Clinical Aspects of 
*Mycoplasma genitalium* Infection

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Disclosures

- **GSK**: External Advisory Support for Gepotidacin development for treatment of gonorrhoea

- **GARDP-Entasis**: External Advisory Committee member for the planned Phase 3 trial of Zoliflodacin for uncomplicated urogenital gonorrhoea

- **SpeedX**: ResistancePlus™ MG assay research samples, video production
Mycoplasma genitalium

- Very small 580 kb genome = fastidious bacterium
- 140 kDa Mg surface adhesin (Mg Pa)
- Mg Pa is similar to the major adhesin protein (P1) of Mycoplasma pneumoniae
- Can grow intracellularly and extracellularly
- Potent chemotactic/activating factors for phagocytes released during epithelial cell infection
- Difficult to culture in vitro – impeded research
- Molecular diagnostics have enhanced our understanding of the role of this pathogen
- Molecular assays can now simultaneously detect the bacterium and its susceptibility to antibiotics
Evidence to Support Sexual Transmission

• High concordance rate of *M. genitalium* infection among sexual partners

• Concordant *M. genitalium* multi-locus genotypes reported among 87% of co-infected sexual partners

• Increased prevalence in high risk groups for STIs

• Association with number of sex partners

• Estimates for chlamydial transmission from men to women per episode of vaginal coitus have been based on observational studies and range from 10% to 39.5%

Mycoplasma genitalium in Men
Population-based Survey Studies

• Wave III, National Longitudinal Study of Adolescent Health, USA
  o Young men (n=1,218) and women (n=1,714) aged 18-27 yrs enrolled in 2001-2002
  o *M. genitalium* prevalence: 1.1% in men, 0.8% in women
  o Strongly associated with increasing numbers of sexual partners and black race

• Third National Survey of Sexual Attitudes and Lifestyles (NATSAL-3), UK
  o Probability sample survey (2010-2012)
  o Sexually experienced men and women between aged 16-44 (n=4,507, no gender split)
  o *M. genitalium* prevalence: 1.2% in men, 1.3% in women
  o Risk factors: black race, numbers of total & new sex partners, unsafe sexual practices

• Population-based survey, Aarhus County, Denmark
  o Survey undertaken in 1997-1998
  o Population: young adults (731 males, 921 females) aged 21-24 yrs
  o *M. genitalium* prevalence: 1.1% in men, 2.3% in women
  o Detection associated with increasing number of sexual partners

\[\text{Manhart et al., Am. J. Public Health 2007;97:1118–1125; }\]
\[\text{Andersen B et al., Sex. Transm. Infect. 2007;83:237–241}\]
Relative Importance of *M. genitalium* as a Cause of Urethritis

- Prevalence of *M. genitalium* cases varies geographically and by socio-economic status
- *Neisseria gonorrhoeae* - most common cause of urethritis in most developing regions of the world
- *Chlamydia trachomatis* - most common pathogen associated with NGU
- *Mycoplasma genitalium* - main organism associated with persistent NGU following first-line treatment

Lewis *et al.*. Unpublished surveillance data from six aetiological surveys among men attending primary health care clinics with urethral discharge.
Survey Studies in MSM

- MSM have higher rates of *M. genitalium*, *N. gonorrhoeae* and *C. trachomatis* infections than are observed in population-based surveys
  - Prevalence highest at ano-rectal sites for all three bacteria
  - Oro-pharyngeal *M. genitalium* uncommon

<table>
<thead>
<tr>
<th>Site of Infection</th>
<th>MSM attending six SOPVs, Melbourne, 2001-2002 (N = 521, 24% response rate)</th>
<th>MSM attending Western Sydney Sexual Health Centre, 2017 (N = 508, consecutive)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
<td>Anal</td>
</tr>
<tr>
<td><em>M. genitalium</em></td>
<td>2.1%</td>
<td>1.6%</td>
</tr>
<tr>
<td><em>C. trachomatis</em></td>
<td>8.1%</td>
<td>6.2%</td>
</tr>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>4.8%</td>
<td>2.2%</td>
</tr>
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</table>

