An Australia-wide molecular study of *Neisseria gonorrhoeae* identifies frequent occurrence of a key cephalosporin resistance mechanism


QPID Laboratory, QCMRI, The University of Queensland, Australia

WHO estimates: approx. 100 million cases of gonorrhoea globally.
→ Disease burden is highest in low income settings.

Rates are increasing (15,675 notifications in Aust. in 2014)

**Background**

- Rise in ceftriaxone MICs → decreased susceptibility (DS)
- Increasing reports of ESC treatment failures, primarily cefixime but also ceftriaxone in cases of pharyngeal infections.
  → Mostly due to the dissemination of resistant clones
- Predominantly mosaic Penicillin Binding Protein 2 (PBP2) 7363 and 1901 MLST types.
Mosaic PBP2; HOW?

- Altered PBP2 protein → Mosaic PBP2; arising from recombination events.

H041

N. cinerea PBP2

N. perflava PBP2

1 200 400 600 Amino acids

N. flavescens PBP2

F89

Ceftriaxone resistant strain; A8806 Australia 2014

Similar to H041; MIC = 0.5mg/L

H041

N. gonorrhoeae mosaic PBP2 (CRO DS and CEF R)

Where are the H041 and F89 strains?

H041 – No more reports

F89 – No more reports

H041

N. flavescens PBP2

F89

Ceftriaxone resistant strain; A8806 Australia 2014

Similar to H041; MIC = 0.5mg/L

H041

N. gonorrhoeae mosaic PBP2 (CRO DS and CEF R)

associated with MLST types 1901 and 7363

HOWEVER, NG with mosaic-PBP2 is now spreading worldwide.

Associated with MLST types 1901 and 7363

H041

N. gonorrhoeae mosaic PBP2 (CRO DS and CEF R)

N. flavescens PBP2

F89

Ceftriaxone resistant strain; A8806 Australia 2014

Similar to H041; MIC = 0.5mg/L
Phase 1: To better understand the spread of NG and resistance.
- Genotyping isolates from throughout Australia.

Phase 2: Molecular AMR testing of NG NAAT-positive clinical samples
- Focus on the NT.

David Whiley, Tuesday 2pm; Molecular aspects of antimicrobial resistant Neisseria gonorrhoeae

Phase 1:
To better understand the spread of NG and resistance.
- Genotyping isolates from throughout Australia.
  - AMR mutations
  - SNP-MLST = Strain

Sequenom Massarray platform
- Uses MALDI-TOF MS.
- Multiplexing 12 or 14 SNPs per single reaction
- 384 well plate; 10 hours
- Cost per isolate = approx. $15.0 per isolate

Total isolates = 2218 (90.5% isolates in first half of 2012)

Total isolates = 271 (90.5% isolates in first half of 2012)
Dominated by certain strains
- Top 40: 80% of isolates
- Top 10: 50% of isolates
- Top 3: 25% of isolates

Heterosexual
MSM

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total isolates</th>
<th>Gender</th>
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Strain | Total isolates | Gender | male | female |
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1/10/2015
Mosaic-1901; geographic distribution?

Mosaic-1901, geographic distribution?

Mosaic-1901; gender distribution?

Mosaic-1901; gender distribution?

**Strain**

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<th>Gender</th>
<th>Susceptibility profile</th>
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<td>NG10</td>
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<td>LS  neg  S  S  S  S  S</td>
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**AMR Profile**

- **SNP** - **MLST**
- Strain = Strain

**Total isolates**

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**Pen.** - **Tet.** - **Spect.** - **Ceft.** - **Cip.** - **Azith.**

- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S

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- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S

**LS**

- neg  S  S  S  S  S
- neg  S  S  S  S  S
- neg  S  S  S  S  S
- neg  S  S  S  S  S
- neg  S  S  S  S  S
- neg  S  S  S  S  S
- neg  S  S  S  S  S
- neg  S  S  S  S  S
- neg  S  S  S  S  S

**susceptibility profile**

- Pen.  Tet.  Spect  Ceft  Cip.  Azith
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S
- LS  neg  S  S  S  S  S

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**# mPBP2-1901**

- NG1: 84
- NG2: 82
- NG3: 17
- NG4: 4
- NG5: 1
- NG6: 0
- NG7: 188

**%**

- NG1: 11%
- NG2: 13%
- NG3: 13%
- NG4: 1.5%
- NG5: 0.7%
- NG6: 0%
- NG7: 8.1%

**Metropolitan**

- Dominates metropolitan regions
- Mainly found in VIC and NSW

**Aus. States and territories**

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<td>818</td>
<td>210</td>
<td>269</td>
<td>146</td>
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**Mosaic-1901; geographic distribution?**

- Dominates metropolitan regions
- Mainly found in VIC and NSW

**Mosaic-1901; gender distribution?**

- Dominates MSM population
- However, isolated from female patients as well

<table>
<thead>
<tr>
<th>Gender</th>
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<td>178</td>
<td>8</td>
<td>3</td>
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</tbody>
</table>

**Heterosexual networks as well**
Also typically: resistance to Penicillin (67.5%) and ciprofloxacin (98.4%).

**Azithromycin**

Most isolates MICs 0.12 - 0.25mg/L. However; → 15% with MIC of 0.5mg/L (breakpoint = 1.0)

Concern:

- These strains just require two mutations to be resistant to both ceftriaxone and azithromycin
- A501P (PBP2) + C2611T (23S) = ceftriaxone and azithromycin resistant.

Concern:

- mPBP2-1901 is a highly successful strain in our population.
  - First described in Aust. ~ 10 years ago;
  - Now comprises 8.1% of the isolate population in Australia
  - Most states and territories.

- Bacterial culture doesn’t ‘flag’ a large proportion of these strains

**STRENGTHENING AMR SURVEILLANCE CAPABILITIES IS CRUCIAL**
Acknowledgements

Study investigators and collaborators include:

- Baker IDI, NT
- James Ward
- PathWest, WA
- Dr David Speers
- Melbourne Sexual Health Centre, Vic
- Prof Christopher Fairley
- A/Prof Marcus Chen
- MDU, The University of Melbourne, Vic
- Kerrie Stevens
- Women's and Children's Hospital, SA
- Andrew Lawrence
- Royal Darwin Hospital, NT
- Robert Baird
- Kevin Freeman
- PathWest, Royal Perth Hospital, WA
- Julie Pearson
- Queensland Health Forensic and Scientific Services
- Dr Nathan Ryder
- Dr Jiunn-Yih Su
- Kirby Institute, UNSW
- A/Prof Rebecca Guy
- Prof John Kaldor
- Dr Handan Wand
- Dr David Regan
- Miles Beaman
- QPID laboratory, QCMRI, Qld
- A/Prof David Whiley
- Cameron Buckley
- A/Prof Michael Nissen
- A/Prof Theo Sloots
- Prince of Wales Hospital, NSW
- A/Prof Monica Lahra
- Athena Limnios
- Dr Tiffany Hogan
- Ratan Kundu
- Rodney Enriquez
- Namraj Goire
- Pathology Queensland
- Prof Graeme Nimmo
- Dr Cheryl Bletchly
- Fleur Francis
- Sexual Health and Blood Borne Virus Unit, NT
- Dr Thomas Sisley
- NT Sex Health
- Nicky Institute, ADMA
- Dr Michelle Ham</