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

| Journal:                      | BMJ Open  |
|-------------------------------|---|
| Manuscript ID                 | bmjopen-2021-050475   |
| Article Type:                 | Original research   |
| Date Submitted by the Author: | 24-Feb-2021   |
| Complete List of Authors:     | Stafford, Irene; The University of Texas Health Science Center at<br>Houston, Department of Obstetrics and Gynecology<br>Hummel, Kelsey; Baylor College of Medicine, Department of Pathology<br>and Immunology<br>Dunn, James J.; Baylor College of Medicine, Department of Pathology<br>and Immunology<br>Muldrew, Kenneth; Baylor College of Medicine, Department of Pathology<br>and Immunology<br>Berra, Alexandra; Baylor College of Medicine, Obstetrics and Gynecology<br>Kravitz, Elizabeth; Baylor College of Medicine, Obstetrics and Gynecology<br>Gogia, Soumya; Baylor College of Medicine,<br>Martin, Irene; Public Health Agency of Canada,<br>Munson, Erik; Marquette University, Clinical Laboratory Science |
| Keywords:                     | BACTERIOLOGY, Reproductive medicine < GYNAECOLOGY, INFECTIOUS DISEASES, Maternal medicine < OBSTETRICS, OBSTETRICS  |
|                               |   |





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

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women Irene A. Stafford  $MD^1$ irene.stafford@uth.tmc.edu, Kelsey Hummel MD<sup>2</sup>, Kelsey.hummel@bcm.edu, James J. Dunn PhD<sup>2</sup> jjdunn@texaschildrens.org, Kenneth L. Muldrew, MD<sup>2</sup> muldrew@bcm.edu, Alexandra Berra MD<sup>3</sup> alexandra.berra@bcm.edu, Elizabeth S. Kravitz, BS<sup>3</sup> elizabeth.kravitz@bcm.edu, Soumya Gogia, BS<sup>3</sup> soumya.gogia@bcm.edu, Irene Martin BSc<sup>4</sup> irene.martin@canada.ca, Erik Munson PhD<sup>5</sup> erik.munson@marquette.edu (1) Department of Obstetrics and Gynecology, University of Texas Health Science Center, McGovern Medical School, 6431 Fannin MSB 3.286, Houston, TX 77030, (2) Department of Pathology and Immunology; Baylor College of Medicine, MC315 1 Baylor Plaza Houston, TX 77030 832-824-2662, 615-429-6825, (3) Department of Obstetrics and Gynecology; Baylor College of Medicine, 6651 Main Street, Houston, TX 77030 (4) JC Wilt Infectious Diseases Research Centre 745 Logan Avenue, Winnipeg, Manitoba 1 204-789-2000, (5) College of Health Sciences, Marguette University, Schroeder Complex, Room 244 Milwaukee, WI 53233 (414) 288-5053 **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.

# 23 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 ina 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 transcriptionmediated 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. **Funding Statement:** This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. **Competing Interest Statement:** 

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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<sup>1-</sup> <sup>10</sup>. Due to its fastidious nature, culture technique methods have not proven to successfully identify organism in the clinical environment<sup>1-8</sup>. Fortunately, with the recent developments of highly sensitive molecular platforms, *M. genitalium* can expeditiously be detected in urogenital samples with > 97% sensitivity <sup>11-20</sup>. 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<sup>21-28</sup>. 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)<sup>22-28</sup>. The meta-analysis by Lis et. al<sup>28</sup> 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<sup>4-7,20-29-33</sup>. 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 <sup>29-33</sup>. The objective of this study was to determine these characteristics among a cohort of pregnant women in a large tertiary obstetrical care center.

94 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<sup>11-20.</sup> 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<sup>2,20,21,26,27</sup>.

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<sup>34</sup>. 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. 

- <sup>0</sup> 125 **Patient and public involvement:**
- <u>,</u> 126

There was no patient involved for this study.

5 127 7 128 **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%)

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 = .003) .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)

149 Of the samples with detectable *M. genitalium* RNA, 26 (63.4 %) were of sufficient quantity 150 to undergo conclusive sequencing analysis for azithromycin resistance. Of these, 8 / 26 (30.7%) 151 were found to have 23S rRNA mutations (A2059G) associated with azithromycin resistance. Of 152 the 18 samples that were of sufficient quantity to undergo sequencing analysis for the parC gene 153 mutation, one (5.6%) was found to have the parC (Ser $\rightarrow$ Asn83) gene mutation. Of the 26 samples 154 that were of sufficient quantity to undergo sequencing analysis for the gyrA gene mutation, one

**Discussion:** 

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(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.

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 nonpregnant patients approach 50%, azithromycin resistance was detected in 30% of isolates collected from the cohort and 5.6% demonstrated moxifloxacin resistance<sup>29-33,35</sup>.

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<sup>2-10</sup>. 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<sup>10</sup>. 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<sup>6,7,9</sup>. 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*<sup>11-9</sup>. 

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<sup>11-19, 22-28</sup>. 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<sup>29-33,35</sup>. In some countries, strains of multi-drug resistant M. genitalium strains exist, limiting therapeutic options<sup>29-33,35</sup>. 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<sup>10,35-38</sup>. 

198 Data on which to determine whether prenatal treatment of *M. genitalium* can reduce the 199 incidence of pelvic complaints, preterm birth or any other adverse perinatal outcome is still Page 11 of 33

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200 lacking. Future research is warranted to examine relationships between mycoplasmas and 201 pregnancy, given that some of these organisms may be mechanistically related in their ability to 202 induce inflammatory cytokines, potentially leading to preterm labor<sup>11-19, 22-28</sup>. This gap in 203 knowledge is a significant impediment for implicating this organism as a notifiable cause of 204 reproductive tract disease, and for evidence-based improvement of the current prenatal STI-205 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<sup>1-10,20-22</sup>. 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.

221 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 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 his ST1. patient harboring this STI. Word Count: 1,863 

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| 53        | 217 | regimen should be used for treating Muconlasma ganitalium A meta analysis Sex  |
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497 (69.4)

134 (18.7)

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| 41<br>42   |   |  |  |  | p-value**   |   |
| 42<br>43   |   |  | (N=41)   | (N=678)  |   | (N=719)   |
| 44   |   |  |  |  |   |   |
| 45   |   |  |  |  |   |   |
| 46<br>47   |   | Age, mean (std)  | 24.9 (4.89)  | 28.1 (6.93)  | 0.004   | 27.9 (6.87)   |
| 48   |   | < 20   | 5 (12.2)   | 80 (11.9)  | 0.021   | 85 (11.9)   |

20-34

35 or more

462 (68.4))

133 (19.7)

35 (85.4)

1 (2.4)

| Race/ethnicity   | 41           | 675           | 0.003* | 716           |
|--|--------------|---------------|--------|---------------|
|  |              |               | 0.004^ |               |
| 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<br>Hawaiian/Pacific Islander,<br>American Indian/Alaskan<br>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<br>pregnancy   | 0/38 (0)     | 25/654 (3.8)  | 0.390  | 25/692 (3.6)  |
| Tobacco use during<br>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<br>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<br>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)   |

59

| 351<br>352<br>353 | exact test or W<br>^^Any of the for<br><b>Bolded</b> if signif | <ul> <li>*p-value compares Hispanic (including Mexican and unknown) vs. non-Hispanic. **p-value from Fisher exact test or Wilcoxon Rank Sum test.</li> <li>*Any of the following symptoms: pelvic pressure, vaginal discharge or lower abdominal cramping</li> <li>Bolded if significantly different</li> </ul> |                                   |  |  |  |  |
|-------------------|--|---|-----------------------------------|--|--|--|--|
| 354               |  |   |                                   |  |  |  |  |
| 355               | Table 2: My  | coplasma genitalium RNA Detection ra  | ates from genital swab collection |  |  |  |  |
| 356               | Race/Ethnicit  | y   |                                   |  |  |  |  |
|                   |  | 0   | Detection of Mycoplasma genitaliu |  |  |  |  |
|                   |  |   | [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   | 0/21 (0)                          |  |  |  |  |
|                   |  | Islander, American Indian/Alaskan Native)   |                                   |  |  |  |  |
|                   |  |   | 4                                 |  |  |  |  |
|                   |  | 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)                     |  |  |  |  |
| 357<br>358        | p-value from F   | ïsher's exact test.   |                                   |  |  |  |  |

| 359 | 59 Table 3. Co-Infections with <i>M. genitalium</i> |                 |                  |         |                |  |
|-----|---|-----------------|------------------|---------|----------------|--|
|     |   | M. genitalium   | M. genitalium    |         | Total          |  |
|     |   | Positive (N=41) | Negative (N=678) |         | Population     |  |
|     |   |                 |                  | p-value |                |  |
|     |   | n/N1 (%)        | n/N1 (%)         |         | (N=719)        |  |
|     | Human papillomavirus 16,<br>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           |             |
| <i>M. genitalium</i> 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 |