- *C. trachomatis* and *M. genitalium* prevalence lower among MSM with NGU than in heterosexual men - more NGU cases in MSM are idiopathic
- *M. genitalium* is reportedly more prevalent in men with HIV infection

M. genitalium and Non-Gonococcal Urethritis

- **M. genitalium** was first isolated from urethral samples of two men with NGU
- Strong + consistent associations with NGU
- Higher bacterial load in symptomatics
- Pro-inflammatory cytokines released during infection – biologically plausible
- Taylor-Robinson & Jensen reviewed literature and reported:
  - **M. genitalium** detected in 15%–25% of men with symptomatic NGU
  - **M. genitalium** detected in 5%–10% of those without disease
  - Association with NGU: OR 5.5; 95%CI, 4.3–7.0
**M. genitalium** has a Lower Infectivity than **C. trachomatis**

- Incubation period for development of *M. genitalium* NGU is unknown

- Likely *M. genitalium*-associated NGU has a prolonged incubation period compared with chlamydia - slower replication

- Leung *et al* reported on the relative likelihood, by bacterial aetiology, of NGU patients having a new partner or >1 partner compared to controls:
  - *M. genitalium* NGU cases were similar to controls regarding these characteristics
  - *C. trachomatis* NGU cases were associated a new partner or >1 partner

- Wetmore *et al*. assessed the mean duration of relationship for the most recent partner for men with NGU
  - 75 days for *M. genitalium*-positive men
  - 16 days for *C. trachomatis*-positive men

M. genitalium and other Conditions in Men - I

1. PROCTITIS

- Bissessor et al. reported 12% *M. genitalium* prevalence among 154 MSM with proctitis (c.f. *N. gonorrhoeae*, 25%; chlamydia, 19%; HSV, 18%)

- Rectal *M. genitalium* bacterial load was significantly higher in men with proctitis vs. asymptomatic men.

- We lack treatment effectiveness studies for *M. genitalium* proctitis

2. EPIDIDYMO-ORCHITIS

- *M. genitalium* may be a cause of acute epididymitis in some patients – however, still not proven and requires further study

- Ito et al. reported *M. genitalium* was detected in 8% of 56 cases of epididymitis in men <40 years old (c.f. *C. trachomatis* in 50%)
3. PROSTATITIS

- Sparse evidence that *M. genitalium* is associated with chronic prostatitis
  - *M. genitalium* not detected by PCR in prostatic biopsy specimens from 50 patients with chronic abacterial prostatitis
  - *M. genitalium* detected by PCR in prostatic biopsy specimens from 5/135 (4%) men
  - *M. genitalium* detected in semen from 2/18 men with chronic abacterial inflammatory prostatitis, compared to 0/20 controls (non-significant)
  - Mo et al. evaluated 235 Chinese men with prostatitis and 152 asymptomatic STI clinic controls who underwent specimen collection procedures including prostate massage – the *M. genitalium* PCR assay was positive in 10% men with clinical prostatitis and 3% of controls (p=0.005)

4. BALANOPPOSTHITIS

- *M. genitalium* associated with balanoposthitis in 114 men with acute NGU
- Further studies required

Mycoplasma genitalium in Women
Mycoplasma genitalium Prevalence in Women

- *M. genitalium* prevalence is on par with other STIs of public health concern
- In low-risk female populations, *M. genitalium* prevalence is around 2% (most cohorts report a prevalence of <1% to 5%)
- US National Longitudinal Study of Adolescent Health demonstrated a *M. genitalium* prevalence of 0.8% (c.f. gonorrhoea 0.4%, chlamydia 4.2%)

Manhart et al., Am. J. Public Health 2007;97:1118-1125
Vaginal Discharge

- *M. genitalium* is not thought to cause vaginal discharge
  - positive vaginal swab NAATs likely reflect endocervical infections
  - no association with other organisms causing vaginitis (*T. vaginalis*, *Candida* spp.)
  - relationship with bacterial vaginosis (BV) inconsistent between studies

Female Urethritis

- Most studies have been conducted in Scandinavia – data lacking elsewhere
- Moi *et al.* reported a significant association between *M. genitalium* and microscopic urethritis in one large Scandinavian study **BUT** three other Scandinavian studies failed to show any significant associations
- Need to exclude patients with concomitant cervicitis as inflammation at other sites may cause urethral contamination and false positive diagnosis of urethritis
Mucopurulent Cervicitis

