

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

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# Disclosures

(Research Support, Consulting or Honorarium)

## Research Grants to my Institution

- Abbott Molecular
- BD Diagnostics
- binx Health
- BioFire
- Cepheid
- Hologic
- NeuMoDx
- Rheonix
- Roche Molecular
- Speedx

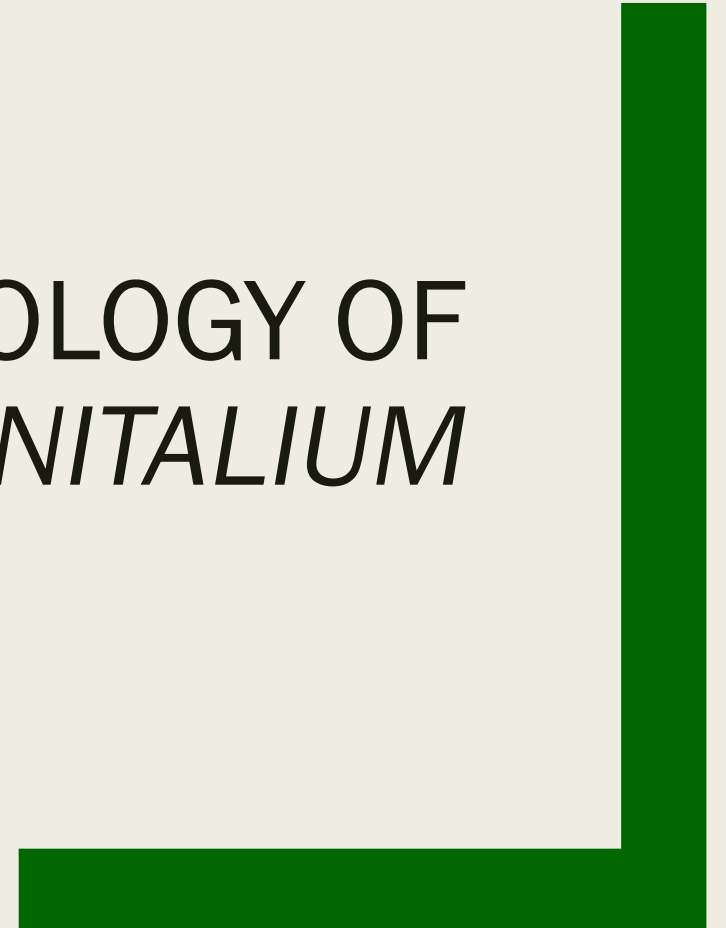
## Salary/Consulting Honoraria

- University of Alabama at Birmingham
- NIH
- FDA
- BD Diagnostics
- BioFire
- Roche Molecular

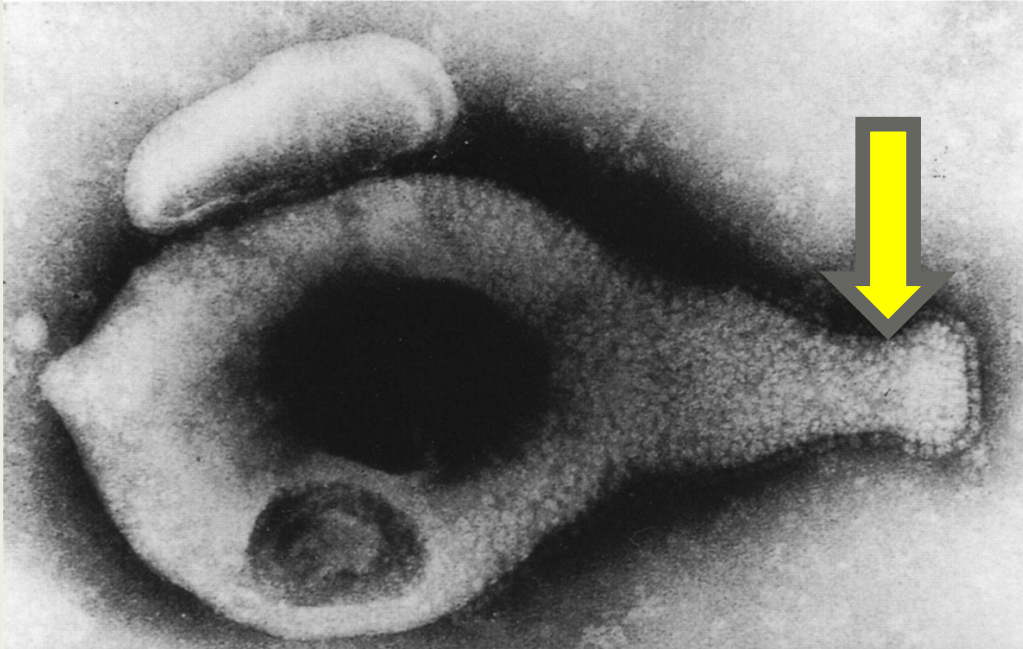
# Topics

- Epidemiology of *Mycoplasma genitalium*
- Treatment
- Diagnosis
  -
- Using diagnostic results to guide treatment