Mycoplasma genitalium and pregnancy For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

 

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|                          |   |   |   |
|                          | Mutation related to<br>azithromycin resistance <sup>^</sup>   | 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**   |
|                          | <i>M. genitalium</i> positive   | 1 (5.9)   | 0.15 - 28.7   |
|                          | M. genitalium Negative  | 16 (94.1)   | 71.3 - 99.9   |
| 365<br>366<br>367<br>368 | Data presented as N (%)<br>*Percent of positive for <i>M. gen</i><br>Exact 95% confidence intervals<br>^ Denominator is positive samp | <i>hitalium</i><br>(CI) except for **<br>les with conclusiv | which are based on the normal approximation ve sequencing results |
| 369                      | ^^ Wild type  |   |   |
| 370                      |   |   |   |
| 371                      |   |   |   |
| 372                      |   |   |   |
| 373                      |   |   |   |
|                          |   |   |   |
|                          |   |   |   |
|                          |   |   |   |

| C-PROTOCOL                   | PROTOCOL<br>Harris Health Administrative Review<br>Research Application<br>Harris Health System | Protocol # 18-09-2029<br>Date Printed:<br>02/20/2021 |
|------------------------------|---|--|
| Continuing Review            |   | 1  |
| Personnel Information        |   | 2  |
| Study Affiliate and Location |   |  |
| Funding                      | <b>.</b>  |  |
| District Resources and Metho | odology   |  |
| Recruiting and Advertising   |   | 7  |
| Informed Consent             |   |  |
| Attachments                  |   | 9  |
| Assurance                    |   |  |
|                              |   |  |
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Page 21 of 33 **BMJ Open** e-Protocol PROTOCOL Protocol # 18-09-2029 Date Printed: 02/20/2021 Harris Health Administrative Review **Research Application** Harris Health System **Protocol Title:** H-44123: A Point Prevalence Study of Mycoplasma Genitalium among Pregnant Women in Houston, TX Protocol Type: Harris Health Administrative Review Research Application **Date Submitted:** 05/29/2020 **Approval Period:** 07/13/2020-06/16/2021 This Print View may not reflect all comments and contingencies for approval. Important Note: Please check the comments section of the online protocol. Questions that appear to not have been answered may not have been required for this submission. Please see the system application for more details. \* \* \* Continuing Review \* \* \* **Continuing Review** 1) Is this the submission of translated Spanish consent documents following the Ν initial 3-month approval period? If yes, please upload the IRB-approved documents in the Attachments section. 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? Ν If no, why should study remain active? ii. Y Have changes been made since last approval? 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 Total number of Harris Health System participants enrolled or patient records 1300 iv. reviewed since last approval? Please provide a summary of any interim findings and/or publication citations since last approval. ۷. 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. Ν vi. Any serious adverse events reported? If yes, please describe 57 58 59

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| <mark>e</mark> -Protocol  | PROTOCOL<br>Harris Health Administrative<br>Research Application   | Protocol # 18-09-2029<br>Date Printed: 02/20/2021  |
|---|--|--|
|   | Harris Health System   | n  |
| Protocol Title:   | H-44123: A Point Prevalence Stu<br>Pregnant Women in Houston, TX   | idy of Mycoplasma Genitalium among   |
| Protocol Type:<br>Date Submitted:   | Harris Health Administrative Rev<br>05/29/2020   | iew Research Application   |
| Approval Period:  | 07/13/2020-06/16/2021  |  |
| Important Note:   | This Print View may not reflect all co<br>Please check the comments section<br>Questions that appear to not have be<br>for this submission. Please see the s | mments and contingencies for approval.<br>of the online protocol.<br>een answered may not have been required<br>system application for more details. |
|   |  |  |
| Please upload all IRB renew<br>consent documents).  | al documents in the Attachments s  | section (e.g. approval letter, updated   |
|   | * * * Personnel Information  | * * *  |
|   |  |  |
|   |  |  |
| Principal Investigator  |  |  |
| Also referred to as the principal in<br><b>Name of Principal Investigator</b><br>Kenneth Muldrew  | vestigator.<br>Degree (MD/PhD)<br>MD, MPH  | <ul> <li>Title</li> <li>Medical Director</li> </ul>  |
| Email<br>muldrew@bcm.edu  | Phone<br>615-429-6825  | Fax  |
| Department  | Mailing Address  |  |
| Pathology/Lab   |  |  |
| Is this personnel credentialed/auth<br>the procedure(s) required for this<br>provider number? If you answered<br>Monique.Okeke@harrishealth.org | norized by Harris Health System to<br>study and been assigned a Harris<br>I no, please contact Monique Oke   | o perform Y<br>Health<br>ke at   |
| Study Coordinator (edit access)   |  |  |
| Harris Health System defines a "s<br>conduct of research.   | tudy coordinator" as an individual   | who assists the investigator in the  |
| Name of Study Coordinator<br>Irene Stafford   | Degree (MD/PhD)  | Title  |
| Email   | Phone  | Fax  |
| petrouia@yahoo.com  |  |  |
| <b>Department</b><br>Obstetrics/Gynecology  | Mailing Address  |  |
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| Protoco  | ol Title:  | H-44123: A Point   | Prevalence Study of Myco   | oplasma Genitalium among  |              |
| Protoco  | ol Type:   | Harris Health Adm  | in nousion, 1A   | rch Application   |              |
| Date Si  | ubmitted:  | 05/29/2020   |  |   |              |
| Approv   | al Period:   | 07/13/2020-06/16/  | /2021  |   |              |
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|  |  |  |  | Obstetrics/Gynecolog  | y<br>        |
| Soumya Gogia   | БА   |  |  | Programs  | reo          |
| Mary Fang  |  |  | Medical Student  | Other   |              |
| Savannah Bryce   | BS   |  | medical student  | Obstetrics/Gynecolog  | y            |
| Kelsey Hummel  |  |  |  | Pathology/Lab   |              |
| Study Affiliate and L<br>Please select y<br>Please attach<br>Baylor College<br>UTHealth - Ho<br>MD Anderson<br>Texas Woman<br>Prairie View A<br>University of H<br>University of T | ocation<br>your Harris He<br>affiliate IRB a<br>of Medicine<br>ouston<br>Cancer Cente<br>'s University<br>&M University<br>louston<br>louston - Clea | ealth System affiliat<br>pproval letter in Atta<br>er<br>v<br>arlake<br>Branch - Galvestor | e institution:<br>achments section.  |   |              |
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|   | C-PROTOCOL   | PROTOCOL<br>Harris Health Administrative Review<br>Research Application<br>Harris Health System   |
|---|--|---|
|   | Protocol Title:  | H-44123: A Point Prevalence Study of Mycoplasma Genitalium among<br>Pregnant Women in Houston, TX   |
|   | Protocol Type:   | Harris Health Administrative Review Research Application  |
|   | Date Submitted:  | 05/29/2020  |
|   | Approval Period:   | 07/13/2020-06/16/2021   |
|   | Important Note:  | This Print View may not reflect all comments and contingencies for approval.<br>Please check the comments section of the online protocol.<br>Questions that appear to not have been answered may not have been required<br>for this submission. Please see the system application for more details. |
|   |  | $\land$   |
| Х | Ben Taub General Hospital  |   |
|   | Unit/Specific Clinic(s)  | Pathology   |
|   | Lyndon B. Johnson Genera<br>Unit/Specific Clinic(s)  | I Hospital  |
|   | Quentin Mease Hospital<br>Unit/Specific Clinic(s)  |   |
|   | The Ambulatory Surgery Ce<br>Thomas Street Health Center<br>Acres Home Health Center<br>Aldine Health Center<br>Baytown Health Center<br>Casa De Amigos Health Ce<br>Gulfgate Health Center<br>MLK Health Center<br>Northwest Health Center<br>Vallbona Health Center<br>Settegast Health Center<br>E.A. Squatty Lyons Health C<br>Strawberry Health Center<br>School Based Clinics<br>Unit/Specific Clinic(s) | er<br>Inter<br>Center   |
|   | Homeless Clinics   |   |
|   |  |   |
|   | El Franco Lee Health Cente   | er  |
|   | El Franco Lee Health Cente<br>Dental Center  |   |
|   | El Franco Lee Health Cente<br>Dental Center<br>Riverside Dialysis Center   | er  |