- Characterised by clinical signs (mucopulent discharge, friability at the cervical os) or by elevated PMNLs in endocervical smears
- Research hampered by the lack of a generally-accepted case definition
- *M. genitalium* has been positively associated with microscopically-proven cervicitis but, only in some studies, with cervical discharge
- Controlling for concomitant chlamydial infection in several studies found significant associations between *M. genitalium* and cervicitis
- Lack of studies controlling for the effect of concomitant gonorrhoea
- The magnitude of the increased risk is comparable to those of other STI pathogens causing cervicitis (i.e. *C. trachomatis* and *N. gonorrhoeae*)

Pelvic Inflammatory Disease

- *M. genitalium* serologically linked to PID in 1984 and later detected by NAATs in both endometrial/fallopian tube samples from women with acute PID
- Several NAAT-based studies have found a +ve association between *M. genitalium* and clinically diagnosed PID in geographically diverse populations
- *M. genitalium* has been significantly associated with endometritis in a small number of studies where the endometrium was sampled
- Studies are hampered by the sub-optimal specificity of some of the clinical signs used to diagnose PID and the recognized lack of correlation with laparoscopic findings
- No clear trend has been observed when comparing studies that excluded co-infections with *C. trachomatis* or controlled for co-infections in multivariate analyses

Prevention of Pelvic Infection (POPI) Trial

- Trial to assess if chlamydia screening can prevent PID in female students recruited in London (2004-2006)

- Students randomly allocated to ‘screen and treat for chlamydia’ or ‘deferred chlamydia testing’ at 12 months

- 2,378 students provided 2 x self-collected vaginal swabs at baseline
  - 1 for chlamydia screening
  - 1 for end-of-study *M. genitalium* testing

- 2,246 (94%) were followed up after 12 months and assessed for incidence of clinical PID

Source: iStock
Prevention of Pelvic Infection (POPI) Trial

- Baseline *M. genitalium* prevalence = 3.3% (78/2378; 95% CI: 2.6%-4.1%)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Pathogen detected % (n/N)</th>
<th>Pathogen not detected % (n/N)</th>
<th>Risk ratio (95% CI)</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. genitalium</em></td>
<td>3.9% (3/77)</td>
<td>1.7% (36/2,169)</td>
<td>2.35 (0.74-7.56)</td>
<td>No</td>
</tr>
<tr>
<td><em>C. trachomatis</em></td>
<td>10.0% (7/70)</td>
<td>1.5% (31/2,114)</td>
<td>6.82 (3.11-14.95)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Conclusion: *M. genitalium* is unlikely to be a major risk factor for clinical PID

Study limitations:
1. Study not powered to investigate *C. trachomatis* and not *M. genitalium*
2. Clinical diagnosis of PID is imprecise (PPV 65%-90% c.f. laparoscopy)
3. Sample bias: student sample; higher risk students were less likely to enrol or provide follow-up specimens
4. Lack of geographical representation
Pregnancy Complications and Infertility

- *M. genitalium* independently associated with pre-term birth in some studies and a recent meta-analysis

- Consistent strong correlations reported between serological evidence of *M. genitalium* infection and laparoscopically-confirmed tubal infertility
  - Association is maintained after excluding women with prior chlamydia

- Grzesko *et al.* reported NAAT detection of *M. genitalium* in endocervical samples was more frequent in infertile women vs. healthy fertile women

- Other studies has indicated that endocervical testing is not useful in predicting upper genital tract infection

- Experimental models (monkeys, mice, fallopian tube organ cultures) indicate *M. genitalium* can colonize upper genital tract leading to salpingitis and/or endometritis

**Mycoplasma genitalium** Binds to Fallopian Tube *in vitro* Organ Cultures (FTC)

Immunofluorescence demonstrates *M. genitalium* binding
Effect of *Mycoplasma genitalium* on Fallopian Tube *in vitro* Organ Cultures (FTC)

