

Mycoplasma genitalium and macrolide resistance detection rates in urogenital samples at a national reference laboratory

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#IDLabCon

Disclosure

- Speaker fees, study reagents (Hologic)

Objectives

- Provide an overview of *Mycoplasma (Mycoplasma) genitalium* (MG or MGen)
- Discuss macrolide resistance and detection of mutations associated with resistance
- Discuss the rates of *M. genitalium* detection and macrolide resistance in samples received for screening and testing of sexually transmitted infections

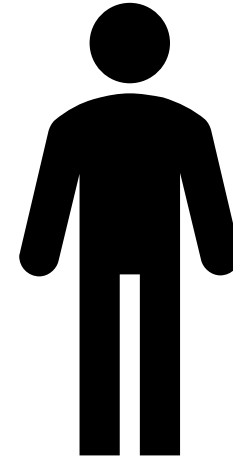
Mycoplasma genitalium

- Mollicutes class, smallest replicating bacteria
- Lack a cell wall, β -lactam antibiotics are ineffective
- STI with hallmarks of Chlamydia
- **No current recommendations for *M. genitalium* screening in any population in the United States**
- **Testing indicated for patients with persistent urethritis or cervicitis, or pelvic inflammatory disease**
- CDC Watch List of Antimicrobial Resistance Threats

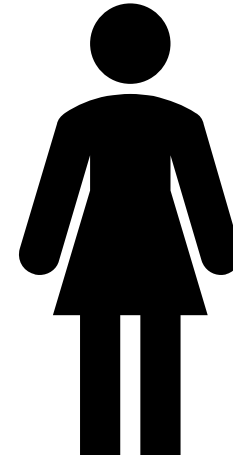


M. genitalium Infections

- **Asymptomatic mostly**
- Urethritis with painful urination, urethral discharge
 - Misdiagnosed as UTI in women, dysuria common
- Cervicitis with vaginal discharge, irregular bleeding
- Co-infections with other STIs are common



Epididymitis
Proctitis in MSM



Pelvic inflammatory
disease
Infertility
Preterm delivery
Endometritis

CDC Recommendations for Screening and Testing

- No recommendations for screening asymptomatic men and women
- Testing recommended for:
 - Men with persistent or recurrent non-gonococcal urethritis
 - Women with persistent or recurrent cervicitis
- Testing should be considered for:
 - Women with PID
- Extragenital testing is not recommended
- Testing should be accompanied with resistance testing, if available
- Treatment guided by resistance testing

M. genitalium Prevalence and Consequences

1.7% in the general population, 10-40% in high-risk populations

In men

- 15%-30% of non-gonococcal urethritis cases
- 40% of persistent or recurrent urethritis
- Reported among 1%-26% of MSM

In women

- Detected among 10%-30% of women with cervicitis
- High prevalence of 12%-18% in pregnant women
 - Increase the risk of PID, endometritis, and infertility

Discerning independent role of *M. genitalium* is challenging

Prevalence – Multicenter US Study 2013-2014

TABLE 1 Prevalence of *M. genitalium* by subject age group, self-identified demographic status, and health care facility type

Category	No. with <i>M. genitalium</i> /total no. (% [95% CI])	
	Female	Male
Age		
14–17 yr	12/40 (30.0 [18.7–45.4])	No data
18–20 yr	35/140 (25.0 [18.6–32.8])	6/48 (12.5 [5.9–24.7])
21–30 yr	28/210 (13.3 [9.4–18.6])	53/220 (24.1 [18.9–30.2])
31–40 yr	7/73 (9.6 [4.7–18.5])	11/75 (14.7 [8.4–24.4])
41–50 yr	1/34 (2.9 [0.5–14.9])	3/49 (6.1 [2.1–16.5])
51–60 yr	1/16 (6.3 [1.1–28.3])	1/29 (3.4 [0.6–17.2])
61–70 yr	0/2 (0 [0–65.8])	0/9 (0 [0–29.9])
71–78 yr	No data	0/1 (0 [0–79.4])
≤30 yr	75/390 (19.2 [15.6–23.4]) ^a	59/268 (22 [17.5–27.4]) ^b
>30 yr	9/125 (7.2 [3.8–13.1])	15/163 (9.2 [5.7–14.6])
All females (14–70 yr)	83/515 (16.1 [13.2–19.6])	
All males (18–78 yr)		74/431 (17.2 [13.9–21])
Symptomatic status		
Symptomatic ^c	69/327 (21.1 [17–25.8]) ^d	33/171 (19.3 [14.1–25.9])
Asymptomatic	14/188 (7.5 [4.5–12.1])	40/260 (15.4 [11.5–20.3])