EPIDEMIOLOGY OF  
*MYCOPLASMA GENITALIUM*



# *M. Genitalium* Cell Biology & Pathogenesis



- Class Mollicutes – 1<sup>st</sup> 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  $\mu$ m
  - 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 - cytoadherence & persistence
- Immunogenic proteins elicit proinflammatory cytokines

# 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)

\*Tosh, et al. J Adol H 2007

\*\*Slifirski, et al. Emerg Infect Dis 2017

# *M. genitalium* in the General Population

Site	Males			Females		
	<u>MG</u>	<u>CT</u>	<u>GC</u>	<u>MG</u>	<u>CT</u>	<u>GC</u>
U.S. (Add Health; 18-27yo) <sup>1,2</sup>	1.1%	3.7%	0.4%	0.8%	4.7%	0.4%
New Mexico (21-30yo) <sup>3</sup>				4.6%	4.3%	0.3%
Britain (16-44yo) <sup>4,5</sup>	1.2%	1.1%	<0.1%	1.3%	1.5%	<0.1%
Denmark (21-24yo) <sup>6</sup>	1.1%	5.6%	N/A	2.3%	8.4%	N/A

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

1. Manhart, Am J Public Health. 2007; 97:1118-25.

2. Miller, JAMA 2004; 291:2229-36.

3. Gravitt, Patti. EPIC-STI. Unpublished data.

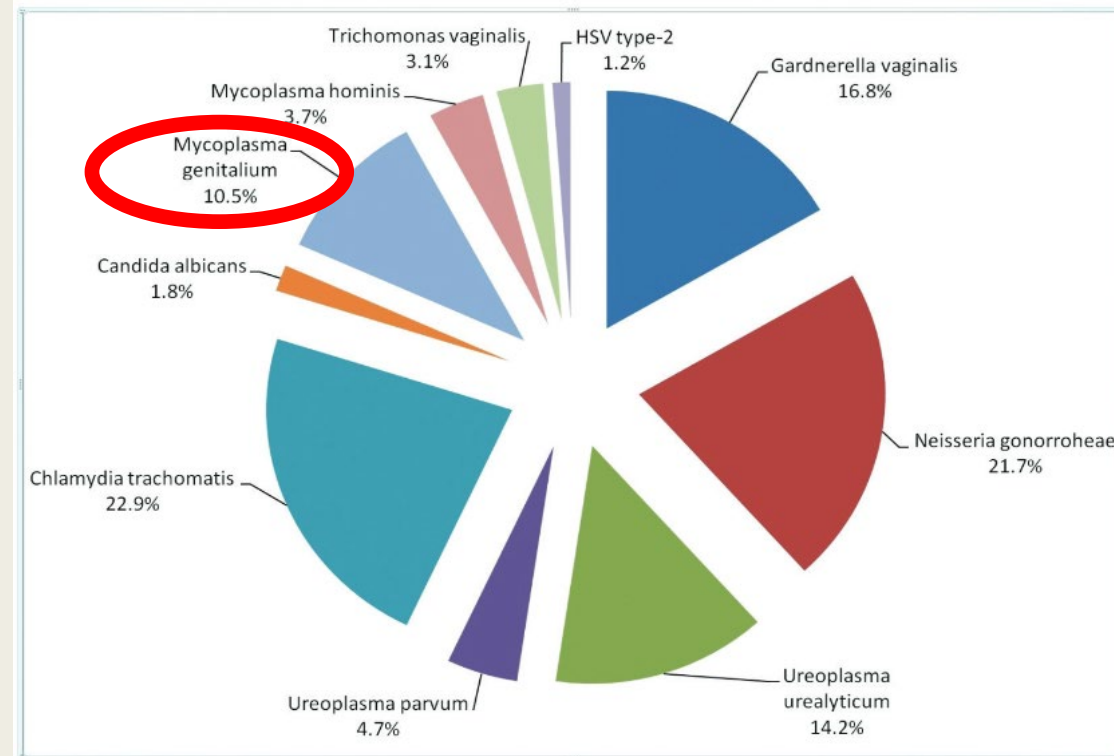
4. Sonnenberg, Int J Epidemiol. 2015; 44:1982-94.

