Resistance Guided Therapy for Mycoplasma genitalium: Application of Macrolide Resistance Testing Results

Barbara Van Der Pol, PhD, MPH
Professor of Medicine and Health Behaviors
University of Alabama at Birmingham
Disclosures
(Research Support, Consulting or Honorarium)

<table>
<thead>
<tr>
<th>Research Grants to my Institution</th>
<th>Salary/Consulting Honoraria</th>
</tr>
</thead>
<tbody>
<tr>
<td>■ Abbott Molecular</td>
<td>■ University of Alabama at Birmingham</td>
</tr>
<tr>
<td>■ BD Diagnostics</td>
<td>■ NIH</td>
</tr>
<tr>
<td>■ binx Health</td>
<td>■ FDA</td>
</tr>
<tr>
<td>■ BioFire</td>
<td>■ BD Diagnostics</td>
</tr>
<tr>
<td>■ Cepheid</td>
<td>■ BioFire</td>
</tr>
<tr>
<td>■ Hologic</td>
<td>■ Roche Molecular</td>
</tr>
<tr>
<td>■ NeuMoDx</td>
<td>■ SpeeDx</td>
</tr>
<tr>
<td>■ Rheonix</td>
<td></td>
</tr>
<tr>
<td>■ Roche Molecular</td>
<td></td>
</tr>
<tr>
<td>■ SpeeDx</td>
<td></td>
</tr>
</tbody>
</table>
Topics

- Epidemiology of *Mycoplasma genitalium*
- Treatment
- Diagnosis
  - Using diagnostic results to guide treatment
EPIDEMIOLOGY OF MYCOPLASMA GENITALIUM
**M. Genitalium** Cell Biology & Pathogenesis

- **Class Mollicutes** – 1st isolated in 1981 from 2 men with NGU
  - No cell wall
  - Trilaminar cell membrane
- **Smallest self-replicating organism**
  - Genome 580 kb; < 500 genes
  - Cellular dimensions ~ 0.3 x 0.6 µm
  - Generation time ~ 18 hrs
- **Flask shape with terminal structure**
  - MgPa - adheres to RBCs, sperm, epithelial cells of urogenital tract & rectum
  - Antigenic variation of MgPa & P110 - cytadherence & persistence
- **Immunogenic proteins** elicit proinflammatory cytokines

Figure from Taylor-Robinson & Jenson Clin Microbiol Rev 2011
[Slide courtesy of W. Geisler]
Transmission

- Among 383 women in a longitudinal study, 13.6% tested positive for Mgen*
  - Mgen positivity among sexual partners was more common if the female partner had Mgen (25% vs 2.8%, p=0.02)

- Study of sexual contacts**
  - 48% of women, 31% of men who reported sex with women only (MSW) and 42% of MSM were (+) for Mgen
  - Within dyads, concordance was among heterosexuals 47% and 27% among men who have sex with men (MSM)

**Slifirski, et al. Emerg Infect Dis 2017
# M. genitalium in the General Population

<table>
<thead>
<tr>
<th>Site</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S. (Add Health; 18-27yo)¹,²</td>
<td>1.1% MG</td>
<td>3.7% CT</td>
<td>0.4% GC</td>
<td>0.8% MG</td>
</tr>
<tr>
<td>New Mexico (21-30yo)³</td>
<td>4.6% MG</td>
<td>4.3% CT</td>
<td>0.3% GC</td>
<td></td>
</tr>
<tr>
<td>Britain (16-44yo)⁴,⁵</td>
<td>1.2% MG</td>
<td>1.1% CT</td>
<td>&lt;0.1% GC</td>
<td>1.3% MG</td>
</tr>
<tr>
<td>Denmark (21-24yo)⁶</td>
<td>1.1% MG</td>
<td>5.6% CT</td>
<td>N/A GC</td>
<td>2.3% MG</td>
</tr>
</tbody>
</table>

- No current US recommendations for *M. genitalium* screening in any population

---

² Miller, JAMA 2004; 291:2229-36.
³ Gravitt, Patti. EPIC-STI. Unpublished data.
⁶ Andersen, Sex Transm Infect. 2007; 83:237-41.