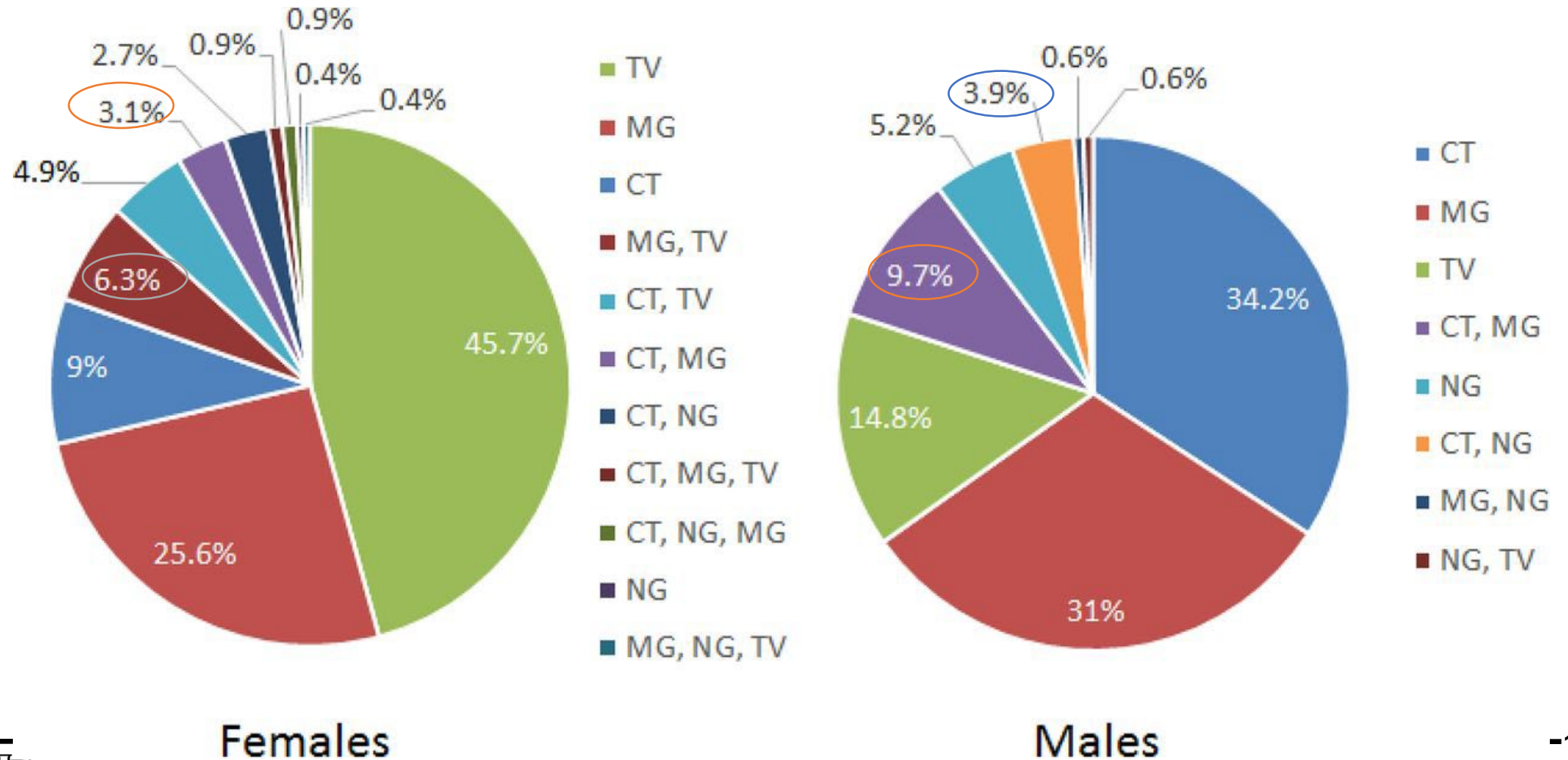
Prevalence – Multicenter US Study 2013-2014

TABLE 1 Prevalence of *M. genitalium* by subject age group, self-identified demographic status, and health care facility type

Category	No. with <i>M. genitalium</i> /total no. (% [95% CI])	
	Female	Male
Race/ethnicity		
Black	76/323 (23.5 [19.2–28.5]) ^e	76/272 (27.9 [22.9–33.6]) ^f
White	8/163 (4.9 [2.5–9.4])	5/117 (4.3 [1.8–9.6])
Asian	0/5 (0 [0–43.5])	0/2 (0 [0–65.8])
Unknown race	0/24 (0 [0–13.8])	4/40 (10.0 [4–23.1])
Hispanic	1/49 (2.0 [0.4–10.7])	4/75 (5.3 [2.1–12.9])
Non-Hispanic	77/401 (19.2 [15.6–23.3]) ^g	63/294 (21.4 [17.1–26.5]) ^h
Unknown ethnicity	6/65 (9.2 [4.3–18.7])	6/62 (9.7 [4.5–19.6])
Enrollment site		
Family medicine/OB-GYN, northeastern USA	2/84 (2.4 [0.6–8.3])	1/66 (1.5 [0.3–8.1])
Family planning clinic, southwestern USA	0/24 (0 [0–13.8])	14/127 (11.0 [6.7–17.7])
Public health clinic, southeastern USA	13/78 (16.7 [10–26.5])	35/153 (22.9 [16.9–30.2])
Public health clinic, mid-Atlantic USA	8/39 (20.5 [10.8–35.5])	20/79 (25.3 [17–35.9])
Hospital system high-risk STD clinics, midwestern USA	10/54 (18.5 [10.4–30.8])	3/6 (50.0 [18.8–81.2])
Adolescent gynecology clinic, midwestern USA	39/135 (28.9 [21.9–37])	No data
Family planning clinic, midwestern USA	4/22 (18.2 [7.3–38.5])	No data