5. Sonnenberg, Lancet. 2013; 382:1795-806.

6. Andersen, Sex Transm Infect. 2007; 83:237-41.

# 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*





# *Mgen* in Women

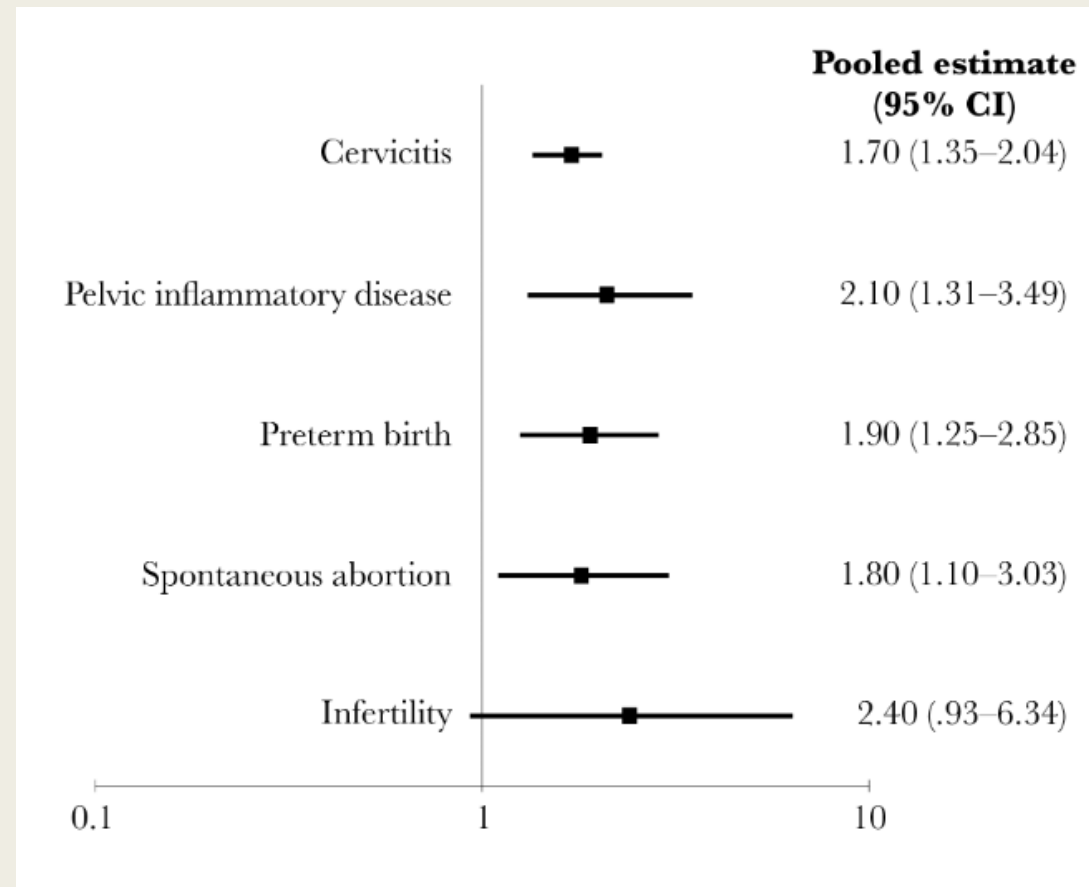
- Prevalence in settings with high STI risk range from 9-12%
- Up to 70% of women with infections have no symptoms\*
  - *Symptoms are more common in co-infections*
  - *Key point for screening recommendations*
- Untreated infections often (25-55%) persist\*\*
- Women with pelvic inflammatory disease (PID) are often positive for *Mgen*