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|  | C-PROTUCU   | Harris Health Administrative Review<br>Research Application<br>Harris Health System  | )29<br>)21 |
|  | Protocol Title:   | H-44123: A Point Prevalence Study of Mycoplasma Genitalium among<br>Pregnant Women in Houston, TX  |            |
|  | Protocol Type:<br>Date Submitted:   | Harris Health Administrative Review Research Application 05/29/2020  |            |
|  | Approval Period:<br>Important Note:   | 07/13/2020-06/16/2021<br>This Print View may not reflect all comments and contingencies for approval.<br>Please check the comments section of the online protocol.<br>Questions that appear to not have been answered may not have been required<br>for this submission. Please see the system application for more details. |            |
|  |   |  |            |
|  |   | * * * Funding * * *  | -          |
|  |   |  |            |
| NON<br>unch  | EThis project does r<br>eck "NONE."   | not have any external funding. If you want to add funding for the study, plea  | ase        |
| Funding  |   |  |            |
| Add e<br>extern  | external funding sourc<br>nal funding for the stu   | e(s) below: Sponsor, Federal, or Other. Select "None" above if there is no dy.   |            |
|  |   |  |            |
| Sponsor  |   |  |            |
| Lalagia C  |   | . Flease specify   |            |
| Hologic C  |   |  |            |
| Hologic C  |   | 0  |            |
| Hologic C  | * *   | * * District Resources and Methodology * * *   | -          |
| Hologic C  | alth System Resource  | * * District Resources and Methodology * * *   | -          |
| Hologic C<br><br>Harris Hea<br>Stud  | alth System Resources   | * * District Resources and Methodology * * *<br>s and Methodology  | -          |
| Hologic C<br>Harris Hea<br>Stud<br>H-44<br>TX                                    | alth System Resource:<br>y Title<br>4123: A Point Prevale   | * * District Resources and Methodology * * *<br>s and Methodology<br>nce Study of Mycoplasma Genitalium among Pregnant Women in Houston  | -          |
| Hologic C<br>Harris Hea<br>Stud<br>H-44<br>TX<br>Study Info                      | alth System Resources<br>y Title<br>4123: A Point Prevale   | * * District Resources and Methodology * * *<br>s and Methodology<br>nce Study of Mycoplasma Genitalium among Pregnant Women in Houston  | -          |
| Hologic C<br>Harris Hea<br>Stud<br>H-44<br>TX<br>Study Infor<br>Pleas            | alth System Resources       y Title       4123: A Point Prevale       rmation       se attach affiliate IRB a             | * * District Resources and Methodology * * *<br>s and Methodology<br>nce Study of Mycoplasma Genitalium among Pregnant Women in Houston  | -          |
| Hologic C<br>Harris Hea<br>Stud<br>H-44<br>TX<br>Study Infor<br>Pleas<br>Affilia | alth System Resources<br>y Title<br>4123: A Point Prevale<br>rmation<br>se attach affiliate IRB a<br>ate IRB Protocol Num | ** District Resources and Methodology *** s and Methodology nce Study of Mycoplasma Genitalium among Pregnant Women in Houston application/summary in Attachments section. ber: H-44123  | -          |

| <del>С</del> -Ркотосоі                                 | - PROTOCOL Protocol #<br>Harris Health Administrative Review<br>Research Application<br>Harris Health System   | : 18-09-2029<br>: 02/20/2021    |
|--|--|---------------------------------|
| Protocol Title:  | H-44123: A Point Prevalence Study of Mycoplasma Genitaliur<br>Pregnant Women in Houston, TX  | n among                         |
| Protocol Type:<br>Date Submitted:                      | Harris Health Administrative Review Research Application 05/29/2020  |                                 |
| Approval Period:<br>Important Note:                    | 07/13/2020-06/16/2021<br>This Print View may not reflect all comments and contingencies for a<br>Please check the comments section of the online protocol.<br>Questions that appear to not have been answered may not have bee<br>for this submission. Please see the system application for more detail | pproval.<br>In required<br>ils. |
|  |  |                                 |
| Sample Size: (Harris Healt<br>ONLY)                    | h participants 1300  |                                 |
|  |  |                                 |
| Will any Harris Health resources                       | rces<br>ources or services be utilized for research purposes?  | Y                               |
|  |  |                                 |
| Indicate which of the follow apply.                    | ing services will be used for research-specific procedures only. S   | select all that                 |
| Investigational I                                      | Drug Services  |                                 |
| X Pathology/Labo                                       | ratory Services  |                                 |
| a. Data R  | eport Search   |                                 |
| X b. Block/S   | Slide/Sample Retrieval   |                                 |
| c. Stain/T   | est or Procedure   |                                 |
| d Other  |  |                                 |
|  | 0  | ]                               |
|  |  |                                 |
| Nursing Service  |  |                                 |
| Radiology Serv   | ice  |                                 |
| Patient data pro<br>request an IT R<br>Nuclear Medicir | ovided by Information Technology (IT). Email research@harrishea<br>esearch Report Request.<br>ne Service   | alth.org to                     |
| Health Informat  | ion Management (Chart Review)  |                                 |
| Other (specify):                                       |  |                                 |
| Methodology  |  |                                 |
| Please check here if the p                             | rotocol does not involve patient care or clinical interventions (e.g.  | medical                         |
| record review, employee's                              | urvey research).   |                                 |
| For nee  | r review only - http://bmjopen.bmj.com/site/about/quidelines.xhtml   | Pa                              |
| . o. pec   |  | i di                            |

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|         | e-Protocol  | PROTOCOL P  | rotocol # 18-09-2029                                      |
|---------|---|---|---|
|         |   | Harris Health Administrative Review   | ; Pfiffled. 02/20/2021                                    |
|         |   | Hesearch Application  |   |
|         | Protocol Title:   | H-44123: A Point Prevalence Study of Mycoplasma G   | enitalium among   |
|         |   | Pregnant Women in Houston, TX   |   |
|         | Protocol Type:<br>Date Submitted:                       | Harris Health Administrative Review Research Applica  | ation   |
|         | Approval Period:  | 07/13/2020-06/16/2021   |   |
|         | Important Note:   | This Print View may not reflect all comments and contingen-<br>Please check the comments section of the online protocol.<br>Questions that appear to not have been answered may not<br>for this submission. Please see the system application for m | cies for approval.<br>have been required<br>lore details. |
|         |   |   |   |
|         |   |   |   |
|         |   |   |   |
| a) S    | Specify which protocol pro-<br>he patient or insurance. | cedures and/or tests are considered routine clinical care   | and will be billed to                                     |
| Ī       | Nothing will be billed to the                           | e patient on insurance. We will be testing residual, other  | wise discarded  |
| Ŀ       | urogenital samples from p                               | regnant women that have been stored   |   |
|         |   |   |   |
| b) S    | Specify which protocol pro                              | cedures and/or tests are being done solely for research   | purposes using  |
| Ĺ       | arris Health resources. It                              | ems included here will be included in the Harris Health fi  | nancial agreement   |
| s<br>II | n the Attachments section                               | y sponsor. If a coverage analysis has been completed,   | please upload a copy                                      |
| Ī       | We will be using previous!                              | v analyzed and stored urogenital samples (Hologic NG/0  | CT NAAT) collected  |
| 1       | from pregnant women at E                                | en Taub between July 1, 2020 and June 30, 2021. Thes  | se samples will be  |
|         | de-identified, assigned a s                             | tudy identification number, and shipped to Dr. Erik Muns  | on with the Clinical                                      |
| i       | for M. genitalium testing us                            | sing the Hologic transcription-mediated amplification (TN   | IA) Panther system.                                       |
|         | No costo will be apprued a                              | t Pon Touch boonital. We will be collecting stored comple   | a with the help of  |
|         | oathologists Drs. Dunn-Ur                               | bonas and Muldrew. The PI will de-identify, label, and sh   | is with the help of                                       |
| li      | Marquette University. Holo<br>the samples using the TM  | ogic corp will be providing all reagents and supplies to De<br>A Panther system.  | r. Erik Munson to test                                    |
| -       | The PI will be accessing n                              | atient's medical records to record limited demographic d  | ata including age   |
| Ŀ       | race, ethnicity, parity, and                            | STI coinfection.  | ata molduling age,  |
|         |   |   |   |
|         |   |   |   |
|         |   |   |   |
|         |   | * * * Recruiting and Advertising * * *  |   |
|         |   |   |   |
| _       |   |   |   |
| Red     | cruitment and Advertising                               |   |   |
|         |   |   |   |
|         | Are you requesting acce                                 | ss to Harris Health facilities for recruitment purposes ON  | ILY (e.g. N   |
|         | posting of flyers, screeni                              | ng medical records)? Patients will be required to under   | JO all<br>non-Harris                                      |
|         | Health location.  | icidaling the process of informed consent, at an on-site,   |   |
|         |   |   |   |
|         |   |   |   |
|         | Are you requesting oner                                 | oval to nost recruitment flyers in a Harris Haalth System   | facility? If N  |
|         |   |   |   |
|         |   |   |   |
|         |   |   |   |
|         | For peer  | review only - http://bmjopen.bmj.com/site/about/guidelin  | es.xhtml Page 7 o   |