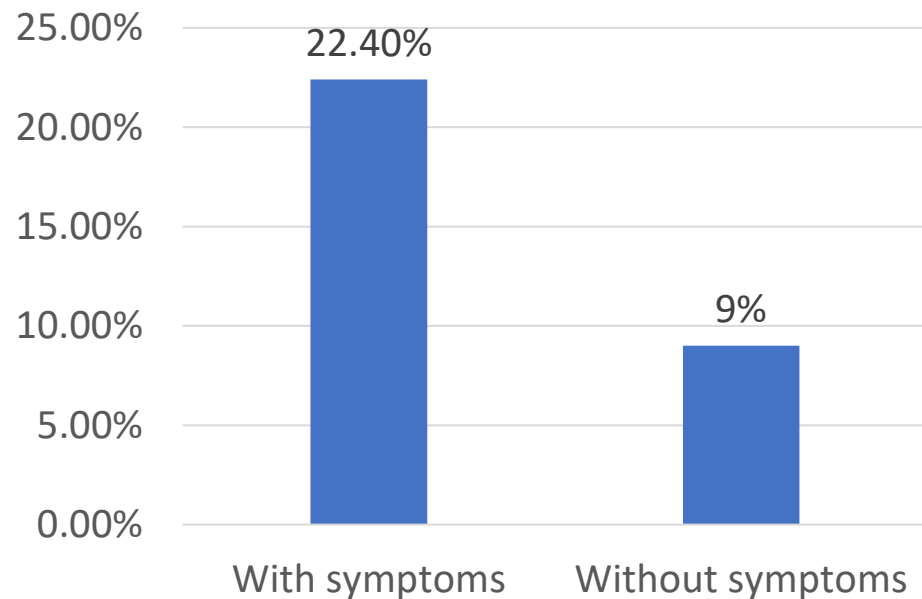
Coinfection with other STIs

Distribution of single-infection and coinfection rates of STIs among 223 women and 155 men who tested positive for a STI

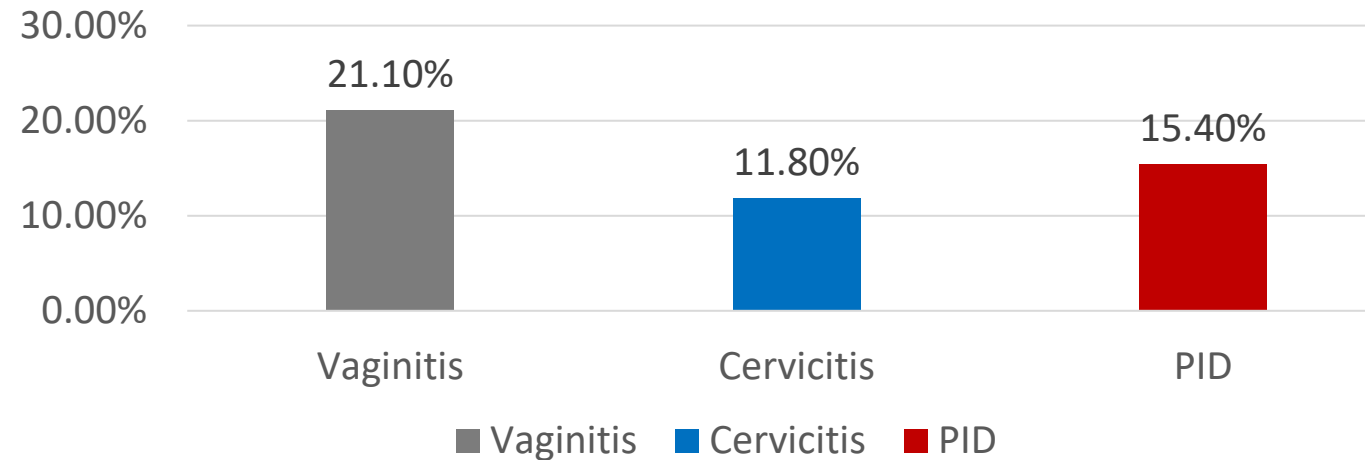


Mycoplasma genitalium in the US (MyGeniUS study)

Prevalence of MG in males



Detection of MG in females with STI syndromes



- Macrolide resistance mutations (MRM)
59.1% prevalence
- MRM were associated with
 - Vaginitis (adjusted prevalence ratio: 1.8; 1.14–2.85)
 - Cervicitis (aPR: 3.5; 1.69–7.30)
 - PID (aPR: 1.8; 1.09–3.08)
- No association between MRMs and CT/GC

***M. genitalium* Treatment**

- Limited antibiotics
- Cell-wall mediated antibiotics are ineffective

Tetracyclines (Doxycycline)

Inhibits protein synthesis
Clinical and microbial response is poor (<40% effective)



Macrolides (azithromycin)