\*Goje, et al. J AIDS Clin Res 2017

\*\*Trent, et al. Sex Transm Inf 2018

# Review of *Mgen* Among Women

Wiesenfeld & Manhart, J Infect Dis, 2017



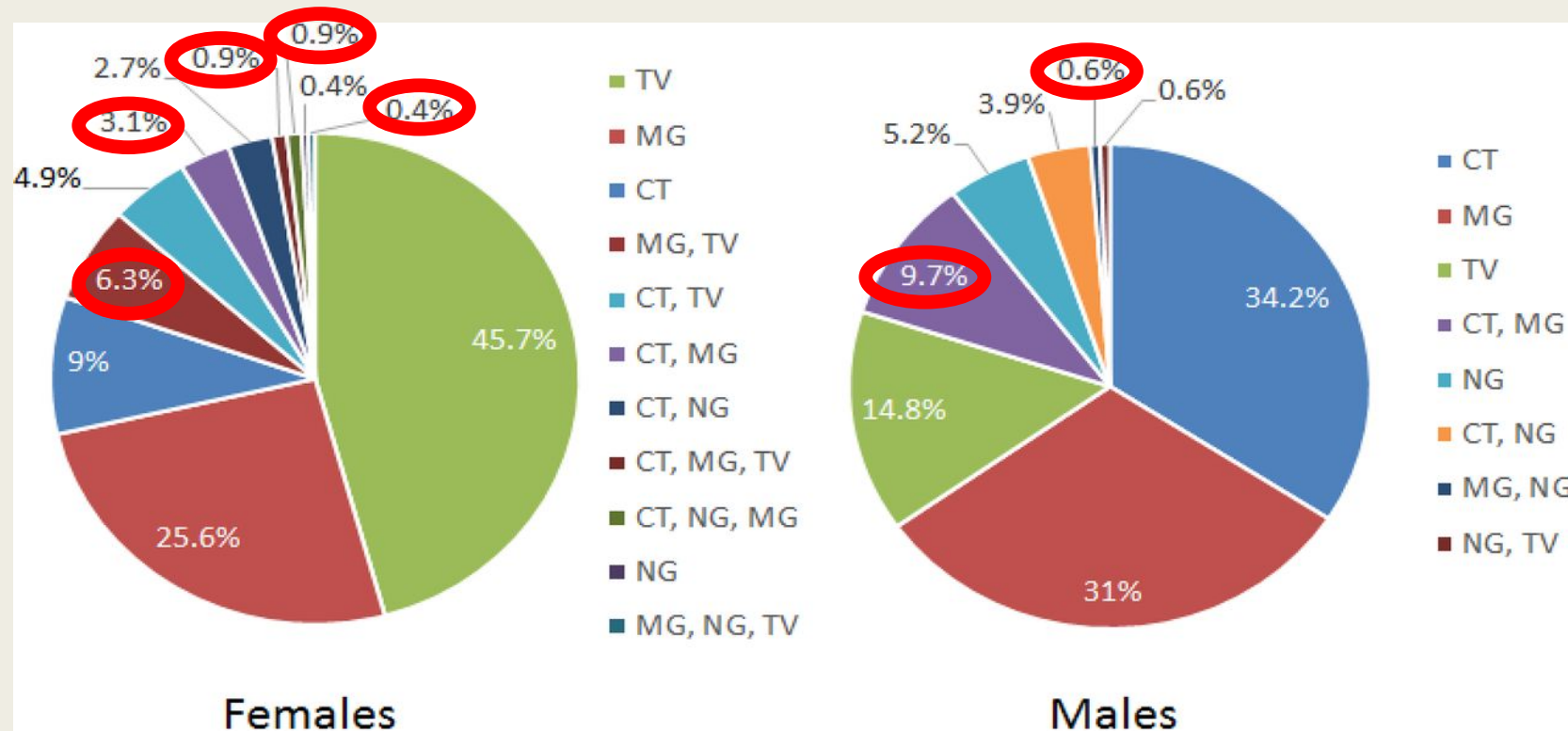
**Figure 1.** Summary effect sizes from meta-analysis of the association between *Mycoplasma genitalium* infection and 5 female reproductive tract disease syndromes