Les mycoplastmes urogénitaux : des agents d’IST ?

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USC EA 3671 Mycoplasmal and chlamydial infections in humans
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Bordeaux University Hospital

National French Reference Center for bacterial IST

December 1st 2017
Characteristics of mycoplasmas

- Smallest free-living eubacteria (500 to 2200 kbp)
- Lack of cell wall (« Mollis cutis » or Mollicutes)
- 18 human species: respiratory or urogenital tract

  5 human pathogenic species:
  - *M. pneumoniae* → respiratory tract infections
  - *M. hominis*
  - *U. urealyticum* → urogenital tract infections
  - *U. parvum* Sexually transmitted infections (Mg)
  - *M. genitalium*
**Ureaplasma spp. and M. hominis**

- **Commensals of the urogenital tract**
  - More frequent in women
  - *Ureaplasma* spp. (30%) >> *M. hominis* (<10%)
  - Variable according to different parameters
    ✓ age, sexual activity, race, pregnancy, socio-economic level

- **Opportunistic pathogens**
  - Challenge to interpret their presence in the lower genital tract

- **Not IST agents**
<table>
<thead>
<tr>
<th>Disease</th>
<th>M. hominis</th>
<th>Ureaplasma spp.</th>
<th>M. genitalium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male genital disease</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nongonocccocal urethritis</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Epididymitis, prostatitis</td>
<td>-</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td><strong>Gynecologic infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>+</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Cervicitis</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PID</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Adverse pregnancy outcomes</strong></td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td><strong>Neonatal infections</strong></td>
<td>±</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td><strong>Extragenital infections</strong></td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>

+ : Confirmed association  
± : Non confirmed association  
- : No association  
?: Unknown
Laboratory detection

• **Culture**: *M. hominis* and *Ureaplasma* spp.
  - Agar or liquid broth
  - Commensal ➔ Quantitative cultures required

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile sites or specimens</td>
<td>No threshold</td>
</tr>
<tr>
<td></td>
<td>Detection = infection</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
</tr>
<tr>
<td>Urethral specimens, sperm</td>
<td>U ≥ 10⁴ CCU/ml</td>
</tr>
<tr>
<td>1&lt;sup&gt;er&lt;/sup&gt; void urines</td>
<td>U ≥ 10³ CCU/ml</td>
</tr>
<tr>
<td><strong>Women</strong> (cervico-vaginal specimens)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Mh ≥ 10⁴ CCU/ml</td>
</tr>
<tr>
<td></td>
<td>- U : no threshold,</td>
</tr>
<tr>
<td></td>
<td>detection non significant</td>
</tr>
<tr>
<td><strong>Neonates</strong></td>
<td></td>
</tr>
<tr>
<td>Endotracheal specimens</td>
<td>U ≥ 10⁴ CCU/ml</td>
</tr>
</tbody>
</table>

U, *Ureaplasma* spp. Mh, *M. hominis*; CCU, color changing unit

• **PCR**: specimens from sterile sites
  - Higher sensitivity
Urogenital mycoplasmas and antibiotics

• Intrinsic resistance related to:
  - the Mollicutes class: ATB targeting the cell wall (β-lactams, glycopeptides, fosfomycin) and rifampicin (mutation in rpoB gene)
  - certain species and macrolides and related ATB:
    ✓ *M. hominis* resistant to 14- and 15-membered macrolides
    ✓ *Ureaplasma* spp. resistant to lincosamides

• Active antibiotics
  - Macrolides and related ATB: macrolides, lincosamides, streptogramin combinations, ketolides (MLSK)
  - Fluoroquinolones
  - Tetracyclines
Antibiotic susceptibility testing

• Phenotypic techniques
  - Broth microdilution
  - Agar dilution
  - E-tests (*M. hominis*)

• CLSI recommendations
  M43-A 31(19), 2011

• In routine, commercialized kits
  *M. hominis, Ureaplasma* spp. only

• Molecular techniques to detect tetracycline, fluoroquinolone or macrolide resistance
Acquired resistance to antibiotics in *Ureaplasma* spp. and *M. hominis*

- **Resistance to tetracyclines +++**
  - acquisition of \textit{tet}(M) gene
  - 15\% Mh, 7.5\% U (France, 2010-2015)
- **Resistance to macrolides**
  - Mutations in domain V of 23S rRNA
  - Very rare cases in Mh and U
- **Resistance to fluoroquinolones**
  - mutations of gyrase and topoisomerase IV genes
  - Patients previously treated by FQ
  - 3\% Mh and 1\% U (France, 2010-2015)