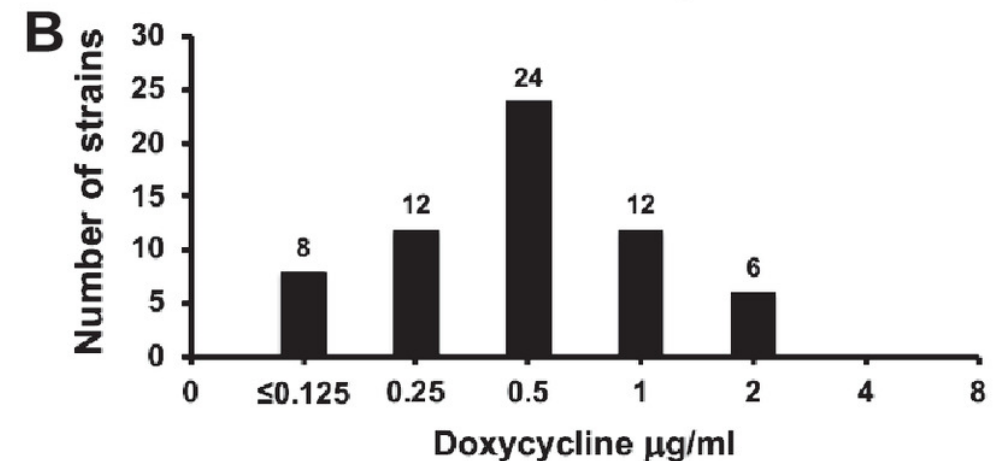
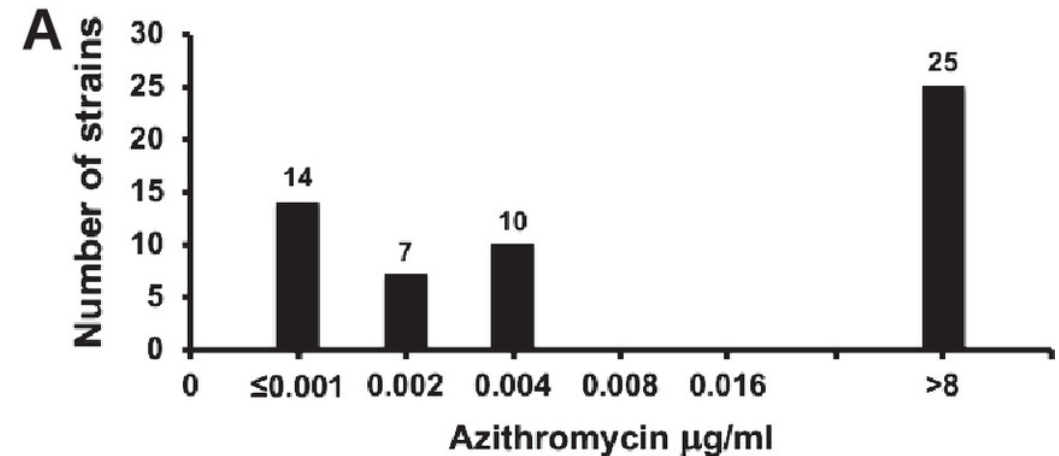
Inhibits protein synthesis
Drug resistance increasingly common

Fluoroquinolone (moxifloxacin)

Inhibits DNA synthesis
Drug resistance increasing

Drug resistance in *M. genitalium*

- Resistance to azithromycin is increasing (44%-90%)
- Mutations in 23S rRNA marker for macrolide resistance (A2058G/C/T and 2059G/C)
- High correlation with treatment failure 44%-90% in the US
- Doxycycline: low cure rates 30%-40%, not resistance dependent
- Quinolone resistance is much lower (0%-15% in US)
 - S83I mutation
 - Correlation with treatment failure is less consistent



Two-step, resistance guided therapy

If macrolide resistance testing is not available

Doxycycline 100 mg BID for 7 days



Moxifloxacin 400 mg for 7 days

If macrolide resistance testing is available

Doxycycline 100 mg BID for 7 days



Macrolide resistant

Moxifloxacin 400 mg for 7 days

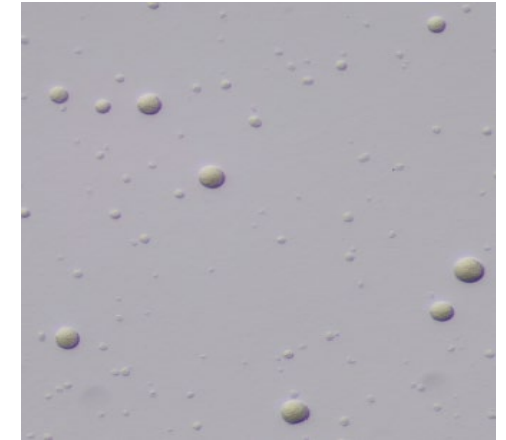


Macrolide susceptible

Azithromycin 1 g initial dose,
followed by 500 mg orally once
daily for 3 additional days

M. genitalium laboratory testing

- Culture can take several weeks, extremely slow growing
- NAAT is preferred
- Three FDA-cleared NAATs available
 - Hologic Aptima Mycoplasma genitalium Assay
 - Abbott Alinity m STI assay for CT, MG, NG, and TV
 - Roche cobas TV/MG
- Preferred specimens are a first-void urine sample in males and a vaginal swab in females
- Serology: not currently a standard or recommended method for routine diagnosis due to cross-reactivity with other Mycoplasma sp
 - Lack of standardized, commercialized assays



Spherical *M. genitalium* colonies (G37) growing on SP4 agar after 10 days of incubation

Lack of Antimicrobial Resistance Detection (AMR)

- No FDA- cleared test, several CE marked AMR tests
 - SpeedDx ResistancePlus MG, AllplexMG
- Laboratory-developed tests
 - Univ of Alabama at Birmingham, Mycoplasma lab
 - Culture
 - NAAT (detection/macrolide resistance)
 - Medical Diagnostic Labs
 - Pyrosequencing for macrolide/quinolone resistance
 - ARUP Laboratories – NAAT detection/macrolide resistance
 - Other reference laboratories

Study Aim

To understand the prevalence of *M. genitalium* in urogenital STI screening and diagnostic samples from diverse geographic regions and to assess the rates of macrolide resistance