Uninfected control FTC

FTC showing cilia swellings due to *M. genitalium*

Damaged cilia with *M. genitalium* attached to secretory cells (arrow)
Systematic Review and Meta-Analysis

*M. genitalium* and female reproductive tract disease

- Examined associations of *M. genitalium* infection and reproductive tract syndromes
  - cervicitis, PID, preterm birth, spontaneous abortion, infertility
- 2 independent reviewers screened for and examined relevant studies
  - 1 Jan 1980 to 25 June 2014; PubMed, Embase, Biosis, Cochrane Library and reference review
- Examined Forest plots and conducted random-effects meta-analysis to estimate prevalence
- Between study heterogeneity examined with the $I^2$ statistic and meta-regression
- Publication bias assessed via funnel plots and Begg & Egger tests; quality of the methods rated
- 1080 records screened (cervicitis, 311; PID, 292; pregnancy outcome, 174; infertility, 203)
Mycoplasma genitalium and Cervicitis

Figure 1. Forest plot of the association between Mycoplasma genitalium and cervicitis. *Adjusted effect estimate (crude effect estimate in all other cases). Abbreviations: CI, confidence interval; OR, odds ratio.
Mycoplasma genitalium and PID

Figure 2. Forest plot of the association between Mycoplasma genitalium and pelvic inflammatory disease. *Adjusted effect estimate (crude effect estimate in all other cases). Abbreviations: CI, confidence interval; OR, odds ratio.
**Mycoplasma genitalium and Preterm Birth**

![Forest plot](image)

**Figure 3.** Forest plot of the association between *Mycoplasma genitalium* and preterm birth. *Adjusted effect estimate (crude effect estimate in all other cases). Abbreviations: CI, confidence interval; OR, odds ratio.
Mycoplasma genitalium and Spontaneous Abortion

Figure 4. Forest plot of the association between *Mycoplasma genitalium* and spontaneous abortion. *Adjusted effect estimate (crude effect estimate in all other cases). Abbreviations: CI, confidence interval; OR, odds ratio.
**Mycoplasma genitalium and Infertility**

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year</th>
<th>OR (95% CI)</th>
<th>Weight</th>
</tr>
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<tbody>
<tr>
<td>Moller</td>
<td>1985</td>
<td>0.65 (.26, 1.62)</td>
<td>21.79</td>
</tr>
<tr>
<td>Clausen*</td>
<td>2001</td>
<td>5.60 (3.28, 9.42)</td>
<td>25.18</td>
</tr>
<tr>
<td>Svenstrup*</td>
<td>2008</td>
<td>4.50 (1.20, 15.60)</td>
<td>18.32</td>
</tr>
<tr>
<td>Haggerty*</td>
<td>2008</td>
<td>1.40 (.60, 2.90)</td>
<td>23.02</td>
</tr>
<tr>
<td>Grzesko</td>
<td>2009</td>
<td>5.37 (.64, 44.70)</td>
<td>11.68</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>2.43 (.93, 6.34)</td>
<td>100.00</td>
</tr>
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</table>

NOTE: Weights are from random-effects analysis

**Figure 5.** Forest plot of the association between *Mycoplasma genitalium* and female infertility. *Adjusted effect estimate (crude effect estimate in all other cases). Abbreviations: CI, confidence interval; OR, odds ratio.
Implications of the Meta-Analysis Findings

- Approx. 2-fold increased risks were demonstrated for all 5 conditions examined – all pooled estimates were significant except for infertility.
- Sub-analyses of studies that accounted for other STI pathogens demonstrated greater and significant pooled estimates for all 5 conditions.
- Although preterm delivery was associated with *M. genitalium* infection, most cases of preterm delivery are not STI-related.
- Prevalence of *M. genitalium* in pregnancy is too low to recommend routine antenatal screening.
- Role for screening women at high risk and the impact of treatment on pregnancy outcomes remains unclear.