*Meygret et al. oral communication, RICAI 2017*
Mycoplasma genitalium

- **1980:** *Mycoplasma genitalium* isolated from 2 of 13 men with nongonococcal urethritis (NGU)
  - Very slow growth (>50 days)
  - Very few isolates available

- **1990’s:** development of PCR assays

- **1995:** smallest genome known (580 kbp, 485 genes)
  - The 2nd bacterial genome fully sequenced (Himmelreich, 1995)
  - Minimal requirements of life, concept of minimal cell

*Tully, Int J Syst Bacteriol 1983*

*Tully Lancet 1981*
**M. genitalium prevalence**

- **Community-based populations 1–3%**
  - Carriage frequently asymptomatic

- **STI testing centers populations (high risk) 4 – 38%**

- **Prevalence in France 2014-2015**
  Urogenital specimens submitted for *C. trachomatis* (Ct) and *N. gonorrhoeae* (Ng) detection (2594 patients)

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**M. genitalium prevalence in France**

- **By sample collection sites**
  Urogenital specimens submitted for *C. trachomatis* and *N. gonorrhoeae* detection in France 2014-2015

![Bar chart showing prevalence of M. genitalium by sample collection sites.](chart.png)

STI centres
Abortion centres
Family planning centres
Penitentiary centres

Gynaecologic practices
Obstetric practices
Reproduction centres

*p = 0.009*

### M. genitalium: disease association

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

34 studies
1993-2010
Europe, America, Asia, Oceania

Pooled OR = 5.5 (4.3-7.0)

2nd cause of NGU after *C. trachomatis*

Manhart et al, Clin Infect Dis 2011
**M. genitalium: disease association**

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<td>Prostatitis</td>
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<td>Proctitis (MSM)</td>
<td><strong>Adverse pregnancy outcomes</strong></td>
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<td></td>
<td>Female infertility</td>
</tr>
<tr>
<td></td>
<td>Increased HIV transmission</td>
</tr>
</tbody>
</table>
Association between *M. genitalium* and female disease
Meta-analysis 1980-2014

![Graph showing association between *M. genitalium* and female disease](image)

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

*M. genitalium*-positive urogenital specimens submitted for *C. trachomatis* and *N. gonorrhoeae* detection in France 2014-2015

- No symptom: 70.9%
- Abnormal vaginal or penile discharge: 16.4%
- Pelvic pain: 7.3%
- Pain, burning or stinging passing urine: 9.1%
- Genital pruritus: 1.8%

An STI agent

- Sexually transmission is established
  - Among heterosexual contacts, women are twice as likely as men to be infected (aOR=2.18, Slifirski, Emerg. Infect. Dis. 2017)
  - Transmission is probably lower than that for *C. trachomatis*
    ✓ Consistent with lower infectious load of Mg
    ✓ men with symptomatic NGU may be more infectious than men with asymptomatic infection

- Among MSM, rectal infection is more common than urethral infection

- Transmission through oral sex is likely to be rare
  - as carriage of Mg in the oropharynx is not frequent

_Horner, J infect Dis 2017; Slifirski, Emerg Infect Dis, 2017_
Diagnostic of *M. genitalium* infections

- **Only direct diagnosis**, no serology kit commercialized

- **Culture extremely fastidious** (co-culture with Vero cells required)
  No routine MIC determination

- **Nucleic acid amplification tests:**
  - A lot of in-house PCRs, real-time PCR ++, TMA
  - MgPa adhesin gene (*mgpB*), 16S rRNA
  - Monoplex and multiplex tests commercialized, some CE-marked

# 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>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>Progenie molecular</td>
<td>MYGE-U, MYGE-G</td>
<td>qPCR</td>
<td><em>M. genitalium</em></td>
</tr>
<tr>
<td>SpeedX</td>
<td>ResistancePlus MG</td>
<td>qPCR</td>
<td><em>M. genitalium and macrolide resistance</em></td>
</tr>
<tr>
<td>BioGX (BD MAX)</td>
<td></td>
<td>qPCR</td>
<td><em>M. genitalium</em> and urogenital mycoplasmas</td>
</tr>
<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>*M. genitalium and several STI pathogens and urogenital mycoplasmas</td>
</tr>
<tr>
<td>Sacace</td>
<td>Several kits</td>
<td>qPCR</td>
<td>*M. genitalium 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>*M. genitalium and several STI pathogens and urogenital mycoplasmas</td>
</tr>
</tbody>
</table>