[Slide courtesy of W. Geisler]
Mgen in Men

- Prevalence estimates range from 5-15% in populations at risk for STI
- Recognized as a cause of non-gonococcal urethritis (NGU) since it was first isolated from men with urethral discharge in the early 1980’s
  - Many microbes play a role in NGU much like bacterial vaginosis (BV)
- Mgen is often found among MSM who present with proctitis
  - Mgen is found more commonly in the rectal than the urethra compartment among MSM

Figure from Sarier. J Urol Surg 2019
Mgen in Women

- Prevalence in settings with high STI risk range from 9-12%

- Up to 70% of women with infections have no symptoms*  
  - Symptoms are more common in co-infections  
  - Key point for screening recommendations

- Untreated infections often (25-55%) persist**

- Women with pelvic inflammatory disease (PID) are often positive for Mgen

**Trent, et al. Sex Transm Inf 2018
Review of *Mgen* Among Women
Wiesenfeld & Manhart, J Infect Dis, 2017

![Graph showing associations between *Mycoplasma genitalium* infection and various reproductive health outcomes.](image_url)

**Figure 1.** Summary effect sizes from meta-analysis of the association between *Mycoplasma genitalium* infection and 5 female reproductive tract disease syndromes Adapted from Li et al [7]. Abbreviation: CI, confidence interval.
Gaps in Epidemiologic Knowledge

■ Importance of asymptomatic infection
  – Women with PID and men with proctitis have Mgen, but were they symptomatic prior to development of consequences?

■ Importance of co-infection
  – Is Mgen playing a different role in the presence of other STI?
Co-Infections

- 10-12% of genital discharge is associated with Mgen co-infections
  - ~1/3 of Mgen infections in women are co-infections
  - ~1/4 of Mgen infections in men are co-infections

TREATMENT
Antimicrobial Classes Active Against Mycoplasmas

**Antimicrobial Mechanism of Action**

- **Inhibition of bacterial folic acid synthesis**
- **Inhibition of bacterial cell wall synthesis**
- **Inhibition of bacterial protein synthesis**
- **Inhibition of bacterial nucleic acid synthesis**

**Antimicrobial Classes**

- **Quinolones**
  - Macrolides*
    - Ketolides (Solithromycin)
    - Lincosamides (Lincomycin)
    - Streptogramins (Pristinamycin)
    - Aminoglycosides (Spectinomycin)
    - Tetracyclines*
      - Pleuromutilins (Lefamulin)
  - Lincosamides (Lincomycin)
  - Streptogramins (Pristinamycin)
  - Aminoglycosides (Spectinomycin)
  - Tetracyclines*
  - Pleuromutilins (Lefamulin)

*Only options in the U.S.*

Slide courtesy of W. Geisler
Antimicrobial Resistance - Macrolides

- Macrolide resistance has been shown to be associated with mutations on 23S rRNA gene of *Mgen*

- Declining cure rates have been seen with Azithromycin (AZ) in areas with heavy AZ use
  - *Empiric treatment for chlamydia*
  - *Use for non-STI treatments (e.g. Z-packs)*
A Randomized Trial of NGU Treatment Outcomes

Table 2. Clinical and Microbiologic Cure at Follow-up in the Modified Intent-to-Treat Population, by Infection at Enrollment

