Mycoplasma genitalium: epidemiology, diagnostics and antimicrobial resistance

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Déclaration d’intérêts de 2012 à 2015

Investigateur principal d’une recherche ou d’une étude clinique :

- Roche Diagnostics,
- Diagenode,
- Hologic,
- SpeeDx
Mycoplasma genitalium

- 1980: *Mycoplasma genitalium* isolated from 2 of 13 men with nongonococcal urethritis (NGU)
  - Mollicutes class: no cell wall
  - Very slow growth (>50 days)
  - Very few isolates available
  - Animal model in chimpanzees


- 1990’s: development of PCR assays, allowed study of disease association

- 1995: smallest genome known (580 kbp, ≈ 480 genes)
  - The 2nd bacterial genome fully sequenced (Himmelreich, 1995)
  - Minimal requirements of life, concept of minimal cell
M. genitalium: prevalence and incidence

• Prevalence
  - Community-based populations 1–3%
    Carriage may be asymptomatic
  - STI testing centers populations (high risk) 4 – 38%

• Incidence
  - University women: 0.9 per 100 WY
  - Kenyan female sex workers: 23 per 100 WY

**M. genitalium: disease association**

<table>
<thead>
<tr>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGU</td>
<td>Urethritis</td>
</tr>
<tr>
<td>Balanoposthitis</td>
<td>Cervicitis</td>
</tr>
<tr>
<td>Epididymitis</td>
<td>Endometritis, Salpingitis (PID)</td>
</tr>
<tr>
<td>Prostatitis</td>
<td></td>
</tr>
<tr>
<td>Proctitis (MSM)</td>
<td>Adverse pregnancy outcomes</td>
</tr>
<tr>
<td></td>
<td>Female infertility</td>
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<tr>
<td></td>
<td>Increased HIV transmission</td>
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</tbody>
</table>
Association between *M. genitalium* and male NGU (1)

- Responsible for 15-20% NGU (pooled OR 5.5),
  20-25% NCNGU,
  30% persistent-recurrent U

- 2nd cause of NGU after *Chlamydia trachomatis*

- Coinfection with *C. trachomatis* not incommon

- 2015 Updated CDC STD guidelines, 2016 European guideline on NGU, 2016 European guideline on *M. genitalium* infection: role of Mg in urethritis and treatment-related implications

Association between *M. genitalium* and male NGU (2)

Manhart et al, *Clin Infect Dis* 2011
Association between *M. genitalium* and female reproductive tract disease (1)

- Fewer studies than in men, small sample sizes
- Commonly asymptomatic
- Mg detected in 10-30% clinical cervicitis, 2-22% PID
- Similar to *C. trachomatis*: Mg can cause PID (proportion of cases unknown), but less frequently than with *C. trachomatis*
- Adverse pregnancy outcomes and female infertility: more research needed
- 2015 Updated CDC STD guidelines, 2016 European guideline on *M. genitalium* infection: role of Mg in cervicitis and treatment-related implications

Association between *M. genitalium* and female disease (2)
Metanalysis 1980-2014
*(Lis et al, Clin Infect Dis 2015, 61:418, PMID: 26042815)*

- *M. genitalium* infection significantly associated with approximately 2-fold increased risk of:
  - Cervicitis (20 included studies): pooled OR, 1.66
  - PID (10 studies): pooled OR, 2.14
  - Pre-term birth (6 studies): pooled OR 1.89
  - Spontaneous abortion (3 studies): pooled OR 1.82

- Elevated risk of female infertility
  - 5 included studies, risk about 2.5-fold
  - Only statistically significant in subanalyses
Diagnosis of *M. genitalium* infections (1)

- **Only a direct diagnosis**, no serology kit commercialized
- **Culture extremely fastidious** (very few strains isolated worldwide, coculture with Vero cells)
- **By nucleic acid amplification tests:**
  - A lot of in-house PCRs, real-time PCR ++, TMA
  - MgpA adhesin gene, 16S rRNA
  - Monoplex and multiplex tests commercialized, some CE-marked, no FDA-approved (RUO tests)
    - Load very low even in symptomatic infections
  - Some specimens better than others:
    - FVU > urethral swabs in men
    - vaginal swabs > cervix > FVU in women

## Commercially available mono and multiplex NAATs for *M. genitalium*

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Kit</th>
<th>Technique</th>
<th>Pathogens targeted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagenode</td>
<td>S-DIAMGTV</td>
<td>qPCR</td>
<td><em>M. genitalium, Trichomonas vaginalis</em></td>
</tr>
<tr>
<td>Fast-track Diagnostics</td>
<td>Several kits</td>
<td>qPCR</td>
<td><em>M. genitalium</em> and several STI pathogens and urogenital mycoplasmas</td>
</tr>
<tr>
<td>Hologic</td>
<td><em>Mycoplasma genitalium</em></td>
<td>TMA</td>
<td><em>M. genitalium</em></td>
</tr>
<tr>
<td></td>
<td>Aptima assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roche/TIB MolBiol</td>
<td>LightMix <em>Mycoplasma genitalium</em></td>
<td>qPCR</td>
<td><em>M. genitalium</em></td>
</tr>
<tr>
<td>Sacace</td>
<td>Several kits</td>
<td>qPCR</td>
<td><em>M. genitalium</em> alone or multiplexed with several STI pathogens and/or urogenital mycoplasmas</td>
</tr>
<tr>
<td>Seegene</td>
<td>Several kits</td>
<td>qPCR</td>
<td><em>M. genitalium</em> and several STI pathogens and urogenital mycoplasmas</td>
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Diagnosis of *M. genitalium* infections (2)