| 1<br>2               | <mark>e</mark> -Protocol  | PROTOCOL<br>Harris Health Administrative Review  | Protocol # 18-09-2029<br>Date Printed: 02/20/2021                                |
|----------------------|---|--|--|
| 3<br>4               |   | Research Application<br>Harris Health System   |  |
| 5<br>6               | Protocol Title:   | H-44123: A Point Prevalence Study of Mycopla<br>Pregnant Women in Houston, TX  | asma Genitalium among  |
| 7<br>8               | Protocol Type:<br>Date Submitted:   | Harris Health Administrative Review Research 05/29/2020  | Application  |
| 9                    | Approval Period:  | 07/13/2020-06/16/2021  |  |
| 10<br>11<br>12       | Important Note:   | This Print View may not reflect all comments and co<br>Please check the comments section of the online pr  | ntingencies for approval.<br>otocol.   |
| 12<br>13<br>14       |   | Questions that appear to not have been answered n<br>for this submission. Please see the system applicati  | nay not have been required<br>on for more details.                               |
| 14<br>15<br>16       |   | <u> </u>   |  |
| 17<br>18             | yes, a copy of the flyer m<br>with the Harris Health app  | ust be uploaded in the Attachments section and proval.   | will be stamped  |
| 19<br>20             |   |  |  |
| 21<br>22             |   |  |  |
| 23                   | In the Attachments section,   | please attach a copy of all subject recruitment m  | aterials that will be used to  |
| 24<br>25             | recruit Harris Health System  | patients.  |  |
| 26                   |   | *** Informed Concert * * *   |  |
| 27<br>28             |   | a a a informed Consent a a a   |  |
| 20                   |   |  |  |
| 30                   | 4. Informed Consent   |  |  |
| 31<br>32             | If your protocol involves a physica   | al intervention that may incur research-related inj  | juries, the injury   |
| 33<br>34<br>25       | be returned if the disclaimer is no   | t present.   |  |
| 36<br>37<br>38<br>39 | "In the event of injury resulting fro<br>of Harris Health facility or facilities<br>medical treatment. However, nec<br>available to you, just as they are t | m this research, (your institution) and/or the Har<br>s) are not able to offer financial compensation no<br>essary facilities, emergency treatment and profe<br>to the general community."   | ris Health System (name<br>or to absorb the costs of<br>ssional services will be |
| 40<br>41             | a) Will WRITTEN informed conse  | ant be obtained from participants in this study?   | Ν  |
| 42                   |   |  |  |
| 43<br>44<br>45       | <ul> <li>b) Will patients who only speak S<br/>policy that this population be in</li> </ul>   | panish be included in this study? Please note, if noted and the study? Please note, if noted and the state is a scientific rationale to extra the state of the st | is Harris Health<br>cclude them.   |
| 46                   | If no, please provide a scient  | fic rationale for excluding this population.   |  |
| 47<br>⊿9             |   |  |  |
| 40<br>49             | Are foreign language con  | sent forms, other than Spanish, being used for th  | nis studv? (e.a.   |
| 50                   | Arabic, Čhinese, Vietnam  | ese)?  |  |
| 51<br>52             |   |  |  |
| 53                   |   |  |  |
| 54<br>55             | In the Attachments section, pleas   | e upload all IKB-approved informed consent doc   | cuments.   |
| 56                   | For studies enrolling Spanish-spe   | aking only participants, please ensure a translat  | ed full Spanish consent  |
| 57<br>59             |   |  |  |
| 50<br>59             |   |  |  |
| 60                   | For peer  | review only - http://bmjopen.bmj.com/site/about/g  | juidelines.xhtml P   |