<table>
<thead>
<tr>
<th></th>
<th>Clinical Cure</th>
<th>Microbiologic Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Azithromycin (n = 216)</td>
<td>Doxycycline (n = 206)</td>
</tr>
<tr>
<td>All participants</td>
<td>79.6 (73.6–84.8)</td>
<td>76.2 (69.8–81.9)</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td>86.8 (74.7–94.5)</td>
<td>76.0 (61.8–86.9)</td>
</tr>
<tr>
<td><em>Mycoplasma genitalium</em></td>
<td>63.2 (46.0–78.2)</td>
<td>48.1 (28.7–68.1)</td>
</tr>
<tr>
<td><em>Ureaplasma urealyticum</em></td>
<td>82.7 (69.7–91.8)</td>
<td>72.7 (59.0–83.9)</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>79.0 (68.5–87.3)</td>
<td>86.7 (76.6–92.5)</td>
</tr>
</tbody>
</table>

MG Macrolide Resistance Markers (MRMs)*

- Worldwide, reported MG MRM prevalence ranges from 4%-100%, mostly in the 15%-60% range

MG MRM prevalence ranges from 44%-90% across U.S. sites

7 U.S. cities
48% (clinic attendees) 5

Seattle, WA
62% (hetero men) 1
69% (men w/urethritis) 2
94% (MSM) 3

Los Angeles, CA
80% (clinic attendees) 4

Durham & Greensboro, NC
61% & 64% (men w/urethritis) 2

Birmingham, AL
44% (STD Clinic) 6
61% (hetero couples) 7
61% (men w/urethritis) 2
74% (HIV+ MSM) 8

New Orleans, LA
60% (men w/urethritis) 2

Pittsburgh, PA
58% (men w/urethritis) 2

*MRMs in the 23S rRNA gene, typically A2071 and A2072 (E.coli numbering 2058 and 2059)


Slide Material Courtesy of Lisa Manhart
Antimicrobial Resistance - Fluoroquinolones

- Fluoroquinolone resistance has been shown to be associated with mutations on the gyrA and ParC genes encoding gyrase A and topoisomererase, respectively.

- These mutations have been linked with clinical outcomes*
  - 6/6 patients with ParC mutations failed Moxifloxacin (MX)
  - 3/48 without ParC mutations failed MX (p<0.001)

*Murray, et al. Emerg Infect Dis 2017*
Antimicrobial Resistance in Alabama

- Samples from 27 men living with HIV
  - 23S rRNA target for RT-PCR for MRM
  - Sequencing for gyrA and ParC mutations

Current CDC Treatment Guidelines

- No screening recommendation

- Diagnostics using molecular methods for people with *persistent/unresolved* symptoms

- AZ 1 g empirically as first-line treatment
  - MX 400 mg x 7-14 days if symptoms persist
Ex-US Treatment Guidelines

■ 2016 European Guidelines*
  - *Test men with symptoms of urethritis; women with mucopurulent cervicitis or abnormal discharge & STI risk*
  - *MRM (-): AZ 500 mg day 1, 250 mg days 2-5*
    - Data do not show improved outcomes over 1 gm single-dose**
  - *MRM (+): MX 400 mg 7-10 days*

■ 2018 BASHH Guidelines***
  - *Test men with symptoms of urethritis, epididymitis or proctitis; women with mucopurulent cervicitis or PID*
  - *MRM (-): Doxycycline (DX) 100 mg 2/day for 7 days followed by AZ 500 mg day 1, 250 mg days 2-5*
  - *MRM (+): MX 400 mg 7-10 days*

---

* Jensen, et al. j European Acad Derm Vener 2013
DIAGNOSIS OF M. GENITALIUM
Culture

- Highly fastidious organism
  - Requires growth in tissue culture
  - Isolates can subsequently be adapted to broth culture
  - Can take 3-6 weeks

- 20-50% sensitivity

- Only method to establish minimum inhibitory concentrations for assessment of antimicrobial sensitivity
Lab Developed Tests

- Nucleic acid amplification tests (NAATs) were developed for this organism in 1991
  - Initially used for epidemiologic research and surveillance
  - Eventually validated for generation results intended for patient management

- Variability of LDTs
  - DNA extraction
  - Primer and probe reagent quality
  - Predominately manual assays
Commercially Available in the US