- No reimbursement
- Need for external quality assessment programs
Indication for Mg testing

Symptoms
- Symptoms or signs of urethritis in men
- Mucopurulent cervicitis
- Cervical or vaginal discharge with risk factor of STI
- Intermenstrual or post coidal bleeding
- Acute pelvic pain and/or PID
- Acute epididymo-orchitis in a male <50 yo
Indication for Mg testing

**Symptoms**

**Risk factors**

- Symptoms in a regular sexual partner

- Persons with high-risk sexual behavior (<40 yo, >3 new sexual contacts in the last year)

The public health value of testing asymptomatic persons for Mg has not been established. Decision on testing should be informed by local epidemiology when available.

- Sexual contact of persons with STI or PID, with Mg-infected persons

- Before termination of pregnancy or other procedures, that break the cervical barrier

- Regular testing of MSM including anal sampling
M. genitalium and tetracyclines

- **Relative potency in vitro**

MIC ranges (µg/ml)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>M. genitalium</th>
<th>M. hominis</th>
<th>Ureaplasma spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tetracyclines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>≤0.01-0.3</td>
<td>0.1-2</td>
<td>0.02-1</td>
</tr>
<tr>
<td>Minocycline</td>
<td>≤0.01-0.2</td>
<td>0.03-1</td>
<td>0.06-1</td>
</tr>
</tbody>
</table>

- **BUT, low eradication rate**
  - Microbiological cure: between 30 and 40 %

### M. genitalium and macrolides

- **Low MICs**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>M. genitalium</th>
<th>M. hominis</th>
<th>Ureaplasma spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MLSK group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≤0.01</td>
<td>32-&gt;1 000</td>
<td>0.02-16</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>&lt;0.01</td>
<td>&gt;16</td>
<td>0.1-2</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>≤0.01-0.06</td>
<td>16-&gt;256</td>
<td>≤0.004-2</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>≤0.01-0.03</td>
<td>4-&gt;64</td>
<td>0.06-4</td>
</tr>
<tr>
<td>Josamycin</td>
<td>0.01-0.02</td>
<td>0.05-2</td>
<td>0.03-4</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.2-1</td>
<td>≤0.008-2</td>
<td>0.2-64</td>
</tr>
<tr>
<td>Pristinamycin</td>
<td>≤0.01-0.02</td>
<td>0.1-0.5</td>
<td>0.1-1</td>
</tr>
<tr>
<td>Quinupristin/ Dalfopristin</td>
<td>0.05</td>
<td>0.03-2</td>
<td>0.05-0.5</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>≤0.015</td>
<td>2-32</td>
<td>≤0.015-0.25</td>
</tr>
<tr>
<td>Solithromycin</td>
<td>≤0.000000063-0.000125</td>
<td>0.002-0.008</td>
<td>0.002-0.063</td>
</tr>
</tbody>
</table>

Macrolide resistance in *M. genitalium*

- **Mutations in domain V of 23S rRNA**
  - Single operon encoding 16S and 23S rRNA
  - A2058G/C/T, A2059G/C/T (*E. coli* numbering)
Detection of macrolide resistance-associated mutations

- Amplification and 23S rRNA sequencing
  - Time-consuming, not adapted to routine

- In-house methods
  - HRM (High Resolution Melting curve analysis) (Twin et al. PloS One 2012)

- Commercial kits
  - ResistancePlus™ MG kit (SpeeDx, Australia) : multiplex real-time PCR
Prevalence of macrolide resistance in *M. genitalium*

Macrolide resistance in *M. genitalium*
Bordeaux, France

**M. genitalium** treatment studies: Azithromycin 1g

- Meta-analysis on the efficacy of AZM 1g for Mg treatment *(Lau Clin. Infect. Dis. 2015)*
  21 studies, 1490 participants, mostly male NGU

- AZM 1g single dose is no more the the 1st line treatment
  - Therapeutic failure if patient infected with a mutated strain
  - Selection of resistant mutants during AZM treatment

M. genitalium treatment studies: Azithromycin 1.5 g/5 days

- Extended 1.5 g AZM (500 mg d1, 250 mg d2-4)
  85% 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