Study Design



611 residual samples in Aptima media previously tested for CT, NG, and TV



Samples deidentified and tested on the Aptima Mycoplasma genitalium assay



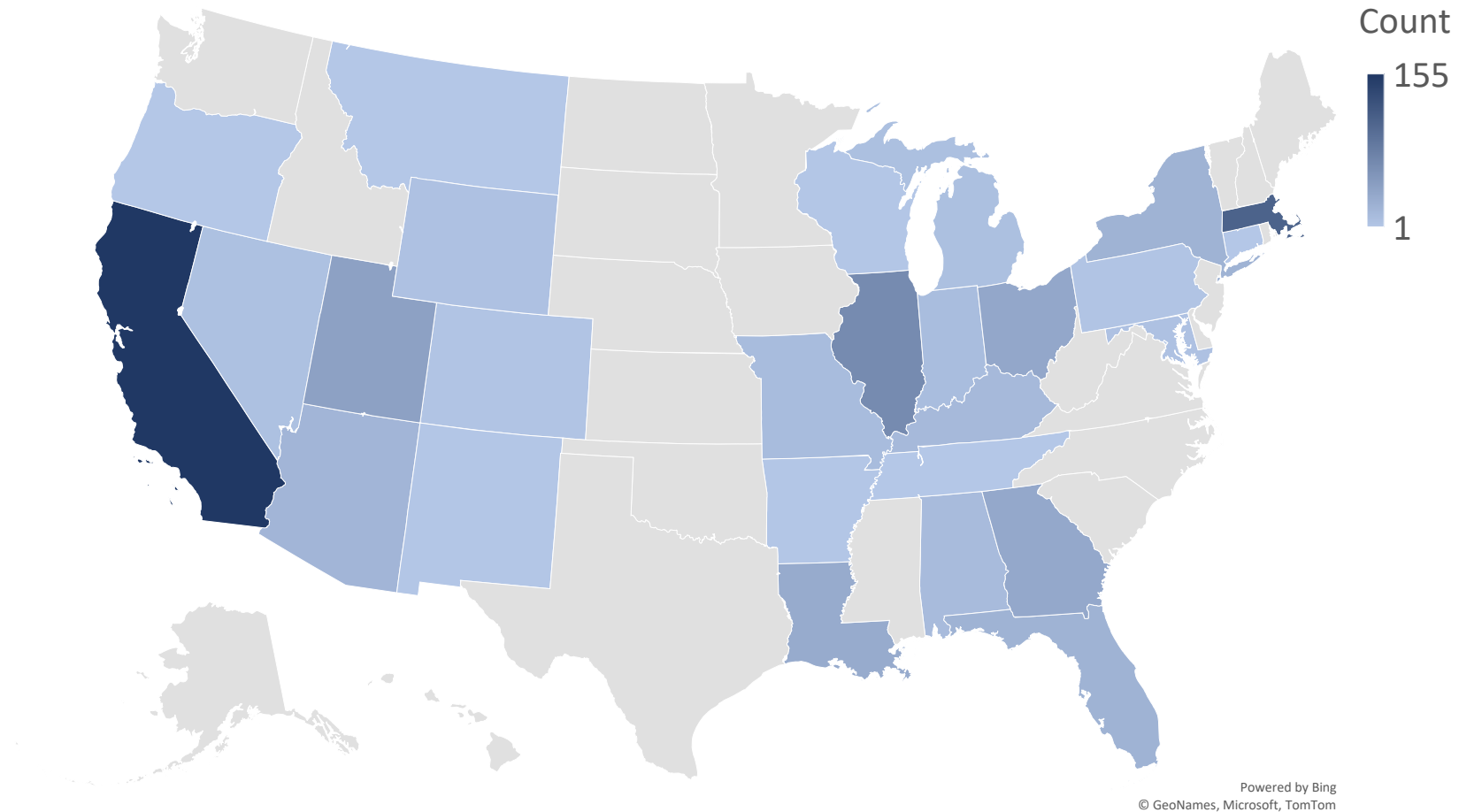
Samples positive for MG were tested on lab-developed *M. genitalium* macrolide resistance PCR