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| <mark>е</mark> -Ркот  | Harris Health Adr<br>Research<br>Harris Health   | TOCOL<br>ministrative Review<br>Application<br>alth System   | Protocol # 18-09-202<br>Date Printed: 02/20/202   |
|---|--|--|---|
| Protocol Title  | e: H-44123: A Point Pr<br>Pregnant Women in  | revalence Study of Mycop<br>Houston, TX  | olasma Genitalium among   |
| Protocol Tvr  | e: Harris Health Admin   | histrative Review Researc  | h Application   |
| Date Submit   | ted: 05/29/2020  |  |   |
| Approval Pe   | riod: 07/13/2020-06/16/20  | 021  |   |
| Important No  | ote: This Print View may no<br>Please check the com<br>Questions that appear<br>for this submission. Pl  | ot reflect all comments and o<br>ments section of the online<br>to not have been answered<br>ease see the system applica   | contingencies for approval.<br>protocol.<br>I may not have been required<br>ation for more details.   |
| locument is uploaded in<br>he affiliate IRB, Harris H<br>locument is required for   | the Attachments section. If the ealth will grant a 3-month app continued approval.   | e Spanish consent docun<br>proval , at which time sub  | nent is pending approval by mission of the translated   |
|   | * * * Attach   | ments * * *  |   |
|   |  |  |   |
|   |  |  |   |
| 5. Attachments<br>Please attach the below i<br>- Affiliate IRB approval le  | items, if applicable.  |  |   |
| 5. Attachments<br>Please attach the below i<br>- Affiliate IRB approval le<br>Affiliate IRB protocol su<br>- Affiliate IRB-approved o<br>- Information Technology<br>- Subject recruitment ma   | items, if applicable.<br>etter<br>mmary/application<br>consent forms (all languages)<br>y Research Report Request<br>iterials used to recruit Harris H   | Health System patients   |   |
| 5. Attachments<br>Please attach the below i<br>- Affiliate IRB approval le<br>Affiliate IRB protocol su<br>- Affiliate IRB-approved o<br>- Information Technology<br>- Subject recruitment ma   | items, if applicable.<br>etter<br>mmary/application<br>consent forms (all languages)<br>y Research Report Request<br>aterials used to recruit Harris H   | Health System patients   | Submitted Date  |
| 5. Attachments<br>Please attach the below i<br>- Affiliate IRB approval le<br>Affiliate IRB protocol su<br>- Affiliate IRB-approved o<br>- Information Technology<br>- Subject recruitment ma   | items, if applicable.<br>etter<br>mmary/application<br>consent forms (all languages)<br>y Research Report Request<br>aterials used to recruit Harris H<br>Attachment Name<br>H-44123 IRB application   | Health System patients          Attached Date         09/24/2018   | Submitted Date  |
| 5. Attachments<br>Please attach the below i<br>- Affiliate IRB approval le<br>Affiliate IRB protocol su<br>- Affiliate IRB-approved o<br>- Information Technology<br>- Subject recruitment ma   | items, if applicable.<br>etter<br>mmary/application<br>consent forms (all languages)<br>y Research Report Request<br>aterials used to recruit Harris H<br><u>Attachment Name</u><br><u>H-44123 IRB application</u><br>Amendment Letter - IRB<br>approval   | Health System patients          Attached Date         09/24/2018         09/24/2018  | Submitted Date<br>09/24/2018<br>09/24/2018  |
| 5. Attachments<br>Please attach the below i<br>- Affiliate IRB approval le<br>Affiliate IRB protocol su<br>- Affiliate IRB-approved o<br>- Information Technology<br>- Subject recruitment ma<br>Subject recruitment ma<br>Fype<br>Affiliate IRB application<br>Affiliate IRB approval<br>etter   | items, if applicable.<br>etter<br>mmary/application<br>consent forms (all languages)<br>y Research Report Request<br>iterials used to recruit Harris H<br>Attachment Name<br>H-44123 IRB application<br>Amendment Letter - IRB<br>approval<br>Human Approval Letter  | Attached Date           09/24/2018           09/24/2018           09/24/2018   | Submitted Date           09/24/2018           09/24/2018           09/24/2018           09/24/2018  |
| 5. Attachments<br>Please attach the below i<br>- Affiliate IRB approval le<br>- Affiliate IRB protocol su<br>- Affiliate IRB-approved o<br>- Information Technology<br>- Subject recruitment ma<br>Subject recruitment ma<br>- Subject recruitment ma | items, if applicable.<br>etter<br>mmary/application<br>consent forms (all languages)<br>y Research Report Request<br>iterials used to recruit Harris H<br>Attachment Name<br>H-44123 IRB application<br>Amendment Letter - IRB<br>approval<br>Human Approval Letter<br>H-44123 Irene Stafford<br>Financial Agreement 9-27-<br>18   | Attached Date           09/24/2018           09/24/2018           09/24/2018           09/24/2018           09/24/2018           09/22/2018  | Submitted Date           09/24/2018           09/24/2018           09/24/2018           09/24/2018           09/24/2018           09/27/2018  |
| 5. Attachments<br>Please attach the below i<br>- Affiliate IRB approval le<br>- Affiliate IRB protocol su<br>- Affiliate IRB-approved o<br>- Information Technology<br>- Subject recruitment ma<br>Type<br>Affiliate IRB application<br>Affiliate IRB approval<br>etter<br>Harris Health Financial<br>Agreement<br>Affiliate IRB approval   | items, if applicable.<br>etter<br>mmary/application<br>consent forms (all languages)<br>y Research Report Request<br>aterials used to recruit Harris H<br>Attachment Name<br>H-44123 IRB application<br>Amendment Letter - IRB<br>approval<br>Human Approval Letter<br>H-44123 Irene Stafford<br>Financial Agreement 9-27-<br>18<br>Human Approval<br>Letter_asp   | Attached Date         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/27/2018         09/27/2018         09/11/2019  | Submitted Date           09/24/2018           09/24/2018           09/24/2018           09/24/2018           09/24/2018           09/24/2018           09/24/2018           09/24/2018           09/24/2018           09/24/2018           09/27/2018           02/10/2020  |
| 5. Attachments<br>Please attach the below i<br>- Affiliate IRB approval le<br>- Affiliate IRB protocol su<br>- Affiliate IRB-approved o<br>- Information Technology<br>- Subject recruitment ma<br>- Subject recruitment  | items, if applicable.<br>etter<br>mmary/application<br>consent forms (all languages)<br>y Research Report Request<br>aterials used to recruit Harris H<br>Attachment Name<br>H-44123 IRB application<br>Amendment Letter - IRB<br>approval<br>Human Approval Letter<br>H-44123 Irene Stafford<br>Financial Agreement 9-27-<br>18<br>Human Approval<br>Letter_asp<br>Consent Waiver<br>Memorandum   | Attached Date         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/27/2018         09/11/2019         05/23/2020   | Submitted Date         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/27/2018         02/10/2020         05/29/2020   |
| 5. Attachments<br>Please attach the below i<br>- Affiliate IRB approval le<br>- Affiliate IRB protocol su<br>- Affiliate IRB-approved o<br>- Information Technology<br>- Subject recruitment ma<br>Subject recruitment ma<br>- Subject recruitment ma | items, if applicable.<br>etter<br>mmary/application<br>consent forms (all languages)<br>y Research Report Request<br>aterials used to recruit Harris H<br>Attachment Name<br>H-44123 IRB application<br>Amendment Letter - IRB<br>approval<br>Human Approval Letter<br>H-44123 Irene Stafford<br>Financial Agreement 9-27-<br>18<br>Human Approval<br>Letter_asp<br>Consent Waiver<br>Memorandum<br>Human Amendment<br>Information                     | Attached Date         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/27/2018         09/11/2019         05/23/2020         05/23/2020  | Submitted Date         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/27/2018         02/10/2020         05/29/2020         05/29/2020   |
| 5. Attachments<br>Please attach the below i<br>- Affiliate IRB approval le<br>- Affiliate IRB protocol su<br>- Affiliate IRB protocol su<br>- Affiliate IRB-approved o<br>- Information Technology<br>- Subject recruitment ma<br>Type<br>Affiliate IRB application<br>Affiliate IRB approval<br>etter<br>- Harris Health Financial<br>Agreement<br>- Affiliate IRB approval<br>etter<br>- Affiliate IRB approval<br>- Affiliate IRB - Approval<br>- Affiliate IRB                                      | items, if applicable.<br>etter<br>mmary/application<br>consent forms (all languages)<br>y Research Report Request<br>aterials used to recruit Harris H<br>Attachment Name<br>H-44123 IRB application<br>Amendment Letter - IRB<br>approval<br>Human Approval Letter<br>H-44123 Irene Stafford<br>Financial Agreement 9-27-<br>18<br>Human Approval<br>Letter_asp<br>Consent Waiver<br>Memorandum<br>Human Amendment<br>Information<br>Amendment Letter | Attached Date         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/27/2018         09/21/2019         05/23/2020         05/23/2020         05/23/2020 | Submitted Date         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/24/2018         09/27/2018         02/10/2020         05/29/2020         05/29/2020         05/29/2020 |

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| C-PROT   | Harris Health Ac<br>Research<br>Harris He  | TOCOL<br>Iministrative Revie<br>Application<br>ealth System   | Protocol # 18-09<br>Date Printed: 02/20  | )-2029<br>)/2021 |
|--|--|---|--|------------------|
| Protocol Title   | H-44123: A Point F<br>Pregnant Women i   | Prevalence Study of M   | lycoplasma Genitalium amor   | ng               |
| Protocol Typ   | e: Harris Health Admi  | nistrative Review Res   | search Application   |                  |
| Date Submit  | (ed: 05/29/2020<br>iod: 07/13/2020-06/16/2   | 0021  |  |                  |
| Important No   | te: This Print View may in<br>Please check the cor<br>Questions that appea<br>for this submission. F       | not reflect all comments<br>nments section of the or<br>ar to not have been answ<br>Please see the system a | and contingencies for approval<br>nline protocol.<br>wered may not have been requi<br>pplication for more details. | l.<br>red        |
|  |  | -   |  |                  |
| Affiliate IRB approval<br>letter   | Human Protocol<br>Report_2020  | 05/29/2020  | 05/29/2020   |                  |
| Affiliate IRB approval<br>letter   | MGen Study Renewal<br>7_7_20-6_16_21_baylor<br>approval letter   | 07/10/2020  | 07/10/2020   |                  |
| Affiliate IRB application  | MGen Study Renewal<br>7_7_20-6_16_21_baylor<br>approved protocol   | 07/10/2020  | 07/10/2020   |                  |
| The PI acknowledges res<br>Administrative Review ap  | ponsibility for the conduct of plication.  | this project as descri  | bed in the Harris Health Systems<br>esources to conduct the stud   | tem<br>dv as     |
| submitted and necessary<br>All co- or sub-investigator<br>study-related responsibilit<br>specific details of study p | to protect subjects who enrors, study coordinators, and or<br>ies will receive thorough trai<br>rocedures. | oll in the study.<br>Ther research personr<br>ning in human subjec  | nel to whom the PI delegates<br>ts protections as well as in th  | s<br>he          |
| The PI will not begin the s<br>approval.   | study until s/he has received  | notification of final Ha  | arris Health System Administ   | trative          |
| The PI acknowledges his<br>System Office of Researc  | /her responsibility for the acc<br>h on his/her behalf.  | uracy of all documen  | ts submitted to the Harris He  | ealth            |
| The PI will comply with al study.  | Harris Health System Office  | e of Research reques  | ts regarding the status of the   | )                |
| The PI will seek and obta  | in Harris Health System Adr  | ninistrative approval fo  | or all study modifications.  |                  |
| The PI will promptly repor<br>or incidents that may occ  | t any unexpected or otherwi<br>ur in the course of this study.   | se significant adverse  | events or unanticipated pro  | blems            |
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Page 10 of 12

