Effects of Metronidazole on *Mycoplasma genitalium* in a High-Risk Population

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Introduction

Mycoplasma genitalium

- *Mycoplasma genitalium* (*Mgen*) is a facultative, intracellular bacteria that is sexually transmitted and causes inflammatory disorders in men and women.
- In men, *Mgen* is one cause of non-gonococcal urethritis (NGU) in addition to rectal infection and proctitis (Wood et al., 2023a).
- In women, *Mgen* infection has been associated with cervicitis, pelvic inflammatory disease (PID), and preterm birth. Infections are generally asymptomatic, and upper reproductive tract sequelae may not present until a long time after initial infection (Wood et al., 2023a).

US rates of *M. genitalium*

- A recent multicenter clinical validation study of an *Mgen* nucleic acid amplification (NAAT) diagnostic test showed a prevalence of 10.3% among those seeking treatment (Manhart et al., 2020).
- Participants reporting urogenital symptoms had a significantly elevated risk of *Mgen* infection compared to asymptomatic individuals (for females, OR 1.53 [95% CI 1.09 to 2.14]; for males, OR 1.42 [95% CI 1.02 to 1.99]).
- Women diagnosed with vaginitis and cervicitis had a higher prevalence of *Mgen* infection than women without those diagnoses, although this was statistically significant only for vaginitis (for vaginitis, OR 1.88 [95% CI 1.37 to 2.58]; for cervicitis, OR 1.42 [95% CI 0.61 to 2.96]).
- A diagnosis of urethritis in men was also significantly associated with *Mgen* infection (OR 2.97; 95% CI 2.14 to 4.13).

***M. genitalium* treatment**

- While the CDC recommends routine *Chlamydia trachomatis* (Ct) and *Neisseria gonorrhoeae* (Ng) NAAT testing in young, sexually active individuals, *Mgen* testing is only recommended for symptomatic individuals, partners of *Mgen* positives, and if urethritis/cervicitis Ct/Ng treatment fails. Recommended treatment is sequential two-step therapy with doxycycline (tetracycline), followed by moxifloxacin (quinolone) or azithromycin (macrolide) (Workowski et al., 2021).
- *Mgen* resistance to macrolides and quinolones is increasing; 50% carry macrolide resistant mutations and > 10% are fluoroquinolone resistant, therefore the need for more effective treatment is increasingly urgent (Wood et al., 2023a).
- A recent, small clinical trial for acute PID unexpectedly revealed that addition of metronidazole (nitroimidazole) to the standard treatment regimen of ceftriaxone and doxycycline reduced *Mgen* incidence (Wiesenfeld et al., 2021).
- While metronidazole was included in this study to treat the gram-negative bacteria associated with bacterial vaginosis (BV) and PID, this result suggested *Mgen* may also be susceptible to metronidazole.
- A subsequent *in vitro* study indicated *Mgen* had susceptibility to nitroimidazoles (Wood et al., 2023b).

Hypotheses

- Rates of *C. trachomatis* and *N. gonorrhoeae* in the young, high-risk women attending our New Orleans Sexual Health Clinic are higher than the national average, and we expect for the *Mgen* rate to follow suit.
- We expect metronidazole treatment for bacterial vaginosis (BV), highly prevalent in this population, to decrease *Mgen* burden, compared to the lack of treatment.

Approach

- Establish a low-cost real-time PCR for the detection and quantification of *Mgen* DNA from vaginal swabs.
- Use archived vaginal samples from a unique longitudinal cohort of young, high-risk women and collected immediately prior to and after 7-day metronidazole treatment of BV to establish *Mgen* rates in untreated individuals and after metronidazole treatment.

Methods

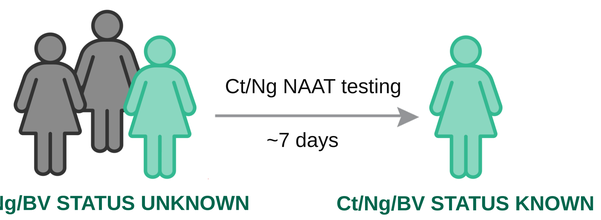

Figure 1. Study design. At the STI screening and enrollment visit (V1), BV was diagnosed using Amsel's criteria by a score of ≥3 or ≥2 when symptomatic. Patients diagnosed with BV were immediately provided a 7-day course of oral metronidazole. Patients testing positive by Ct NAAT (3 to 4-day turnaround) were immediately scheduled to return to clinic for azithromycin treatment and repeat testing (typically 7-9 days after V1).

