High levels of *Mycoplasma genitalium* antibiotic resistance are observed in Australia

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Mycoplasma genitalium (MG)

M. genitalium as an STI

- Men – Non-Gonococcal Urethritis (6-50%)
- Women – Cervicitis, Pelvic Inflammatory Disease (5-20%)

Anti-microbial resistance

- 1st line treatment = Azithromycin (macrolide antibiotic)
- Macrolide resistance associated with 23S rRNA mutations
- A2058G, A2059G, A2058T, A2058C, A2059C (E. coli numbering)

Current testing methods

- MG detection – PCR
- Macrolide resistance detection – Sequencing, HRMA, FRET assays

Clinically relevant rapid NAAT is required
MG macrolide resistance in Europe

GRL 100%

Norway >60%

UK 41%

DK 38%

Sweden <20%

Netherlands 30-39%

FR 18%

Spain 40-50%

Guidelines in Europe now include resistance testing

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Gosse et al JCM. 2016
Salado-Rasmussen et al CID. 2014
Gesink et al Int J Circumpolar Health 2012
(NB not all data from published studies)
**PlexPCR™**

Highly Specific, Extremely Sensitive

Target Gene (human, bacteria, virus)

**ResistancePlus™**

**Mutation Specific PlexPrimer**
- Mismatched to target
- Mismatch increases specificity
- Matches mutation

- Increases SPECIFICITY
- Differs for each mutant

**PlexPrime Amplicon**

**Mutation specific amplification AND mutation specific detection**

*Tan LY et al PLOS ONE January 23rd 2017*

*Patent: WO 2013/123552*
**ResistancePlus™**

- **5’ region**
- **3’ region**

- *Mutation 1 = PlexPrimer 1*
- *Mutation 2 = PlexPrimer 2*
- *Mutation 3 = PlexPrimer 3 etc*

**Channel 1**
- Probe 1
- *PlexZyme 1*

**Channel 2**
- Probe 2
- *PlexZyme 2*

**Channel 3**
- Probe 3
- *PlexZyme 3*

**Amplicon 1**
- **Amplicon 2**
- **Amplicon 3**

**Highly sensitive multiplex assay for clustered mutations**
**ResistencePlus™**

- **5’ region**
  - Mutation 1 = PlexPrimer 1

- **3’ region**
  - Mutation 2 = PlexPrimer 2
  - Mutation 3 = PlexPrimer 3 etc

**Channel 1**

- **Amplicon 1**
  - PlexZyme 1
  - Probe 1

- **Amplicon 2**
  - PlexZyme 2
  - Probe 1

- **Amplicon 3**
  - PlexZyme 3
  - Probe 1

**Highly sensitive multiplex assay for clustered mutations**
5 most common 23S rRNA mutations related to macrolide resistance

Rapid qPCR results (<1.5 hours)
Allows actionable clinical information

Multiple Specimen Types
Swabs (Urogenital & Rectal), Urine (Male, Female)

<table>
<thead>
<tr>
<th>Channel</th>
<th>Pathogen</th>
<th>7 Targets/1 test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Pathogen:</strong> <em>M. genitalium</em> (MgPa)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><strong>5 Resistance Markers:</strong> 23S rRNA mutations (A2058T, A2058C, A2058G, A2059C, A2059G)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Internal Control</td>
<td></td>
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</tbody>
</table>
Prospective Clinical Study
Melbourne, Australia

1089 samples received Nov 2015-Dec 2015

Melbourne Sexual Health Centre
Catriona Bradshaw

Symptomatic male & female

- NGU,
- Cervicitis
- Proctitis
- PID
- Sexual contacts

Royal Womens Hospital
Sepehr Tabrizi

Non-symptomatic female

- Contraceptive advice
- Insertion of intra-uterine contraceptive device
- Termination of pregnancy

<table>
<thead>
<tr>
<th></th>
<th>Urine/urethral swab</th>
<th>Anal swab</th>
<th>Cervical/vaginal swab</th>
<th>Sample numbers</th>
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<tbody>
<tr>
<td>Male</td>
<td>354</td>
<td>34</td>
<td>n/a</td>
<td>388</td>
</tr>
<tr>
<td>Female</td>
<td>203</td>
<td>2</td>
<td>496</td>
<td>701</td>
</tr>
<tr>
<td>Total</td>
<td>557</td>
<td>36</td>
<td>496</td>
<td>1089</td>
</tr>
</tbody>
</table>
Results of Prospective Study

**MG prevalence 6.0%**
- Male 10.8%
- Female 3.3%

**MG 23S rRNA mutant prevalence 63.1%**
- Male 81.0% (34/42)
- Female 30.4% (7/23)
- (Male rectal 100%)

<table>
<thead>
<tr>
<th>SpeeDx</th>
<th>Mutant</th>
<th>Wild type</th>
<th>Total</th>
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<tbody>
<tr>
<td>Mutant</td>
<td>38</td>
<td>1</td>
<td>39</td>
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<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>
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</table>

*S* Only includes MG positive samples by both methods

<table>
<thead>
<tr>
<th>In house qPCR (16S rRNA)</th>
<th>%</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>98.5</td>
<td>91.7 to 99.9</td>
</tr>
<tr>
<td>Specificity</td>
<td>100.0</td>
<td>99.6 to 100.0</td>
</tr>
<tr>
<td>PPV</td>
<td>100.0</td>
<td>94.4 to 100.0</td>
</tr>
<tr>
<td>NPV</td>
<td>99.9</td>
<td>99.5 to 100.0</td>
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</table>

<table>
<thead>
<tr>
<th>Sanger Sequencing</th>
<th>%</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>100.0</td>
<td>90.8 to 100.0</td>
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<tr>
<td>Specificity</td>
<td>96.2</td>
<td>80.4 to 99.9</td>
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<tr>
<td>PPV</td>
<td>97.4</td>
<td>86.5 to 99.9</td>
</tr>
<tr>
<td>NPV</td>
<td>100.0</td>
<td>86.3 to 100.0</td>
</tr>
</tbody>
</table>

High Clinical Sensitivity and Specificity
Improved patient care with ResistancePlus™ MG

Symptomatic Urethritis; Empirical treatment
Doxycycline (or Azithromycin)

Test for CT/GC/MG

~ 3 weeks TOC or failure
Second line treatment

• Patient has MG+AMR ~ 6 weeks
• Community spread of AMR MG

Test for CT/GC/MG+AMR

2 days - call back if resistant
Second line treatment

• Removes MG+AMR from community within days
• IUSTI management guidelines

Improved patient outcome & reduced spread AMR
Collaborators

- Sepehr Tabrizi
- Catriona Bradshaw
- Christopher Fairley
- Suzanne Garland
- Jenny Su
Thank you!

For further queries:

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