Adapted from Lis et al [7]. Abbreviation: CI, confidence interval.

# 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

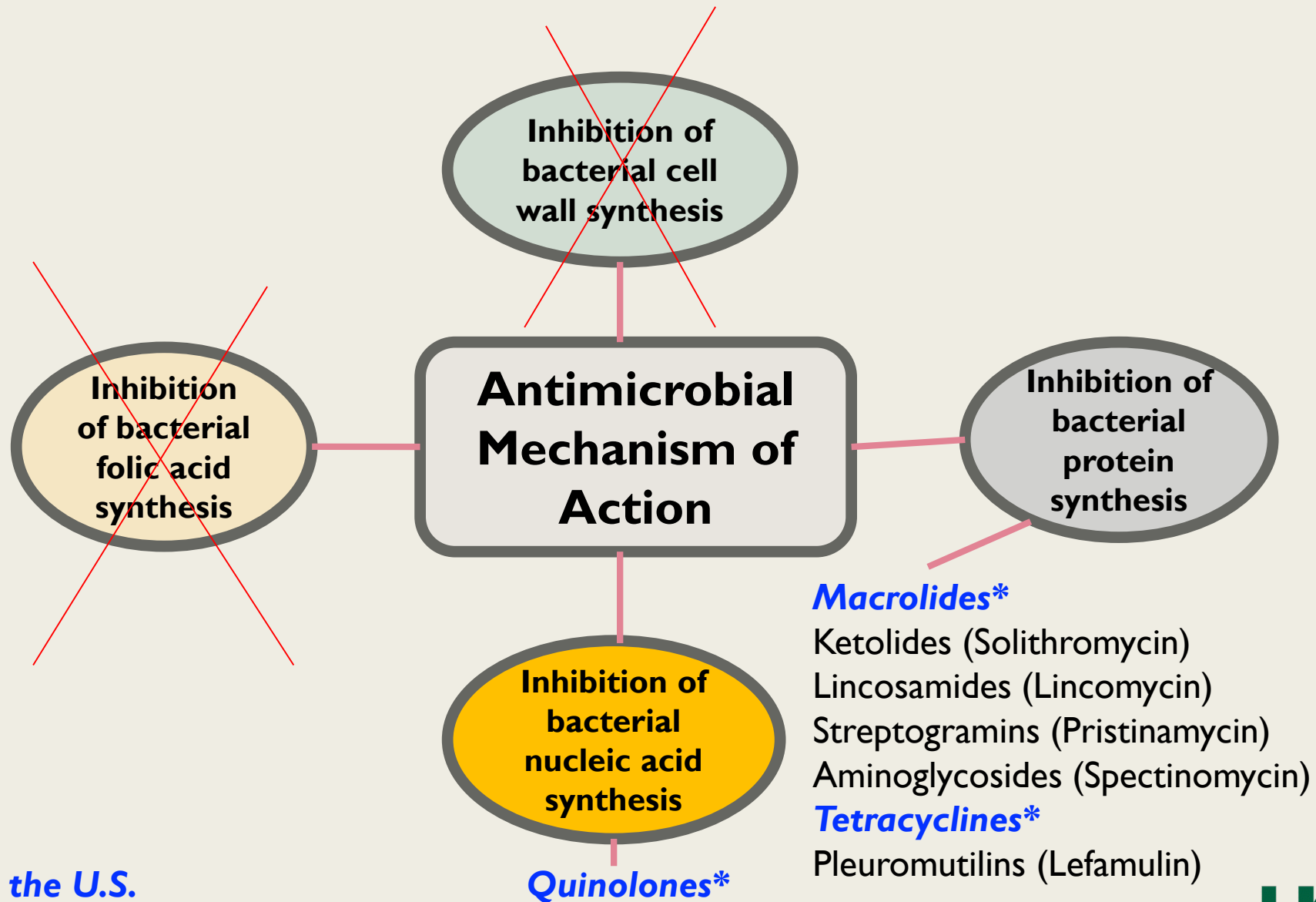
- 10-12% of genital discharge is associated with *Mgen* co-infections
  - ~1/3 of *Mgen* infections in women are co-infections
  - ~1/4 of *Mgen* infections in men are co-infections