Subset of samples confirmed by sequencing of the 23S rRNA gene by Sanger method

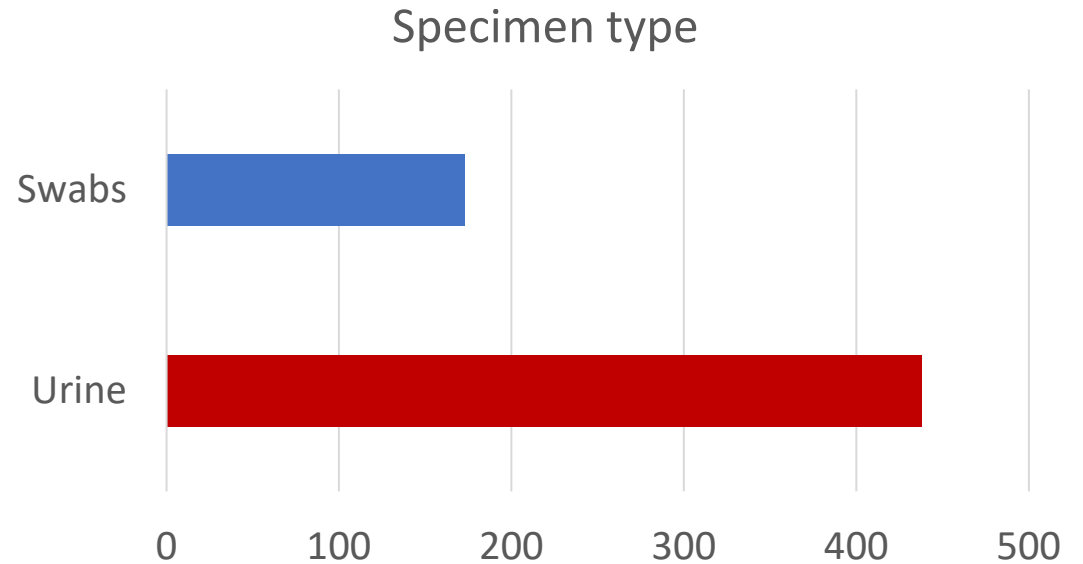
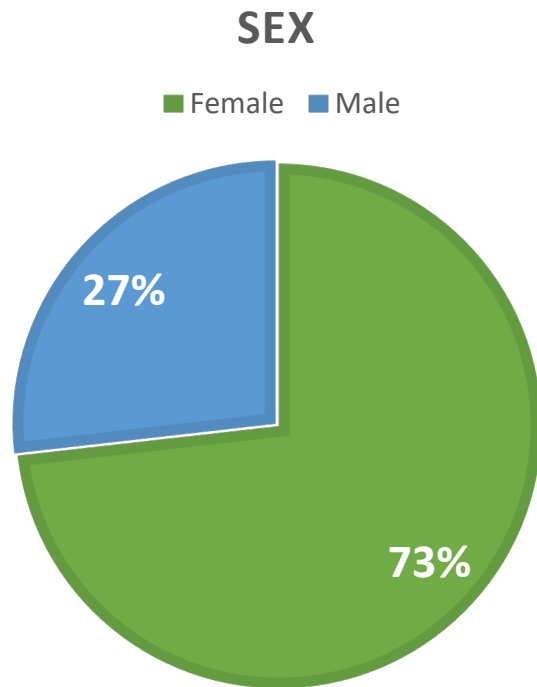
Results

Distribution of Residual Samples by State



Demographic information

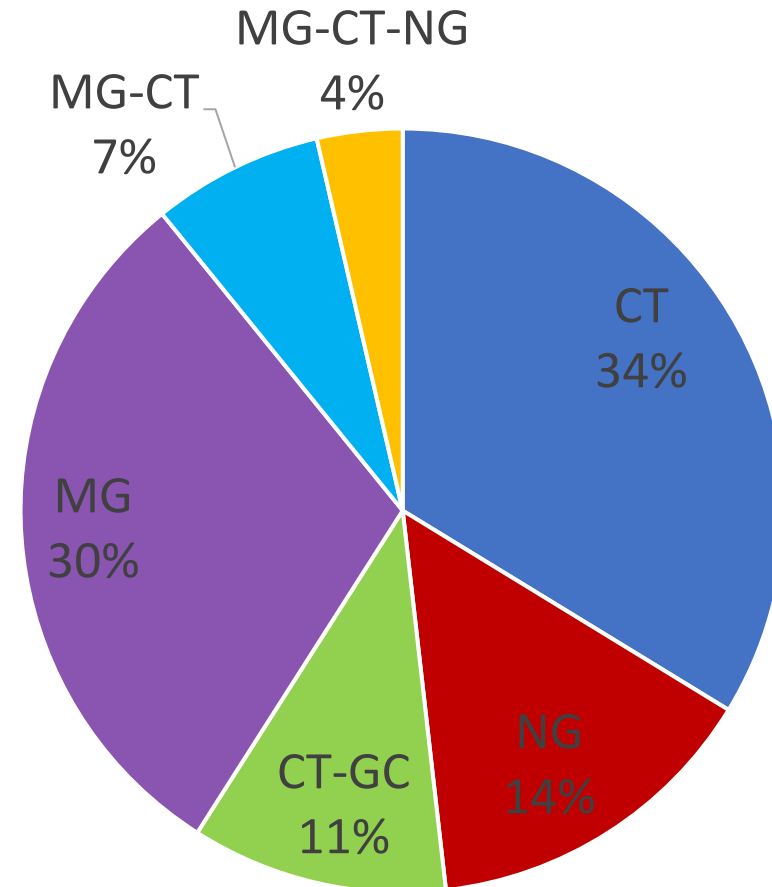
- 515/611 residual samples tested for CT and NG by the Aptima Combo2 assay
- 377 (73%) were female and 138 (26.7%) were male
- The median(range) age of females was 28 y (14-78 y) and 30 y (14-76 y) for males
- Urine (76.3%) and urogenital swab specimens (23.6%)



Detection Rates of CT, NG, and MG in residual Aptima samples

- N=515 tested on the Aptima MG assay
- Positives for all targets =19% (n=98)
- Negatives for targets = 80.9% (n=417)

Target	Detection Rate N (%)
MG	25 (4.9%)
CT	28 (5.4%)
NG	12 (2.3%)
CT-NG	6 (1.1%)
MG-CT	6 (1.1%)
MG-CT-NG	3 (1.1%)