DNA isolation from vaginal swabs

- DNA was extracted from a 0.2 ml aliquot of vaginal Copan swab eluate (1/5 total volume) using a m2000sp Liquid Handler system (Abbott Molecular, Des Plaines, Illinois) according to the instrument set-up prompts and protocol, then eluted in 50 µl of nuclease-free water.

PCR quantification of *M. genitalium*

- We adapted and optimized an internally controlled real-time qPCR assay targeting a 92-bp region of the MG190 gene, *mgpA* (McGowin et al., 2012).
- Two primers (190F and 190R3) and one FAM-labeled TaqMan probe (MG190P) were based on a highly conserved region of *mgpA* (Table 1).
- An internal positive control (IPC) was comprised of an 86-bp fragment corresponding to nucleotides 343 to 428 of pET28a(+) plasmid flanked by sequences of primers 190F and 190R3 (136 bp), and the IPC probe sequence was complementary to nucleotides 373 to 402 of the pET28a(+) plasmid (Table 1). This design allows for the IPC to be amplified with the same primers as the *M. genitalium* target DNA but detected by a different probe.
- The PCR setup was 12.5 µl SsoAdvanced Universal Probes Supermix 2X (Bio-Rad, Hercules, California), 0.4 µM forward primer (190F), 0.4 µM reverse primer (190R3), 0.25 µM *mgpA* probe (MG190P), 0.25 µM IPC probe (IPC2P), 1000 copies of IPC gBlock, and 5 µl of template.
- Real-time PCR was performed using a CFX 96 Real-Time PCR Detection System (Bio-Rad, Hercules, California) with two-step cycling parameters: 50°C for 2 minutes, initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.
- A standard curve was generated using 3-fold serial dilutions of a *mgpA* gBlock based on the G37 type strain.

Table 1. The primers (190F and 190R3), IPC probe (IPC2P), *mgpA* gBlock gene fragment, and IPC gBlock gene fragment were synthesized by Integrated DNA Technologies (Coralville, Iowa). The *mgpA* probe (MG190P) was synthesized by Thermo Fisher Scientific (Waltham, Massachusetts).

Name	Oligo type	Sequence (5' to 3')
190F	Forward primer	GAACTGAGGAGTAATGGGATTAATGTC
190R3	Reverse primer	TTAGTAATGATCGCTCCACTTGC
MG190P	Probe	/FAM/ AGATATAGCCATTAAGTATGGTGGG /MGBNFQ/
IPC2P	Probe	/HEX/ **AGTGAGT**CG /ZEN/ TATTAATTTCGCGGG**ATCGAG** /IBFQ/

Results

***M. genitalium* rates and burden**

- Tested vaginal samples from 76 patients for both Visit 1 and Visit 2 by qPCR (152 samples total).
- Reaction efficiency was within acceptable range (92.7%), and the assay could reliably detect 85 copies per reaction (Figure 2).
- Unexpectedly, we were unable to detect *Mgen* in any of the vaginal DNA samples taken from our cohort at Visit 1 or Visit 2.
  - The IPC always amplified in sample reactions, ruling out potential false negatives due to polymerase inhibition.

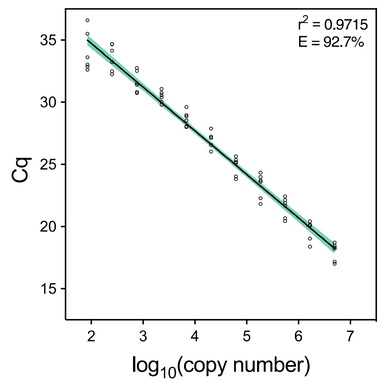

Figure 2. Real-time quantitative PCR standard curve. Data shown are the average of several independent experiments.

Conclusions

- A sensitive and cost-effective qPCR was adapted for detection and quantitation of *Mgen* in genital secretions. The standard curve demonstrates that the qPCR reaction can be used for *Mgen* concentrations between 85 to 5,000,000 genome copies with an average reaction efficiency of 92.7%.
- Unexpectedly, we were unable to detect *Mgen* in any of the vaginal DNA samples taken from our cohort at Visit 1 or Visit 2.
- This is likely due to the DNA isolation method, which was originally used to detect BV-associated bacteria. *Mgen* is the smallest known bacteria, with a size of 200-300 nm (for reference, the size of an average bacterium is 2,000-8,000 nm), and our protocol may have lost *Mgen* due to insufficient centrifugal forces.
- Alternatively, insufficient cell lysis could explain the inability to detect *Mgen*, which is predominantly found intracellularly.
- BV is a chronic condition with low MTZ treatment success rates. While one of our enrollment criteria was the lack of any antibiotic treatment in the past 2 months, many of the women in our cohort have been MTZ-treated multiple times due to chronic, recurrent BV. It is therefore possible that multiple MTZ treatments may have decreased *Mgen* rates in this cohort below normal rates.
- Vaginal samples were used in this study, but previous *Mgen* burden studies have been undertaken using cervical samples, which may be more sensitive in the detection of *Mgen* infection (Manhart et al., 2008).
- We plan to pursue this project due to its clinical significance and the important finding by Wiesenfeld et al. (2020), first by modifying our DNA isolation method and testing archived cervical samples in addition to vaginal samples.