Page 31 of 33 **BMJ Open** e-Protocol PROTOCOL Protocol # 18-09-2029 1 Date Printed: 02/20/2021 Harris Health Administrative Review 2 **Research Application** 3 Harris Health System 4 5 H-44123: A Point Prevalence Study of Mycoplasma Genitalium among **Protocol Title:** 6 Pregnant Women in Houston, TX 7 Protocol Type: Harris Health Administrative Review Research Application 8 **Date Submitted:** 05/29/2020 9 **Approval Period:** 07/13/2020-06/16/2021 10 **Important Note:** This Print View may not reflect all comments and contingencies for approval. 11 Please check the comments action of the online protocol. Questions that appear to not have been answered may not have been required for this submission. Please see the system application for more details. 12 13 14 15 16 17 The PI will notify the Harris Health System Office of Research when his/her research has been completed or 18 terminated. 19 20 The Principal Investigator has read and agrees to abide by the above obligations. Х 21 22 23 Please click on the 'Check for Completeness' button in the left navigation to check if your application is complete. 24 25 \_\_\_\_\_ 26 27 \* \* \* Event History \* \* \* 28 29 **Event History** 30 31 Date Status **View Attachments** Letters 32 09/24/2018 NEW FORM CREATED 33 **NEW FORM** 09/24/2018 34 SUBMITTED 35 **NEW FORM PANEL** 09/24/2018 36 ASSIGNED 37 09/24/2018 NEW FORM 38 REVIEWER(S) 39 ASSIGNED 40 09/27/2018 **NEW FORM** Y 41 SUBMITTED (CYCLE 1) 42 09/28/2018 NEW FORM APPROVED Y 43 09/05/2019 PROTOCOL EXPIRED 44 09/11/2019 **CONTINUING REVIEW 1** 45 FORM CREATED 46 47 09/11/2019 **CONTINUING REVIEW 1** Y FORM SUBMITTED 48 09/11/2019 **CONTINUING REVIEW 1** 49 FORM PANEL 50 REASSIGNED 51 09/17/2019 **CONTINUING REVIEW 1** Y Y 52 FORM APPROVED 53 02/10/2020 AMENDMENT 1 FORM 54 CREATED 55 56 57 58 59 For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml Page 11 of 12 60

|                          | BMJ Open   |
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| <mark>C</mark> -PROTOCOL | PROTOCOL<br>Harris Health Administrative Review<br>Research Application<br>Harris Health System  |
| Protocol Title:          | H-44123: A Point Prevalence Study of Mycoplas<br>Pregnant Women in Houston, TX   |
| Protocol Type:           | Harris Health Administrative Review Research A   |
| Date Submitted:          | 05/29/2020   |
| pproval Period:          | 07/13/2020-06/16/2021  |
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| AMENDMENT 1<br>SUBMITTED | FORM Y   |

Protocol # 18-09-2029 Date Printed: 02/20/2021

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| 02/10/2020 | AMENDMENT 1<br>SUBMITTED    | FORM  |  | Y   |  |  |
| 02/10/2020 | AMENDMENT 1<br>APPROVED     | FORM  |  | Y   |  | Y  |
| 02/18/2020 | AMENDMENT 2<br>CREATED      | FORM  |  |   |  |  |
| 04/03/2020 | AMENDMENT 2<br>SUBMITTED    | 2 FORM  |  | Y   |  |  |
| 04/03/2020 | AMENDMENT 2<br>APPROVED     | 2 FORM  |  | Y   |  | Y  |
| 04/23/2020 | CONTINUING F<br>FORM CREATE | REVIEW 2<br>D   |  |   |  |  |
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# STROBE Statement—Checklist of items that should be included in reports of cohort studies

|                        | Item<br>No | Recommendation  | Page<br>No |
|------------------------|------------|---|------------|
| Title and abstract     | 1          | (a) Indicate the study's design with a commonly used term in the title or the       | 1          |
|                        |            | abstract  |            |
|                        |            | (b) Provide in the abstract an informative and balanced summary of what was         | 2-3        |
|                        |            | done and what was found   |            |
| Introduction           |            |   |            |
| Background/rationale   | 2          | Explain the scientific background and rationale for the investigation being         | 3          |
|                        |            | reported  |            |
| 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           | 4-6        |
|                        |            | recruitment, exposure, follow-up, and data collection                               |            |
| 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           | 4-6        |
|                        |            | unexposed   |            |
| Variables              | 7          | Clearly define all outcomes, exposures, predictors, potential confounders, and      | 4-6        |
|                        |            | effect modifiers. Give diagnostic criteria, if applicable                           |            |
| Data sources/          | 8*         | For each variable of interest, give sources of data and details of methods of       | 4-6        |
| measurement            |            | assessment (measurement). Describe comparability of assessment methods if           |            |
|                        |            | there is more than one group  |            |
| 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,     | 4-6        |
|                        |            | describe which groupings were chosen and why  |            |
| Statistical methods    | 12         | (a) Describe all statistical methods, including those used to control for           | 4-6        |
|                        |            | confounding   |            |
|                        |            | (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                      |            |
|                        |            | ( <i><u>e</u></i> ) Describe any sensitivity analyses                               |            |
| Results                |            |   |            |
| Participants           | 13*        | (a) Report numbers of individuals at each stage of study—eg numbers potentially     | 6-7        |
| 1                      |            | eligible, examined for eligibility, confirmed eligible, included in the study,      |            |
|                        |            | completing follow-up, and analysed  |            |
|                        |            | (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)   | 6-7        |
| -                      |            | and information on exposures and potential confounders                              |            |
|                        |            | (b) Indicate number of participants with missing data for each variable of interest |            |
|                        |            | (c) Summarise follow-up time (eg, average and total amount)                         |            |
|                        | 1 5 4      |   | 67         |

| 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their       | 6-7   |
|----|---|---|
|    | 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  |   |
| 17 | Report other analyses done-eg analyses of subgroups and interactions, and sensitivity           |   |
|    | analyses  |   |
|    |   |   |
| 18 | Summarise key results with reference to study objectives  | 7-10  |
| 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. | 7-10  |
|    | Discuss both direction and magnitude of any potential bias                                      |   |
| 20 | Give a cautious overall interpretation of results considering objectives, limitations,          | 7-10  |
|    | multiplicity of analyses, results from similar studies, and other relevant evidence             |   |
| 21 | Discuss the generalisability (external validity) of the study results                           | 10  |
| on |   | -   |
| 22 | Give the source of funding and the role of the funders for the present study and, if            | N/a   |
|    |   |   |
|    | 16<br>17<br>17<br>18<br>19<br>20<br>21<br>21<br>00<br>22  | <ul> <li>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</li> <li>(b) Report category boundaries when continuous variables were categorized</li> <li>(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period</li> <li>17 Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses</li> <li>18 Summarise key results with reference to study objectives</li> <li>19 Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias</li> <li>20 Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence</li> <li>21 Discuss the generalisability (external validity) of the study results</li> <li>22 Give the source of funding and the role of the funders for the present study and, if</li> </ul> |

\*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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# **BMJ Open**