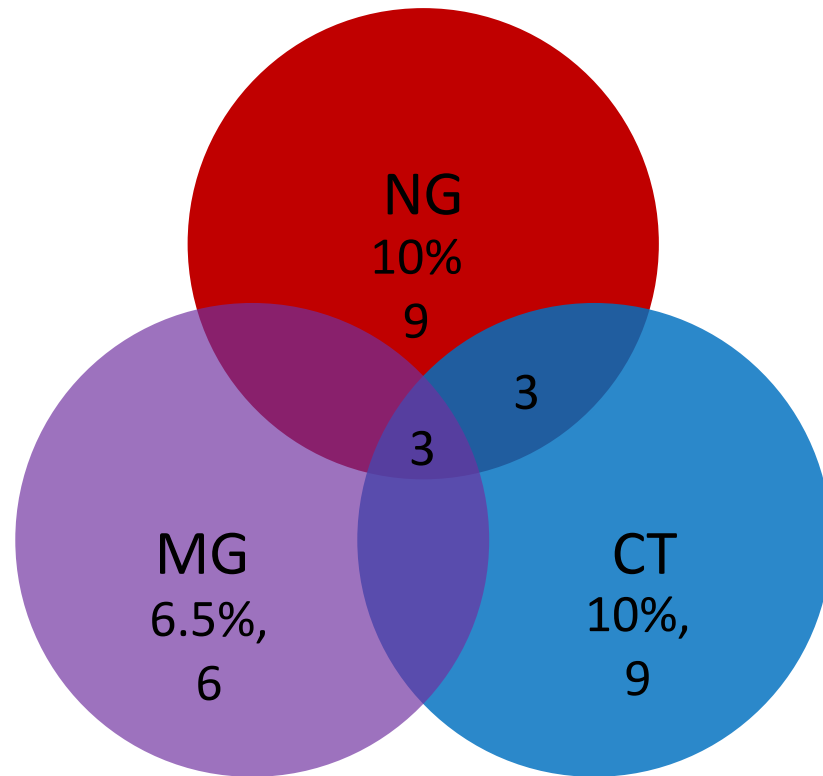


Distribution of CT, NG, and MG in positive samples

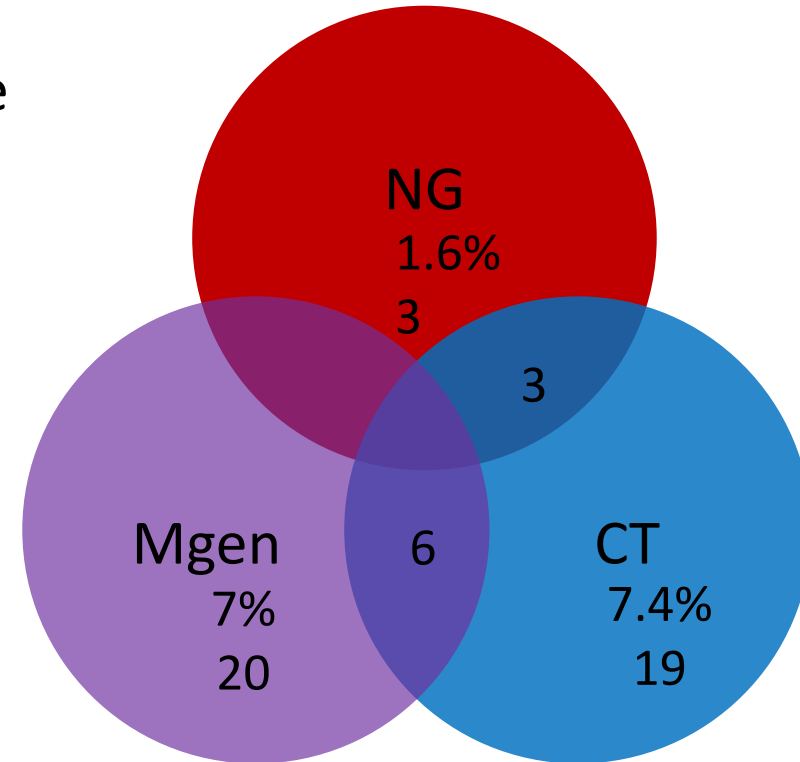
Detection of MG, CT, and NG by Sex

Percent CT, NG, and MG in residual Aptima samples testing positive for an STI

Male



Female



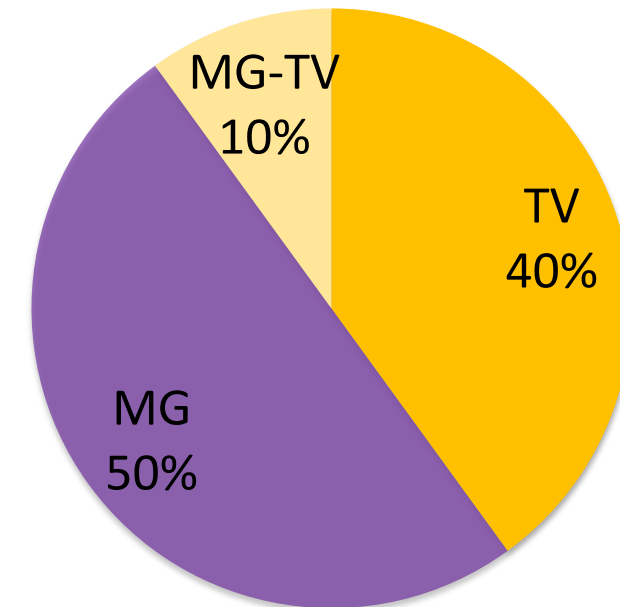
Detection Rates of MG and TV in residual Aptima samples

- Endocervical, vaginal, and urine specimens previously tested on the Aptima TV assay (n=96)
- Demographics: 84 females, 12 males
- 45% urine and 55% cervical/vaginal swab

- Positive for any target= 20 samples
- Negative for all = 76 samples (80.2%)