TREATMENT



# Antimicrobial Classes Active Against Mycoplasmas



**\*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**

	Clinical Cure		Microbiologic Cure	
	Azithromycin (n = 216)	Doxycycline (n = 206)	Azithromycin (n = 216)	Doxycycline (n = 206)
All participants	79.6 (73.6–84.8)	76.2 (69.8–81.9)	...	...
<i>Chlamydia trachomatis</i> <sup>a</sup>	86.8 (74.7–94.5)	76.0 (61.8–86.9)	86.3 (73.7–94.3)	90.0 (78.2–96.7)
<i>Mycoplasma genitalium</i> <sup>b</sup>	63.2 (46.0–78.2)	48.1 (28.7–68.1)	39.5 (24.0–56.6)	29.6 (13.8–50.2)
<i>Ureaplasma urealyticum</i> <sup>c</sup>	82.7 (69.7–91.8)	72.7 (59.0–83.9)	75.0 (61.1–86.0)	69.1 (55.2–80.9)
Idiopathic <sup>d</sup>	79.0 (68.5–87.3)	85.7 (76.6–92.5)	...	...

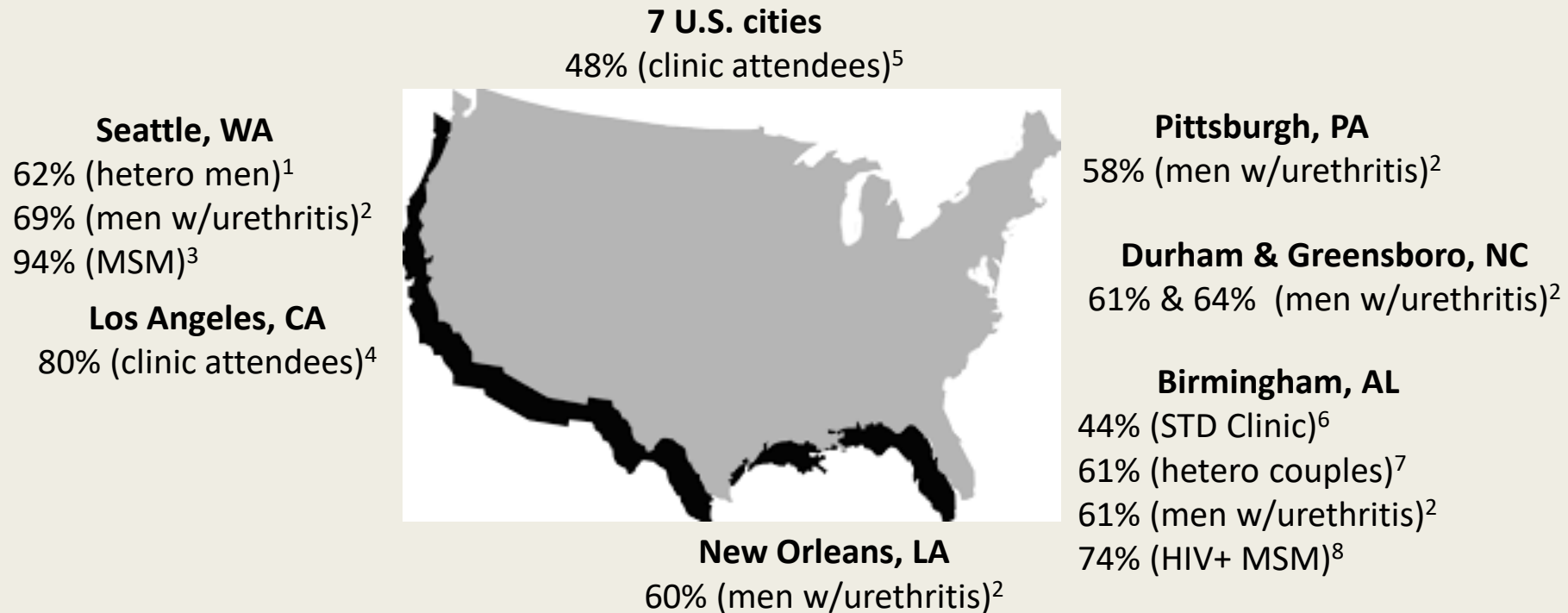
\*Manhart, et al. Clin Inf Dis 2013



# 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



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

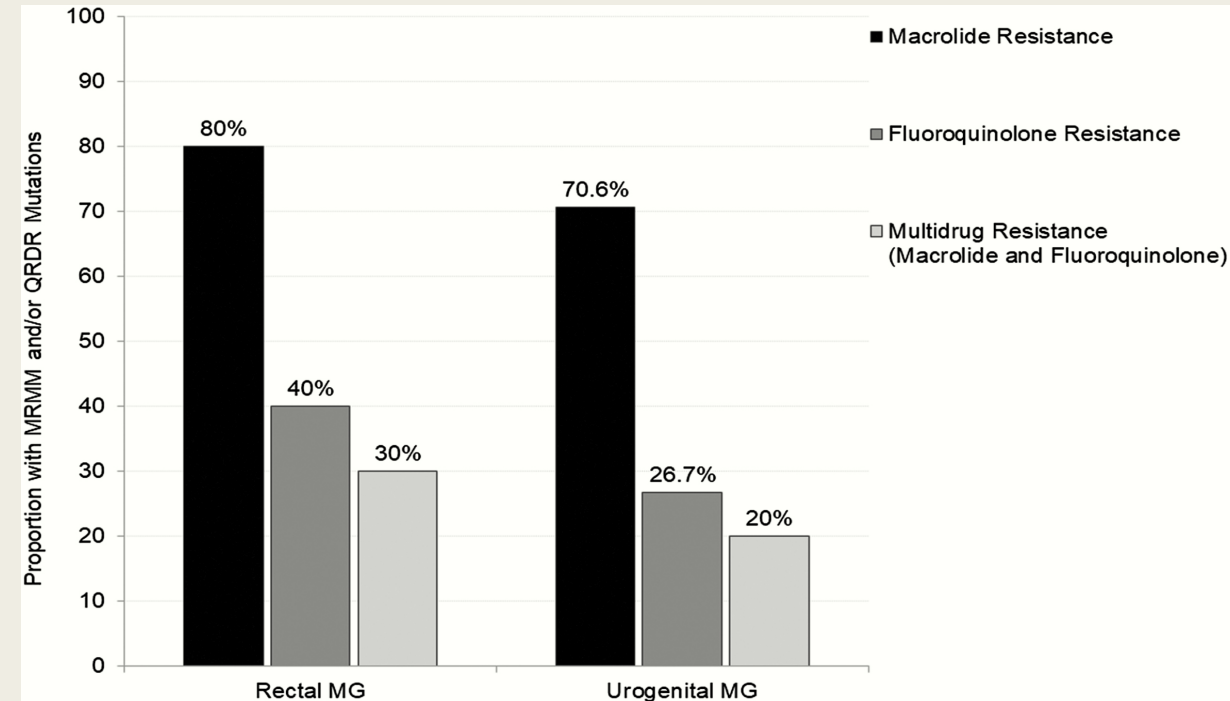
1. Romano 2018, 2. Bachmann (unpublished), 3. Chambers 2019, 4. Allan-Blitz 2018,  
5. Getman 2016, 6. Xiao 2018, 7. Xiao 2019, 8. Dionne-Odom 2018

# Antimicrobial Resistance - Fluoroquinolones

- Fluoroquinolone resistance has been shown to be associated with mutations on the *gyrA* and *ParC* genes encoding gyrase A and topoisomerase, 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 ( $p < 0.001$ )*