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

| Journal:                             | BMJ Open  |
|--------------------------------------|---|
| Manuscript ID                        | bmjopen-2021-050475.R1  |
| Article Type:                        | Original research   |
| Date Submitted by the Author:        | 27-Apr-2021   |
| Complete List of Authors:            | Stafford, Irene; The University of Texas Health Science Center at<br>Houston, Department of Obstetrics and Gynecology<br>Hummel, Kelsey; Baylor College of Medicine, Department of Pathology<br>and Immunology<br>Dunn, James J.; Baylor College of Medicine, Department of Pathology<br>and Immunology<br>Muldrew, Kenneth; Baylor College of Medicine, Department of Pathology<br>and Immunology<br>Berra, Alexandra; Baylor College of Medicine, Obstetrics and Gynecology<br>Kravitz, Elizabeth; Baylor College of Medicine, Obstetrics and Gynecology<br>Gogia, Soumya; Baylor College of Medicine,<br>Martin, Irene; Public Health Agency of Canada,<br>Munson, Erik; Marquette University, Clinical Laboratory Science |
| <b>Primary Subject<br/>Heading</b> : | Obstetrics and gynaecology  |
| Secondary Subject Heading:           | Infectious diseases, Sexual health  |
| Keywords:                            | BACTERIOLOGY, Reproductive medicine < GYNAECOLOGY, INFECTIOUS<br>DISEASES, Maternal medicine < OBSTETRICS, OBSTETRICS   |
|                                      |   |

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# A retrospective analysis of infection and antimicrobial resistance patterns of *Mycoplasma genitalium* among pregnant women in the southwestern United States Irene A. Stafford MD<sup>1</sup> irene.stafford@uth.tmc.edu, Kelsey Hummel MD<sup>2</sup>, Kelsey.hummel@bcm.edu, James J. Dunn PhD<sup>2</sup> jjdunn@texaschildrens.org, Kenneth L. Muldrew, MD<sup>2</sup> muldrew@bcm.edu, Alexandra Berra MD<sup>3</sup> alexandra.berra@bcm.edu, Elizabeth S. Kravitz,

6 BS<sup>3</sup> elizabeth.kravitz@bcm.edu, Soumya Gogia, BS<sup>3</sup> soumya.gogia@bcm.edu, Irene Martin BSc<sup>4</sup>

7 irene.martin@canada.ca, Erik Munson PhD<sup>5</sup> erik.munson@marquette.edu

8 (1) Department of Obstetrics and Gynecology, University of Texas Health Science Center,

9 McGovern Medical School, 6431 Fannin MSB 3.286, Houston, TX 77030, (2) Department of

10 Pathology and Immunology; Baylor College of Medicine, MC315 1 Baylor Plaza Houston, TX

11 77030 832-824-2662, 615-429-6825, (3) Department of Obstetrics and Gynecology; Baylor

12 College of Medicine, 6651 Main Street, Houston, TX 77030 (4) JC Wilt Infectious Diseases

13 Research Centre 745 Logan Avenue, Winnipeg, Manitoba 1 204-789-2000, (5) College of Health

14 Sciences, Marquette University, Schroeder Complex, Room 244 Milwaukee, WI 53233 (414)

*288-5053* 

)

# 17 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.

# 23 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 ina 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 transcriptionmediated 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. **Funding Statement:** This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. **Competing Interest Statement**: 

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| 2              |    |   |
|----------------|----|---|
| 2<br>3<br>4    | 64 | The lead author, Irene A Stafford, MD affirms that this manuscript is an honest, accurate and                     |
| 5<br>6<br>7    | 65 | transparent account of the study being reported; that no important aspects of the study have been                 |
| /<br>8<br>9    | 66 | omitted. The authors report no conflict of interest.  |
| 10<br>11       | 67 | The corresponding author confirms on behalf of all authors that there have been no involvements                   |
| 12<br>13       | 68 | that might raise the question of bias in the work reported or in the conclusions, implications, or                |
| 14<br>15<br>16 | 69 | opinions stated.  |
| 17<br>18       | 70 | Data Sharing:   |
| 19<br>20       | 71 | Extra data can be accessed via the Dryad data repository at http://datadryad.org/ with the doi:                   |
| 21<br>22<br>22 | 72 | 10.5061/dryad.qrfj6q5fq   |
| 23<br>24<br>25 | 73 |   |
| 26<br>27       | 74 | Word Count: 2021  |
| 28<br>29       | 75 |   |
| 30<br>31<br>22 | 76 |   |
| 32<br>33<br>34 | 77 | Introduction:   |
| 35<br>36       | 78 | Mycoplasma genitalium is an emerging cause of sexually transmitted disease in women <sup>1-</sup>                 |
| 37<br>38       | 79 | <sup>10</sup> . Due to its fastidious nature, culture technique methods have not proven to successfully identify  |
| 39<br>40<br>41 | 80 | organism in the clinical environment <sup>1-8</sup> . Fortunately, with the recent developments of highly         |
| 42<br>43       | 81 | sensitive molecular platforms, <i>M. genitalium</i> can expeditiously be detected in urogenital samples           |
| 44<br>45       | 82 | with $> 97\%$ sensitivity <sup>11-20</sup> . As a result, contemporary studies have demonstrated this organism to |
| 46<br>47       | 83 | 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<sup>21-33</sup>.

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)<sup>22-28</sup>. The meta-analysis by Lis et. al<sup>28</sup> 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<sup>4-7,20-33</sup>. 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 <sup>22-33</sup>. The objective of this study was to determine these characteristics among a cohort of pregnant women in a large tertiary obstetrical care center. R. **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<sup>11-20.</sup> Only one sample

105 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 protocoland guidelines.

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*M. genitalium* positive specimens were shipped to the Public Health Agency of Canada, 109 National Microbiology Laboratory for additional testing. DNA was extracted from the specimens 110 using the MagNA Pure DNA and Viral Nucleic Acid kit (Roche, Laval, Quebec) per 111 manufacturer's instruction. Specimens with detectable *M. genitalium* DNA were subsequently 112 analyzed by sequencing the 23S rRNA gene to identify mutations associated with azithromycin 113 resistance and *parC* and *gyrA* genes associated with resistance to moxifloxacin<sup>20,29-33</sup>.

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<sup>34</sup>. 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 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 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 $\rightarrow$ 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. 

# 167 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 nonpregnant patients approach 50%, azithromycin resistance was detected in 30% of isolates collected from the cohort and 5.6% demonstrated moxifloxacin resistance<sup>29-33,35-41</sup>.

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  $1\%^{2-10,20-33,38,41}$ . 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<sup>2-10</sup>. 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<sup>10</sup>. 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<sup>6,7,9</sup>. 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<sup>11-20</sup>. 

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<sup>11-19, 22-33</sup>. 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<sup>22-33,35,39</sup>. In some countries, strains of multi-drug resistant *M*. *genitalium* strains exist, limiting therapeutic options<sup>22-33,35,39</sup>. 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 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<sup>10,36-39</sup>.

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<sup>39</sup>. 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<sup>39</sup>.

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<sup>11-19,22-28</sup>. 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

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% <sup>22-33,40</sup>. 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<sup>1-10,20-22,40,41</sup>. 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