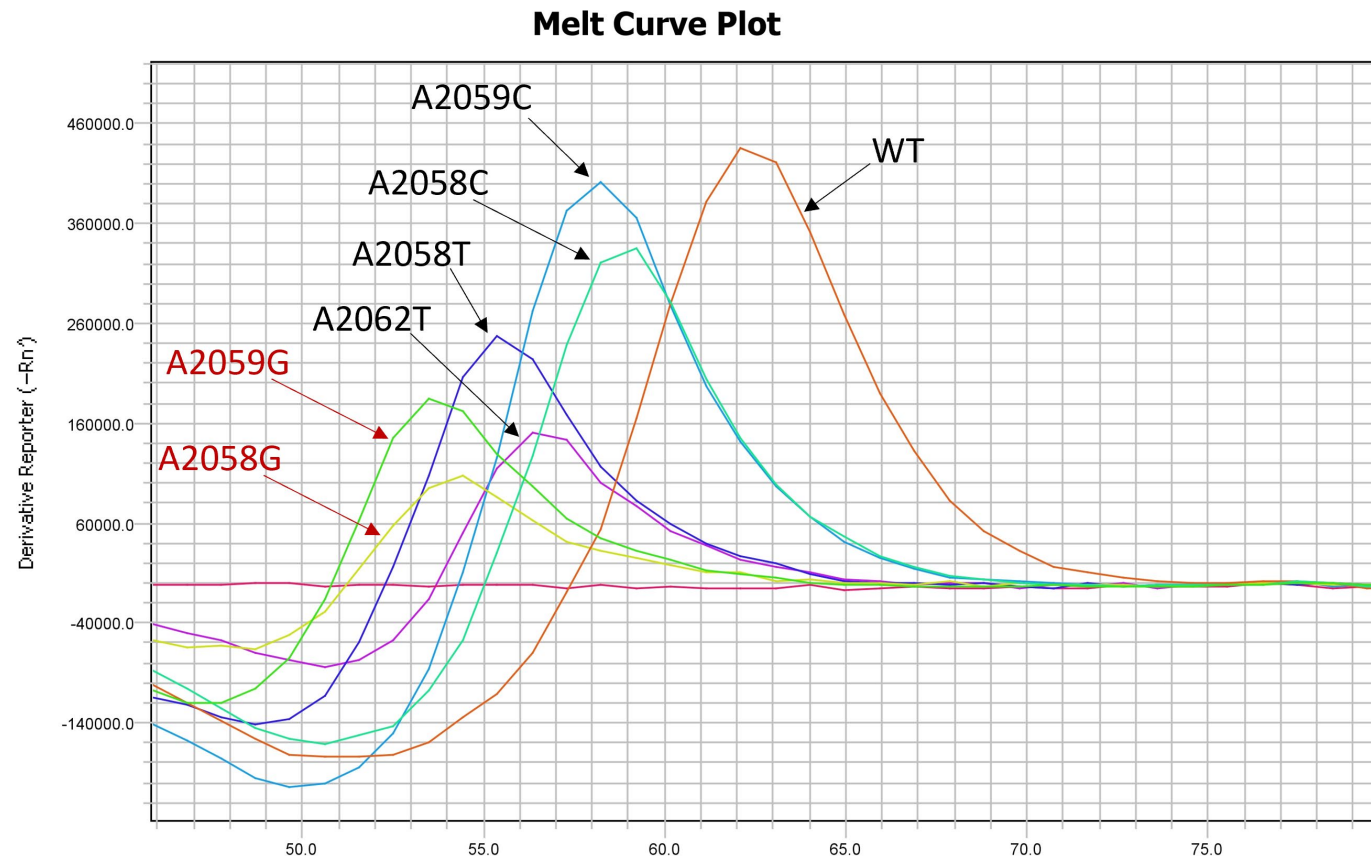
- TV n=8 (8.3%)
- MG n=10 (10.4 %)
- MG-TV n=2 samples

Distribution of TV and MG in positive samples



M. genitalium Macrolide resistance PCR

- Approx 115 bp region of 23S rRNA gene
- 3 mutation sites, 6 resistance associated mutations
- TaqMan probe for detection, Pleiades probe for mutation detection by melt curve



- 15.3% MG Positivity
- 67.9% Macrolide resistant
- A2058G and A2059G most common

Macrolide resistance in all residual samples

Detected in 21 out of 46 specimens (45.6%) by melt curve analysis

Sex	MG Positive	Mutation present (non-WT)	% Predicted Resistant
Female	34	13	38.2%
Male	12	8	66.6%
Total	46	21	45.6%

MG Macrolide resistance detected in 8/12 samples sent for TV testing

23S rRNA mutations Detected

Mutation	Number	Frequency (%)
A2059G	7	33.3
A2058G	6	28.5
A2058C	2	9.5
No Sequence	6	28.5
Total	21	100.0

Conclusions

- Detection rates of *M. genitalium* were in between those of general population and high-risk facilities
- Co-detection of *M. genitalium* was observed with CT and TV, but not NG
- Identified high rates of macrolide resistance-associated mutations in *M. genitalium* from urogenital samples
- A2059G and A2058G mutations being detected most frequently
- Resistance testing is important before using macrolides

Future Directions

- Additional studies to assess the prevalence of *M. genitalium* and macrolide resistance in individuals seeking care for vaginitis
- Fluroquinolone resistance rates in our sample cohort
 - *gyrA* and *parC*

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