128) 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 STI were accounted for (pooled OR 2.3)[22-28]. The meta-analysis by Lis et. al[28] demonstrated the limitations of prior published data mainly related to varying prevalence rates ranging from 2 – 20 % in women, with scant data concerning rates of infection among pregnant women[4-7,20-33]. 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 [22-33]. The objective of this study was to determine these characteristics among a cohort of pregnant women in a large tertiary obstetrical care center.

Design:

After Institutional Review Board approval from the Baylor College of Medicine, all remnant Aptima Multitest clinician-collected endocervical samples from pregnant women presenting to care between August 30, 2018 and November 30, 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, WI for *M. genitalium* 16S rRNA and *Trichomonas vaginalis* testing by the transcription - mediated amplification technique utilizing Panther System automation (Hologic, Inc., San Diego, CA) 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 *N. gonorrhoeae* and *C. trachamatis* per institutional protocol and guidelines.

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M. genitalium positive specimens were shipped to the Public Health Agency of Canada, National Microbiology Laboratory for additional testing. DNA was extracted from the specimens using the MagNA Pure DNA and Viral Nucleic Acid kit (Roche, Laval, Quebec) per manufacturer's instruction. Specimens with detectable *M. genitalium* DNA were subsequently analyzed by sequencing the 23S rRNA gene to identify mutations associated with azithromycin resistance and *parC* and *gyrA* genes associated with resistance to moxifloxacin[20,29-33].

Demographic variables, obstetrical data, pelvic symptoms consistent with cervicitis (pelvic pressure, vaginal discharge, lower abdominal cramping), and STI co-infection [*Neisseria gonorrhoeae*, *Chlamydia trachomatis,* herpes simplex virus, human immunodeficiency virus, *Trichomonas vaginalis*, human papillomavirus (types 16,18)] Bacterial vaginosis and group *B Streptococcus* (GBS) colonization data were extracted from the chart and recorded by the investigators. Patient characteristics, co-infection with other STI and *M. genitalium* resistance profiles were summarized by means with standard deviations, 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% confidence intervals (CIs) were determined for the resistance profiles. STROBE guidelines were followed for the study design, methods and analysis[34]. All protected health information was removed from discarded samples prior to shipment and all data was 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.

Ethics Approval:

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This study was approved by the Institutional Review Board and Research Review Committee at the Baylor College of Medicine and Harris Health systems, approval number H-1809-2029 renewed 7/14/20.

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 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 72.8% were multiparous. There were no significant differences in gestational or pre-gestational 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 vs. 28.1 years respectively p = .004) and *M. genitalium* was significantly associated with Black race (p =.003) and Hispanic ethnicity (p = .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 to 78 women who tested negative for *M. genitalium* infection (11.8%; p = .307).

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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 *Trichomonas vaginalis* (p = <.001). In addition, the rate of group B *Streptococcus* (GBS) colonization was significantly higher among women infected with *M. genitalium* compared to women who tested negative (58.3% vs. 16.1% respectively p = .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 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%[4-7,20-33]. 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< .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[29-33,35-41].

As described in prior studies, infection with *M. genitalium* was found to be more prevalent among pregnant women compared to N. *gonorrhoeae,* where reported prevalence rates in women remain

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less than 1%[2-10,20-33,38,41.] 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 toward 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, 22-33]. Further understanding of this infection as it relates to pregnancy and adverse perinatal outcomes begins with understanding its characteristics as an STI; its association with obstetrical factors, demographics, co-infection patterns and pelvic symptomatology as described in our analysis.

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[22-33,35,39]. In some countries, strains of multi-drug resistant *M. genitalium* strains exist, limiting therapeutic options[22-33,35,39]. Although the predicted azithromycin resistance is significantly less in this population compared to prior published reports involving

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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[10,36-39].

Pristinamycin, an antimicrobial agent synthesized 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[39]. 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[39].