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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| 49       | 376 |  |
| 50       | 377 | Table 1. Demographics and Baseline Obstetrical Characteristics   |
| 51       |     |  |
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|  | <i>M. genitalium</i><br>Positive<br>(N=41) | <i>M. genitalium</i><br>Negative<br>(N=678) | p-value** | Total<br>Population<br>(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*    | 716                            |
|  | 6  |   | 0.004^    |                                |
| 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<br>Hawaiian/Pacific Islander,<br>American Indian/Alaskan<br>Native) | 0  | 21 (3.1)                                    |           | 21 (2.9)                       |
| Unknown  | 0  | 5 (0.7)                                     | 0         | 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<br>pregnancy   | 0/38 (0)                                   | 25/654 (3.8)                                | 0.390     | 25/692 (3.6)                   |
| Tobacco use during<br>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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|----------------|-----|--|-------------------|-----------------|----------------------|-------------------------|
| 3<br>4<br>5    |     | GA at specimen collection, mean (std)  | 22.4 (10.90)      | 22.2 (10.81     | ) 0.816              | 22.2 (10.81)            |
| 6<br>7<br>8    |     | Previous Preterm (< 37<br>wks)         | 2/39 (5.1)        | 63/664 (9.5     | 0.568                | 65/703 (9.3)            |
| 9<br>10        |     | Previous PROM (< 37 wks)               | 0/39 (0)          | 15/651 (2.3     | ) 1.00               | 15/690 (2.2)            |
| 11<br>12       |     | Cervicitis symptoms^^                  | 7/39 (18.0)       | 78/662 (11.     | 8) 0.307             | 85/701 (12.1)           |
| 13<br>14<br>15 |     | Cerclage in index<br>pregnancy         | 0/39 (0)          | 6/664 (0.9)     | 1.00                 | 6/703 (0.9)             |
| 16<br>17       |     | Twin Pregnancy                         | 0/39 (0)          | 9/670 (1.3)     | 1.00                 | 9/709 (1.3)             |
| 18             | 378 | Data presented as N (%) uple           | ss otherwise sne  | rified          |                      |                         |
| 19             | 379 | *n-value compares black vs r           | on-black          | cirica          |                      |                         |
| 20             | 380 | An-value compares Hispanic             | (including Mexic: | an and unknow   | n) vs. non-Hisnanic  | **n-value from Fisher's |
| 21             | 381 | evact test or Wilcovon Bank            | Sum test          |                 | ny vs. non mspanie.  |                         |
| 22             | 201 | AApy of the following symp             | tome: nolvic proc | sura vaginal d  | icchargo or lower ab | dominal cramping        |
| 24<br>25       | 383 | <b>Bolded</b> if significantly differe | nt                | sure, vaginaru  | ischarge of lower ab | dominal cramping        |
| 25<br>26<br>27 | 384 |  |                   |                 |                      |                         |
| 27             |     |  |                   |                 |                      |                         |
| 29<br>30       | 385 | Table 2: Mycoplasma ge                 | enitalium RNA     | Detection ra    | ates from genital    | swab collections by     |
| 31<br>32       | 386 | Race/Ethnicity                         |                   |                 |                      |                         |
| 33             |     |  |                   | <u> </u>        | Detection of My      | coplasma genitalium     |
| 34<br>25       |     |  |                   |                 |                      |                         |
| 35             |     |  |                   |                 |                      | XIVA                    |
| 37             |     |  |                   |                 | [n/N1 (%             | of subjects)]           |
| 38<br>39       |     | Race/Ethnie                            | city              |                 | 22/545 (12)          |                         |
| 40<br>41       |     | White/His                              | panic             |                 | 23/545 (4.2)         |                         |
| 42<br>43       |     | White/No                               |                   |                 | 4/30 (13.3)          |                         |
| 44             |     | Black/Hisp                             |                   |                 | 0/2 (0)              |                         |
| 45<br>46       |     | BidCK/ NOT                             |                   |                 | 14/113 (12.4)        |                         |
| 47             |     | Other (As                              | Idii, Native Ha   | Wallall/Pacific | 0/21(0)              |                         |
| 48<br>49       |     | Islander, Ar                           | nerican Indian/Al | askan Native)   |                      |                         |
| 50<br>51       |     |  |                   |                 |                      |                         |
| 52             |     | Race, p-val                            | ue*               |                 | 0.003                |                         |
| 53<br>54       |     | Black                                  |                   |                 | 14/115 (12.2)        |                         |
| 55<br>56       |     | Non-Blac                               | k                 |                 | 27/601 (4.5)         |                         |
| 50<br>57       |     |  |                   |                 | 1                    |                         |
| 58             |     |  |                   |                 |                      |                         |

| 1             |     |                 |  |                               | 10 |
|---------------|-----|-----------------|--|-------------------------------|----|
| 2             |     |                 | Ethnicity, p-value*                          | 0.008                         |    |
| 4<br>5        |     |                 | Hispanic                                     | 23/535 (4.3)                  |    |
| 6<br>7        |     |                 | Non-Hispanic                                 | 18/179 (10.1)                 |    |
| 8             | 387 | p-value from Fi | sher's exact test.                           |                               |    |
| )<br>10<br>11 | 388 |                 |  |                               |    |
| 12<br>13      |     |                 |  |                               |    |
| 14            |     |                 |  |                               |    |
| 15<br>16      |     |                 |  |                               |    |
| 17<br>18      |     |                 |  |                               |    |
| 19<br>20      |     |                 |  |                               |    |
| 21            |     |                 |  |                               |    |
| 22<br>23      |     |                 |  |                               |    |
| 24<br>25      |     |                 |  |                               |    |
| 26            |     |                 |  |                               |    |
| 27<br>28      |     |                 |  |                               |    |
| 29<br>30      |     |                 |  |                               |    |
| 31<br>32      |     |                 |  |                               |    |
| 33            |     |                 |  |                               |    |
| 34<br>35      |     |                 |  |                               |    |
| 36<br>37      |     |                 |  |                               |    |
| 38<br>39      |     |                 |  |                               |    |
| 40            |     |                 |  |                               |    |
| 42            |     |                 |  |                               |    |
| 43<br>44      |     |                 |  |                               |    |
| 45<br>46      |     |                 |  |                               |    |
| 47            |     |                 |  |                               |    |
| 48<br>49      |     |                 |  |                               |    |
| 50<br>51      |     |                 |  |                               |    |
| 52<br>53      |     |                 |  |                               |    |
| 54            |     |                 |  |                               |    |
| 55<br>56      |     |                 |  |                               |    |
| 57<br>58      |     |                 |  |                               |    |
| 59            |     |                 | For peer review only - http://bmiopen.bmi.co | m/site/about/quidelines.xhtml |    |
| 00            |     |                 |  | , s, aooaq galacinesi, nitili |    |

| 389 | Table 3. Co-Infections with M. genitalium |                 |                  |         |                |  |  |
|-----|---|-----------------|------------------|---------|----------------|--|--|
|     |   | M. genitalium   | M. genitalium    |         | Total          |  |  |
|     |   | Positive (N=41) | Negative (N=678) |         | Population     |  |  |
|     |   |                 |                  | p-value |                |  |  |
|     |   | n/N1 (%)        | n/N1 (%)         |         | (N=719)        |  |  |
|     |   | A (A A (20 C)   | 422/204 (45 2)   | 0.054   | 47 (205 (45 0) |  |  |
|     | Human papiliomavirus 16,                  | 4/14 (28.6)     | 43 / 281 (15.3)  | 0.251   | 47/295 (15.9)  |  |  |
|     | 18  |                 |                  |         |                |  |  |
|     | Bacterial vaginosis                       | 5/18 (27 8)     | 98/3/0 (28.8)    | 1 000   | 103/255 (28.8) |  |  |
|     |   | 5/10 (27.8)     | 50/540 (20.0)    | 1.000   | 103/233 (20.0) |  |  |
|     | 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)   |  |  |
|     | Hopatitic P                               | 0/20 (0)        | 2/627 (0.2)      | 1 000   | 2/676 (0.2)    |  |  |
|     |   | 0/39(0)         | 2/03/ (0.3)      | 1.000   | 2/0/0 (0.5)    |  |  |
|     | 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)  |  |  |
|     |   | 7/42 (50.2)     |                  | 0.000   | 47 (200 (40 4) |  |  |
|     | Group B Streptococcus                     | //12 (58.3)     | 40/248 (16.1)    | 0.002   | 47/260 (18.1)  |  |  |
|     |   | 1               |                  | 1       |                |  |  |

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

# 

# 

# °Z Table 4. Prevalence of *M. genitalium* and Resistance profiles

|                               | Total (N=726) | 95% CI      | 20 |
|-------------------------------|---------------|-------------|----|
| N with sample tested          | 719           |             |    |
| <i>M. genitalium</i> 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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| 1 of 20                  |   | E   | 3MJ Open  |              |
|--------------------------|---|---|---|--------------|
|                          |   |   |   |              |
|                          | 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  | 4   |   |              |
|                          | 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**   |              |
|                          | <i>M. genitalium</i> positive   | 1 (5.9)   | 0.15 - 28.7   |              |
|                          | M. genitalium Negative  | 16 (94.1)   | 71.3 - 99.9   |              |
| 395<br>396<br>397<br>398 | Data presented as N (%)<br>*Percent of positive for <i>M. gen</i><br>Exact 95% confidence intervals<br>^ Denominator is positive samp | i <i>talium</i><br>(CI) except for **<br>les with conclusiv | which are based on the normal a ve sequencing results | pproximation |
| 399                      | ^^ Wild type  |   |   |              |
| 400                      |   |   |   |              |
| 401                      |   |   |   |              |
| 402                      |   |   |   |              |
| 403                      |   |   |   |              |
|                          |   |   |   |              |
|                          |   |   |   |              |
|                          |   |   |   |              |