# 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

\*\*Read, et al. *Clin Infect Dis* 2017; Horner, et al. *Sex Transm Dis* 2018

\*\* <https://www.bashhguidelines.org/current-guidelines/urethritis-and-cervicitis/mycoplasma-genitalium-2018/>

# 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<sup>®</sup> 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)*

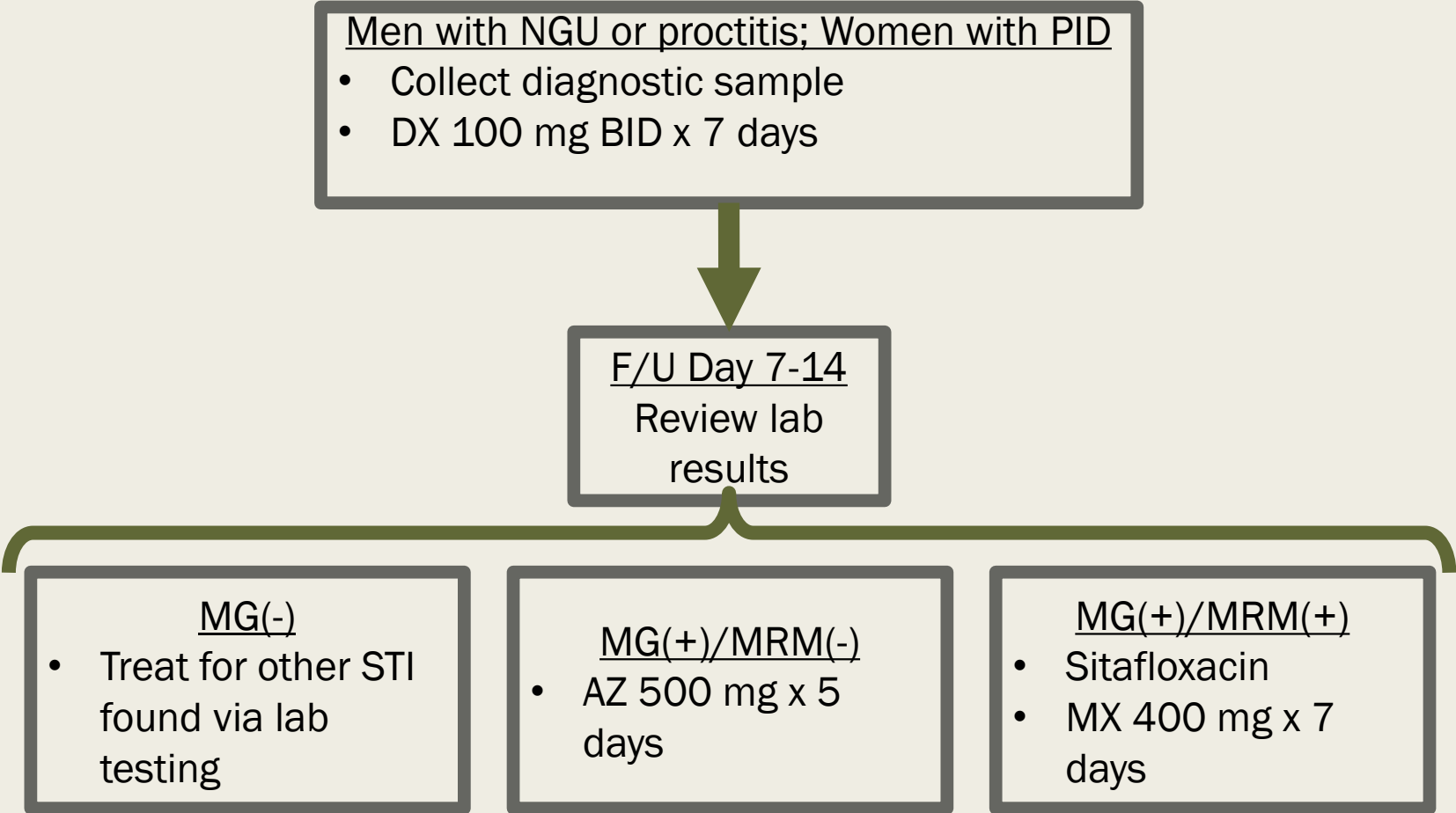
# RESISTANCE GUIDED THERAPY



# 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



# Is 2-stage treatment an improvement?

- Among 47 women with PID; cure rates were above 90%\*
- Among 80 women, 160 MSW and 16 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 (+)

