Detecting antimicrobial resistance in M. genitalium – the key to best therapy

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Mycoplasma genitalium

- Well known but sneaky
- First identified in 1980 – 2 men with non gonococcal urethritis
- Difficulty in culture
- 1980-1990
- 1980-2013 – 843 articles (NG 8,493 : CT 12,612)
- 2015: Emerging Issue - CDC treatment guidelines
What do we know?

• Sexually transmitted

• Acute & chronic urethritis in men
• Vaginal discharge, cervicitis & pelvic inflammatory disease in women

• Focus on *N. gonorrhoeae (NG)* & *C. trachomatis (CT)*
• *M. genitalium (MG)* in the background
What don’t we know?

• Correlation suggested with
  – Endometriosis
  – Spontaneous abortion
  – Pre term delivery
  – Tubal factor infertility

• Suspected facilitation of HIV transmission

• Risk factors for transmission uncertain
How common is it?

- Found globally

- Prevalence varies

- 1 – 4.5% screening populations
- 9% - >50% high risk patients
- CT > MG > NG
How important can it be?

- In men - 15-20% cases non chlamydial non gonococcal urethritis (NCNGU)
- MSM - London
  - Significant number of cases of MG in MSM (4.4% rectal samples)
  - higher rates in HIV infected men
- Brazil – MG detected in 5.8% HIV infected men
- Asymptomatic
  - 40-75% women
  - rectal & pharyngeal infections
## Is it a problem in Australia?

<table>
<thead>
<tr>
<th>Period</th>
<th>Location</th>
<th>Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001-2002</td>
<td>Victoria</td>
<td>MSM</td>
<td>2.1%</td>
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<tr>
<td>2006-2007</td>
<td>Sydney</td>
<td>Men with urethral symptoms STD clinic</td>
<td>4.5%</td>
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<td>(14% MSM)</td>
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<tr>
<td>2007-2008</td>
<td>Victoria</td>
<td>Women primary health centres</td>
<td>2.4%</td>
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<tr>
<td>2007-2011</td>
<td>South Australia</td>
<td>Men with urethral symptoms STD clinic</td>
<td>8.1%</td>
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<tr>
<td>2009-2013</td>
<td>Sydney</td>
<td>STD clinic</td>
<td>218 cases</td>
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<td>(17% MSM)</td>
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<tr>
<td>2012-2013</td>
<td>Victoria</td>
<td>STD clinic</td>
<td>172 cases</td>
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<td>(17% MSM)</td>
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<td>2013-2015</td>
<td>Sydney</td>
<td>STD clinic</td>
<td>16%</td>
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<td>(43% MSM)</td>
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</table>
What do we do about it?

• Collect urine /swabs from patients with symptoms of infection or risk factors associated with infection

• Treatment for NGU on suspicion

• Laboratory detection by PCR
  – NG/CT & MG

• Test of Cure (TOC) –3 weeks after start of treatment
Treatment - Doxycycline

• UK - Recommended as one of the first line treatments for NGU & PID (2015 NGU UK guidelines)

• In vitro susceptibility testing - MG strains susceptible
• But…poor efficacy in clinical practice
• Cure rate between 17 & 94%

• Cannot be recommended as a first line MG treatment
Treatment - Macrolides

- NGU – Azithromycin (AZM) 1g
  - <2008 - Cure rate 72-100%
  - >2008 – Cure rate 50-91%
  - Melbourne (84% efficacy 2005-6; 69% efficacy 2007-9)

- MG positive
  - Extended AZM therapy - 1.5g over 5 days
  - More effective due to slow growth rate of MG
  - Efficacy also declining
Resistance to Macrolides

- Modifications to 23S ribosomal RNA gene

- 30-45% resistance in countries where 1g AZM used routinely for NGU

- Resistance is both transmitted & induced
  - Induced resistance less likely with extended AZM course
Resistance to Macrolides in Australia

• Sydney study 2008-2011
  – 43% patient samples contained resistance causing mutations

• Melbourne study 2012-13
  – 39% failed 1g AZM (high levels pre treatment mutations)
Treatment with Fluoroquinolones

- Second line therapy - Moxifloxacin
- Not tested in clinical trials
- 2008-2012: Cure rate approaching 100%
- More recently: Cure rate of 69-100%
Resistance to Fluoroquinolones

- Mutations in *gyrA* & *parC* genes in the quinolone resistance-determining region

- Treatment failures up to 30% (Asia-Pacific)
Resistance to Fluoroquinolones in Australia

- Sydney study 2008-2011
  - 15% contained potential resistance causing mutations

- First treatment failure reported in Sydney in 2013

- Melbourne study 2012-13
  - 12% failed Moxifloxacin therapy (all contained mutations prior to therapy)
What is left in the cupboard?

- Pristinamycin – 3rd line treatment
  - maximum dose for 10 days
  - Melbourne study 2012-13 – 100% cure rate for 6 samples
  - Treatment failures identified

- 4th line treatment
  - Doxycycline - effective 30% patients, difficulty in treating pregnant contacts
  - Other drugs - newer fluoroquinolones, solithromycin

- Combination therapy
How do we fix this?

• Bring it into the limelight!
  – Increasing incidence
  – Rapid rise in resistance
  – Lack of available therapy options

• Treat patients with the most effective antibiotic the first time

• Identify the presence of infection AND resistance mutations at the same time in order to guide therapy.
Detection and Resistance testing

• Traditionally performed separately
  – PCR for detection
  – Amplification and sequencing of the 23S, parC & gyrA genes
  – HRM assay for macrolide resistance in 2012

• 2016: MG 23S assay (SpeeDx)
  – Multiplex qPCR assay
  – Detection – MgPa gene
  – Macrolide resistance - 5 mutations in the 23S rRNA gene
Does it work?

- Melbourne 2016
- 400 MG positive samples undergoing TOC (2012-13)

- MG detection
  - 99.1% sens; 98.5% spec

- Macrolide resistance genes
  - 97.4% sens; 100% spec
Our evaluation - 2016

• 145 MG positive samples (2007-15)
  – Detection: 100% correlation
  – Resistance: 94.5% correlation (7/145 false positive*; 1/145 false negative)

• 132 unknown samples (2016)
  – Detection: 100% correlation (n=15 MG positive)
  – Resistance – 86% correlation (2/15 false negative)

• Further studies to come for NGU & MSM patients
The key to best therapy

• Screening of high risk groups - asymptomatic infection

• Implementation of assays which allow targeted treatment based on:
  – Rapid identification of the causative organism
  – Identification of resistance markers (macrolide and fluoroquinolone)

• Limit spread of transmitted resistance

• Identify induced resistance - Test of cure
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