- Moxifloxacin 400 mg for 7-10 d in case of AZM failure… but…
**M. genitalium and fluoroquinolones**

- Only moxifloxacin has low MICs

<table>
<thead>
<tr>
<th>Antibiotics</th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluoroquinolones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2</td>
<td>0.1-4</td>
<td>0.1-16</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1-2</td>
<td>0.1-4</td>
<td>0.2-4</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.5-1</td>
<td>0.1-2</td>
<td>0.2-2</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.03-0.06</td>
<td>0.06-0.125</td>
<td>0.125-1</td>
</tr>
<tr>
<td>Sitafloxacin (Japan only)</td>
<td>0.125</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fluoroquinolone resistance in *M. genitalium*

- Mutations in the bacterial target genes of fluoroquinolones
  - Most frequent mutations in *parC* (Topoisomerase IV)
    Primarily Ser83 and Asp87
  - A few mutations in *gyrA* (DNA gyrase)

- Molecular detection only: amplification and sequencing of target genes
Prevalence of fluoroquinolone resistance-associated mutations

Meta-analysis on the efficacy of moxifloxacin for *M. genitalium* treatment \(\text{(Yi et al Int J STD AIDS 2017)}\)

- 17 studies, 252 patients
• **Uncomplicated *M. genitalium* infection:**

- In the absence of macrolide resistance-associated mutations
  Azithromycin 500 mg (day 1), then 250 mg (days 2-5)
  Josamycin 500 mg 3 times daily - 10 days- IV

- Macrolide-resistant *M. genitalium* infection
- Second line treatment for persistent *M. genitalium* infection
  Moxifloxacin 400 mg/d - 7 to 10 days
• Third-line treatment for persistent MG infection after AZM and MXF
  Doxycycline 100 mg x2 daily for 14 days
  Pristinamycin 1 g x4 daily for 10 days

• Complicated MG infection (PID, epididymitis)
  Moxifloxacin: 400 mg - 14 days

• Test of cure: no earlier than 3 weeks after the start of antibiotic treatment
• All *M. genitalium*-positive test should be followed up with an assay capable of detecting macrolide resistance-associated mutations

• The extended azithromycin ttt after failure with 1g single dose will NOT eradicate *M. genitalium*
Ureaplasma spp. and *M. hominis* are NOT STI agents

- **Commensal of the urogenital tract**
  - Quantitative culture to interpret their pathogenic role
    - *Ureaplasma* in men, *M. hominis* in women
    - Adverse pregnancy outcomes and neonates: both
  - PCR: specimens from sterile sites

- **Antibiotic susceptibility testing**
  - Commercialized kits, CLSI breakpoints

- **Prevalence of resistance in France, 2010-2015**
  - Tetracycline: 7.5% (U) to 15% (Mh)
  - Fluoroquinolones: 1% (U), 3% (Mh)
  - Macrolide: very rare

**Take-home message**
M. genitalium, a STI pathogen, has emerged!

- Male NGU, female cervicitis and PID
- Pauci-symptomatic infections
- Highest prevalence in high-risk sexual behavior patients +++
- Diagnostic: NAAT assays
  - Activity is predicted to increase (commercially available NAAT assays)
- Prevalence of resistance in France, 2015-2016
  - Tetracycline: no resistance but 70% treatment failure
  - Azithromycin: 18%
  - Moxifloxacin: 6%
Take-home message

- 1st-line treatment for uncomplicated *M. genitalium* infection
  - Azithromycin 1.5 g on 5 days in absence of macrolide resistance

- Moxifloxacin 400 mg/j 7-10 days in the other cases
  - Under pressure

- Always test of cure after 3-5 weeks

⇒ Superbug? New XDR bacteria?

Might become *untreatable* in certain circumstances
Need for trials of combinations of registered drugs and new antimicrobial compounds
Acknowledgments

USC EA 3671, Univ. Bordeaux
National Reference Center for bacterial STI
Cécile Bébéar
Charles Cazanave
Arabella Touati
Bertille de Barbeyrac
Olivia Peuchant
Chloé Le Roy
Nadège Henin

Statens Serum Institut, Denmark
Jorgen J. Jensen

University of Washington, USA
Lisa L. Manhart

Gynecology, Infect. Diseases clinics, Bordeaux Univ Hospital
Charles Cazanave
Dominique Dallay
Jacques Horowitz
Bordeaux and its surroundings