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 is 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 labor[11-19,22-28]. 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, i.e. preterm birth, after adjusting for prior preterm birth, using a conservative odds ratio of 1.3 per

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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% [22-33,40]. The information provided in this manuscript can inform research scientists for future prospective studies including a large, randomized-controlled treatment trial to prevent preterm birth related to *M. genitalium* infection.

Of note, the co-infection rate of *Trichomonas 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[1-10,20-22,40,41]. An additional interesting result is the significantly higher association of group B streptococcal (GBS) colonization 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 pre-processing 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 center. *M. genitalium* shares features of other STI including common demographic risk factors, such as Black race and

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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 harboring this STI.

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Table 1. Demographics and Baseline Obstetrical Characteristics

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	M. genitalium Positive (N=41)	*M. genitalium* Negative (N=678)	p-value**	Total Population (N=719)
Age, mean (std)	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, 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 pre-gestational 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)

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GA at specimen collection, mean (std)	22.4 (10.90)	22.2 (10.81)	0.816	22.2 (10.81)
Previous Preterm (< 37 wks)	2/39 (5.1)	63/664 (9.5)	0.568	65/703 (9.3)
Previous PROM (< 37 wks)	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

*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.

^^Any of the following symptoms: pelvic pressure, vaginal discharge or lower abdominal cramping

Bolded if significantly different

Table 2: *Mycoplasma genitalium* RNA Detection rates from genital swab collections by Race/Ethnicity

	***Detection of Mycoplasma 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, American Indian/Alaskan Native)	0/21 (0)
Race, p-value*	0.003
Black	14/115 (12.2)
Non-Black	27/601 (4.5)

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Ethnicity, p-value*	0.008
Hispanic	23/535 (4.3)
Non-Hispanic	18/179 (10.1)

p-value from Fisher's exact test.

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Table 3. Co-Infections with *M. genitalium*

	M. genitalium Positive (N=41) n/N1 (%)	*M. genitalium* Negative (N=678) n/N1 (%)	p-value	Total Population (N=719)
Human papillomavirus 16, 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 I/II	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 *M. genitalium* and Resistance profiles

	Total (N=726)	95% CI
N with sample tested	719	
M. genitalium positive	41 (5.7)	4.0 - 7.4**
23S		
A2058G*	3 (7.3)	1.5 - 19.9
A2058T*	2 (4.9)	0.6 - 16.5
A2059G*	3 (7.3)	1.5 - 19.9
No sequence*	15 (36.6)	22.1 - 53.1
WT*	18 (43.9)	28.5 - 60.3

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Mutation related to azithromycin resistance^	8/26 (30.8)	14.3 - 51.8
gyrA		
95MET(ATG)->ILE(ATC)*	1 (2.4)	0.06 - 12.9
Inconclusive*	1 (2.4)	0.06 - 12.9
No sequence*	12 (29.3)	16.1 - 45.5
WT*	25 (60.9)	49.4 - 79.9
gyrA mutation ^	1/26 (3.8)	0.09 - 18.4
parC		
83SER(AGT)->ILE(ATT)*	1 (2.4)	0.06 - 12.9
83SER(AGT)->ASN(AAT)*	0	0 - 8.6
Inconclusive*	9 (22.0)	10.6 - 37.6
No sequence*	14 (34.2)	20.1 - 50.6
WT^^	17 (41.5)	26.3 - 57.9
parC mutation^	1/18 (5.6)	0.14 - 27.3
M. genitalium Negative	678 (94.3)	92.6 - 96.0**
M. genitalium positive	1 (5.9)	0.15 - 28.7
M. genitalium Negative	16 (94.1)	71.3 - 99.9

Data presented as N (%)

*Percent of positive for *M. genitalium*

Exact 95% confidence intervals (CI) except for ** which are based on the normal approximation.

^ Denominator is positive samples with conclusive sequencing results

^^ Wild type

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