Methods

Clinical study design and sample collection

- Identical samples were collected at visit 1 (V1) and visit 2 (V2) using our previously described protocols (Mott et al., 2021).
- Archived DNA from vaginal secretions, originally isolated for use in 16S rRNA gene sequencing studies, were utilized for this summer project.
- Longitudinal samples had been collected for a study designed to determine how BV and its treatment impacts chlamydial growth and clearance in the female genital tract (Figure 1).

Results

Bacterial vaginosis and metronidazole treatment

- 76 patients were recruited at the first screening visit (V1) and returned for the follow-up visit (V2).
- 61 (80%) patients were diagnosed with BV by Amsel's criteria at V1 and immediately given metronidazole, and the remaining 15 (20%) patients were considered normal or indeterminate for BV and not treated with metronidazole.
- These results were confirmed by Nugent scoring, with 55 (72%) positive for BV, 8 (11%) indeterminate for BV, and 13 (17%) normal.

References

Louisiana Department of Health. 2021 STI Data and Rankings, Louisiana. https://ldh.la.gov/assets/oph/HIVSTD/Tables-Profiles/2021-STI-Rankings-Release.pdf

Manhart LE, Mostad SB, Baeten JM, Astete SG, Mandaliya K, Totten PA. High Mycoplasma genitalium organism burden is associated with shedding of HIV-1 DNA from the cervix. J Infect Dis. 2008 Mar 1;197(5):733-6. doi: 10.1086/526501

Manhart, L. E., Gaydos, C. A., Taylor, S. N., Lillis, R. A., Hook, E. W., 3rd, Klausner, J. D., Remillard, C. V., Love, M., McKinney, B., & Getman, D. K. (2020). Characteristics of *Mycoplasma genitalium* Urogenital Infections in a Diverse Patient Sample from the United States: Results from the Aptima *Mycoplasma genitalium* Evaluation Study (AMES). Journal of Clinical Microbiology, 58(7), e00165-20. https://doi.org/10.1128/JCM.

McGowin, C. L., Annan, R. S., Quayle, A. J., Greene, S. J., Ma, L., Mancuso, M. M., Adegboye, D., & Martin, D. H. (2012). Persistent *Mycoplasma genitalium* infection of human endocervical epithelial cells elicits chronic inflammatory cytokine secretion. Infection and immunity, 80(11), 3842–3849. https://doi.org/10.1128/IAI.00819-12

Mott PD, Taylor CM, Lillis RA, Ardizzone CM, Albritton HL, Luo M, Calabresi KG, Martin DH, Myers L, Quayle AJ. Differences in the Genital Microbiota in Women Who Naturally Clear Chlamydia trachomatis Infection Compared to Women Who Do Not Clear; A Pilot Study. Front Cell Infect Microbiol. 2021 Apr 12;11:615770. doi: 10.3389/fcimb.2021.615770. PMID: 33912473; PMCID: PMC8072278.

Wiesenfeld, H. C., Meyn, L. A., Darville, T., Macio, I. S., & Hillier, S. L. (2021). A Randomized Controlled Trial of Ceftriaxone and Doxycycline, With or Without Metronidazole, for the Treatment of Acute Pelvic Inflammatory Disease. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 72(7), 1181–1189. https://doi.org/10.1093/cid/ciaa101

Wood, G. E., Bradshaw, C. S., & Manhart, L. E. (2023). Update in Epidemiology and Management of *Mycoplasma genitalium* Infections. Infectious disease clinics of North America, 37(2), 311–333. https://doi.org/10.1016/j.idc.2023.02.009

Wood, G. E., Kim, C. M., Aguila, L. K. T., & Cichewicz, R. H. (2023). In Vitro Susceptibility and Resistance of *Mycoplasma genitalium* to Nitroimidazoles. Antimicrobial agents and chemotherapy, 67(4), e0000623. https://doi.org/10.1128/aac.00006-23

Workowski, K.A., Bachmann, L.H., Chan, P.A., Johnston, C.M., Muzny, C.A., Park, I., et al. (2021). Sexually Transmitted Infections Treatment Guidelines, 2021. Morbidity and Mortality Weekly Report (MMWR) Recommendations and Reports 70, 83-87.