**YES!**

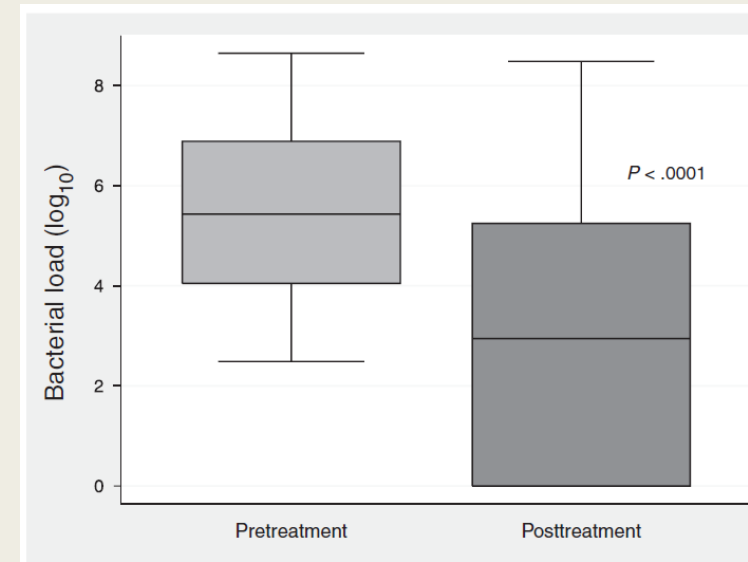
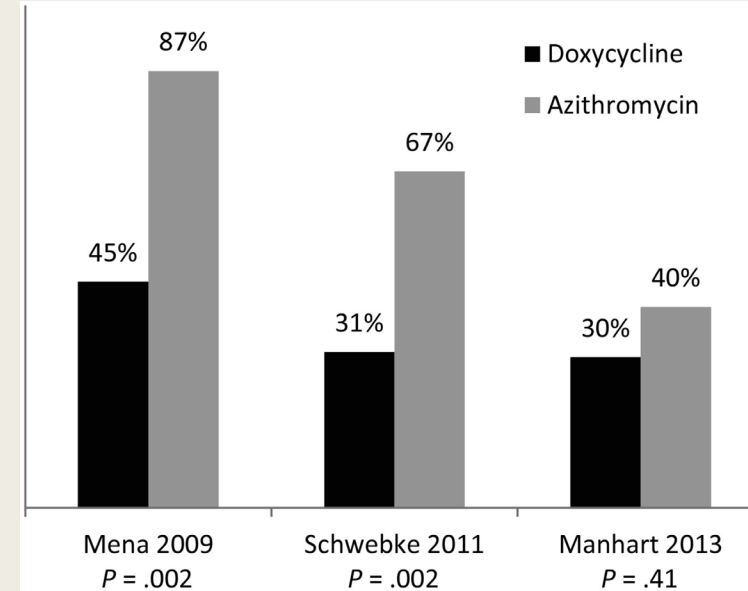
\*Latimer et al. Sex Transm Dis 2019

\*\*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

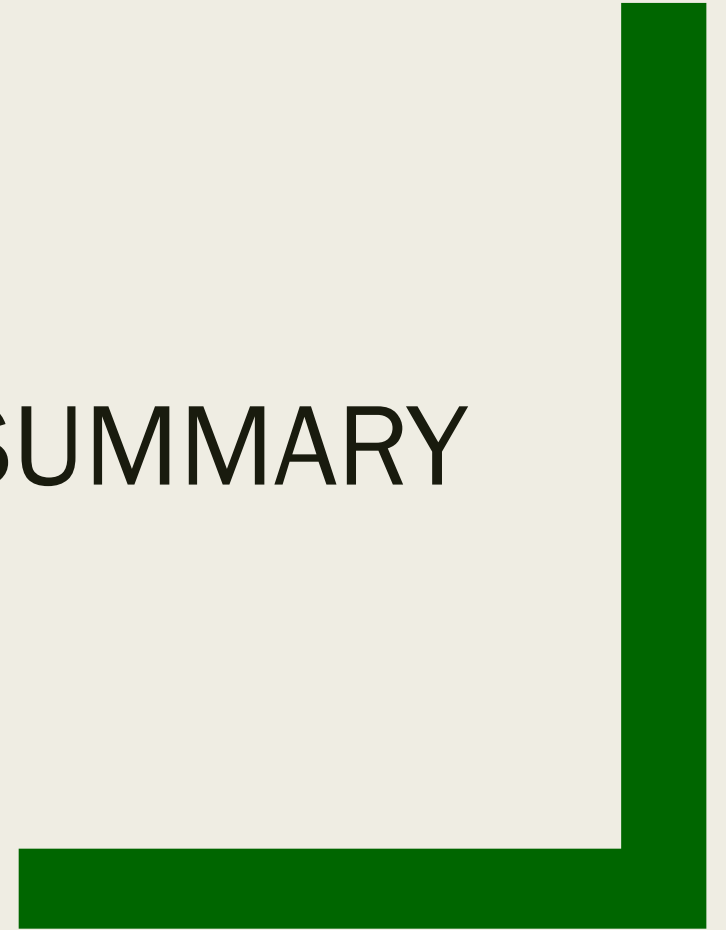


# Impact of Point-of Care (POC) Testing

- 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



# SUMMARY



# 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 *Mgen*
- 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