Lis et al., Sex. Transm. Infect. 2018;94:255-262;
M. genitalium Research Priorities for Women

• Prospective studies in high prevalence M. genitalium populations to better establish a pathogenic role for this pathogen in terms of sequelae

• Further our understanding of M. genitalium pathogenesis and immune activation in both symptomatic and asymptomatic cases

• Improve our understanding as to the importance of eradication of M. genitalium from upper genital tract following antimicrobial therapy
  o current PID guidelines do not properly address M. genitalium infection
  o PID Evaluation and Clinical Health trial demonstrated that 56% of M. genitalium-infected women with PID experienced persistent endometritis after standard therapy

• Primate studies required to investigate whether recurrent M. genitalium infections result in higher risks of tubal infertility

• Determination of whether screening women for M. genitalium reduces the incidence of adverse sequelae

Haggerty et al., Sex. Transm. Infect. 2008;84:338-342
Treatment of Mycoplasma genitalium
Azithromycin Resistant *Mycoplasma genitalium*

- High-level macrolide resistance is a growing problem in countries using azithromycin 1g for chlamydial treatment
- Azithromycin 1g stat. has been shown to select for resistance *in vivo* - 23S rRNA SNPs at positions 2058/2059
- Although no direct head-to-head study exists, the 5-day extended 1.5g azithromycin regimen is preferred to a 1g stat dose
  - 500 mg once daily d1, 250 mg once daily d2-d5
Performance of ResistancePlus™ MG Assay

Prospective study: ResistancePlus™ MG
Clinical results

23S rRNA mutant detection

<table>
<thead>
<tr>
<th>Sanger Sequencing</th>
<th>Mutant</th>
<th>Wild type</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SpeeDx</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>38</td>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>Wild type</td>
<td>0</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>26</td>
<td>64*</td>
</tr>
</tbody>
</table>

* Only includes MG positive samples by both methods

MG 23S rRNA mutant prevalence
- Overall 63.1%, Male 81.0%, Female 30.4%

Highly multiplexed test with excellent clinical sensitivity and specificity

Sensitivity
- 100.0% (90.8 to 100.0)
Specificity
- 96.2% (80.4 to 99.9)
PPV
- 97.4% (86.5 to 99.9)
NPV
- 100.0% (86.3 to 100.0)
Moxifloxacin

- In early studies, all cases of *M. genitalium* infection responded to moxifloxacin

Moxifloxacin resistance was first reported in Sydney in 2013

- Moxifloxacin resistance is now emerging rapidly with treatment failures in 20-30% of cases in Japan and Australia

- Moxifloxacin use has additional safety concerns (e.g. hepatitis, long QT syndrome)
Uncomplicated *Mycoplasma genitalium* infection

**Macrolide resistance status known**
- **Macrolide-resistant**
  - Azithromycin (500 mg day 1, 250 mg day 2–5)
  - TOC after >21 days
  - Moxifloxacin (400 mg for 7–10 days)
  - TOC after >21 days
  - Consider pristinamycin (1 g × 4 for 10 days)
  - TOC after >21 days

**Macrolide-susceptible**
- Azithromycin (500 mg day 1, 250 mg day 2–5)
- TOC after >21 days
- Moxifloxacin (400 mg for 7–10 days)
- TOC after >21 days
- Consider pristinamycin (1 g × 4 for 10 days)
- TOC after >21 days

**Macrolide resistance status unknown**

- Sitafloxacin*
- Lefamulin
- Minocycline

* Give after one week of doxycycline
Challenges in Contact Tracing

• All current partner(s) should be tested and treated with the same treatment as the index patient

• Advised index and partners should not resume sexual activity until all have completed treatment and symptoms have resolved

• This is a complex issue given:
  o lack of access to molecular diagnostic tests for *M. genitalium*
  o limited efficacy of first-line treatment regimens
  o global prevalence of *M. genitalium* macrolide resistance
  o fact that not all sexual partners are infected
Conclusions

• Causative role in NGU now clearly established
• Significant associations recognized with cervicitis, endometritis, pelvic inflammatory disease (PID), spontaneous abortion and pre-term labour
• Evidence of non-significant association with female infertility through a recent meta-analysis
• Role in proctitis, prostatitis, epididymo-orchitis, balanoposthitis remains unclear
• First-line treatment is best guided by novel molecular assays that can ‘detect and predict’ pathogen + antibiotic susceptibility
• Resistant is now a major concern and treatment failures increasingly common
• Improved efforts to ensure effective contact tracing outcomes warranted