• **Barriers for Mg testing:**
  - No reimbursement
  - Lack of validated commercial assays
  - Low throughput in commercial available assays
  - Test diagn. performance varies significantly between labs

⇒ **Need for quality assessment**
⇒ **Diagnostic activity is predicted to increase**
Diagnosis of *M. genitalium* infections (3)

- **Indications for Mg testing:**
  - Symptoms
  - Risk factors
    - Symptoms in a regular sexual partner
    - Persons with high-risk sexual behavior (<40 yo, >3 new sexual contacts, >5 life-time partners)
    - Sexual contact of persons with STI or PID, Mg-infected persons
    - Before termination of pregnancy or other procedures, that break the cervical barrier
    - Regular testing of MSM including anal sampling (role of Mg in increased HIV transmission risk)

**M. genitalium and antibiotics**

- **Intrinsic resistance related to** β-lactams +++, fosfomycin, glycopeptides and rifampicin

- **Active antibiotics in vitro**
  - Macrolides, lincosamides, streptogramins, ketolides (MLSK), tetracyclines, fluoroquinolones
  - Early in vitro Mg studies:
    - highly S to macrolides (azithromycin, AZM), reduced S to tetracyclines and older fluoroquinolones (CIP, OFX)

- No antimicrobial susceptibility testing done in routine

- **Acquired resistance**
  - Genetic support: chromosomal mutations ++
  - Target modification

Most male NGU studies

Tetracyclines not useful, effective in only 30-40% (no acquired R)

AZM 1g single dose = 1\textsuperscript{st} line treatment

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**Efficacy of azithromycin against *M. genitalium* declines**

![Graph showing the efficacy of azithromycin against *M. genitalium* before and after 2009.](image)

- Before 2009: 83.50%
- Since 2009: 67%

Pooled microbial cure rate from meta-analysis if 21 studies (n=1,490)

**M. genitalium treatment studies**

- Most male NGU studies

- Tetracyclines not useful, effective in only 30-40% (no acquired resistance). AZM 1g single dose = 1st line treatment

- Metanalysis on the efficacy of AZM for Mg treatment (Lau Clin Infect Dis 2015)

⇒ Declining treatment efficacy of AZM 1g for the treatment of urogenital *M. genitalium*

⇒ Why? Increasing prevalence of macrolide resistance due to widespread use of AZM 1g single dose

Macrolide resistance in *M. genitalium* (1)

- Mutations in domain V of 23S rRNA
  - A2058G/C, A2059G (*E. coli* numbering)
  - AZM 1g single dose
    - Selection of resistant mutants **during** AZM treatment
    - Therapeutic failure if patient infected with a mutated strain
Macrolide resistance in *M. genitalium* (2)

- **Extended 1.5 g AZM (500 mg d1, 250 mg d2-4) 85-95% effective and associated with lower risk of inducing AZM R**
  

- **Patients failing azithromycin 1g single dose cannot be treated successfully with extended 1.5 g AZM**


- **Macrolide resistance since 2005 in Australia, New-Zealand, Japan, Scandinavia, The Netherlands, France, Spain, Russia, South Africa**

  France: 17.2% of specimens (2013-2014)  
  Denmark, UK: ≈ 40%...Greenland 100%!!

Frequency of macrolide resistance in *M. genitalium*

- No data
- <10%
- 10%-20%
- 30%-40%
- 40-50%
Fluoroquinolone resistance in *M. genitalium*

• **Moxifloxacin 400 mg for 7-10 d in case of AZM failure** but…
  
  www.cdc.gov/std/tg2015;

• **Emerging MXF resistance** with few MXF-R isolates and failures described in Australia, Japan, Scandinavia and Europe:
  
  - **Australia and Japan**: prevalence ranges from 10-47% between 2006 and 2014
  - **France**: prevalence of 7% in 2013-2014
  - **UK**: prevalence of 4.5% in 2011


• **Mutations in the bacterial target genes of fluoroquinolones**
  
  - Most frequent mutations in *parC*
  - A few mutations in *gyrA*
Molecular diagnosis of *M. genitalium* antibiotic resistance

**Molecular detection of macrolide resistance**
- Sanger sequencing, SNP detection using pyrosequencing, qPCR (FRET or TaqMan probes)
- In-house and commercial assays (Speedx)
- Simultaneous detection of Mg and macrolide resistance directly from specimens -> treatment to be adjusted

**Molecular detection of fluoroquinololone resistance**
- amplification and sequencing of the gene targets (*parC*+++)

**Conclusion**

- *M. genitalium*, a STI pathogen, has emerged!!
  An accepted cause of male NGU and female cervicitis,
  Significant association with PID

- Diagnostic activity is predicted to increase (commercially available NAAT tests): testing Mg on symptomatic patients and patients with high STI risk behavior

- Increasing prevalence of macrolide resistance \(\Rightarrow\) Decreasing efficacy of AZM monotherapy

- 2nd line treatment with MXF …under pressure

\(\Rightarrow\) Need to detect Mg and macrolide resistance in the same time
  (implementation of molecular diagnostic tests):
    change from syndromic to etiologic treatment

\(\Rightarrow\) Need for trials of combinations of registered drugs and new antimicrobial compounds

*Horner Clin Infect Dis 2015; Jensen BMC Infect Dis 2015; Manhart Clin Infect Dis 2014*

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