- Hologic Aptima MG – RNA based assay
  - Available as an “analyte specific reagent” for several years
  - FDA approved in 2019 for multiple sample types

- cobas TV/MG – Real time DNA PCR assay
  - FDA approved in 2019

- Automated, mid-high throughput, can be run with samples used for chlamydia/gonorrhea testing
  - Caution when “bundling”!!!
New Assay on the Immediate Horizon

- SpeeDx ResistancePlus MG (RPMG) – PlexZyme® chemistry
  - Results: MG (-); MG(+) / MRM(-); MG(+) / MRM(+)
  - CE-IVD cleared for use in Europe
  - Under evaluation in the US

- Assay is platform agnostic
  - Data shows good performance on the Cepheid GeneXpert platform (CE-IVD cleared for use in Europe)
RESISTANCE GUIDED THERAPY
Linking Resistance Markers to Clinical Outcomes

A study in Australia measured MRM and assessed clinical outcomes among 155 MG(+) patients

- **88/99 (88.9%)** MRM(-) patients responded successfully to AZ
  - 11 patients who failed, all MRM(+) at post-treatment

- **7/56 (12.5%)** MRM(+) patients responded successfully to AZ

- **50.2** times more likely to fail if MRM (+)

**Australian Guidelines**

Men with NGU or proctitis; Women with PID
- Collect diagnostic sample
- DX 100 mg BID x 7 days

F/U Day 7-14
- Review lab results

MG(-)
- Treat for other STI found via lab testing

MG(+)/MRM(-)
- AZ 500 mg x 5 days

MG(+)/MRM(+)
- Sitafloxacin
- MX 400 mg x 7 days
Is 2-stage treatment an improvement?

■ Among 47 women with PID; cure rates were above 90%*

■ Among 80 women, 160 MSW and 166 MSM:
  - 71.5% were MRM(+)
  - Cure rates were 95.4% for MRM(-) and 91.9% for MRM(+) patients**

■ 244 patients with 68% MRM(+)  
  - 94.8% & 92.2% cure rates for MRM(-) and MRM (+)

*Latimer et al. Sex Transm Dis 2019
**Durukan et al. Presented at the International Society for STD Research meeting, July 2019
***Read et al. Clin Infect Dis 2019
Why Does this Work?

- Doxycycline is not highly effective against *Mgen*

- Azithromycin effectiveness is diminishing
  - *Patients MRM(-) often enrich for MRM(+) strains following AZ treatment*

- Organism load may be the answer

Impact of Point-of Care (POC) Testing

■ Rather than wait 1 week for results, POC assays may be a solution that allows immediate targeting of therapy
  - While AZ or MX would still follow 7 days of DX, fewer patients might be lost to follow up

■ For contacts to Mgen who do not have symptoms, treatment cannot be recommended until diagnostic results are available
Take Home Messages (I)

■ In many respects, Mgen is similar to chlamydia
  - Prevalence in the general population
  - Prevalence in high STI risk settings
  - Symptoms and lack of symptoms
  - Complications of untreated infection

■ Co-infection with other treatable STI is common

■ It is unclear what to do about asymptomatic infections
Take Home Messages (II)

- Antimicrobial resistance is common and increasing
  - AMR has evolved quickly
  - AZ is no longer useful as a single drug therapy
  - Resistance to fluoroquinolones is increasing in response to single drug therapy with MX

- Guidelines are (or need to) evolving rapidly
Take Home Messages (III)

■ New diagnostic options are improving our understanding of the epidemiology of *M*gen

■ Genetic MRM are well-correlated with clinical outcomes

■ Resistance guided therapy appears to be effective
  - *DX reduces organism load and f/u with AZ or MX shows excellent clinical cure rates*
  - *Resistant organisms are not being isolated following resistance guided therapy*
THANKS FOR YOUR ATTENTION