 Genetic Analysis of Organisms Isolated From the ICU

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Hosted by Paul Webber
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April 18, 2018

Ingham Institute for Applied Medical Research

- Antibiotic Resistance and Mobile Elements Group

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ESKAPE Pathogens

*Enterococcus faecium* (VRE)
*Staphylococcus aureus* (MRSA)
*Klebsiella pneumoniae*
*Acinetobacter baumannii*
*Pseudomonas aeruginosa*
*Enterobacter species*

- 66% of hospital-acquired infections
- ‘escaping’ the action of antibiotics

Research Areas

- Pathogen evolution and epidemiology
- Mechanisms of antibiotic resistance
- Antibiotic development
- Multiresistance plasmids
- Clinical utility of whole genome sequencing
  - Resistance detection, Outbreak investigation, Role of ICU environment

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Analysis of ICU Isolates

- *Pseudomonas aeruginosa*
  - *Infection Control & Hospital Epidemiology*, 36(9): 1058-1064.

- *Klebsiella pneumoniae*

- *Enterococcus faecium - vanA VRE*

Pseudomonas aeruginosa Outbreak

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P. aeruginosa Outbreak

- Pseudomonas outbreak in a neonatal unit - March 2014
  - babies receiving respiratory support screened weekly
  - marked increase in the number of babies colonised with *P. aeruginosa*
- Enhanced screening of babies was introduced
  - all babies screened weekly
  - 18 colonised with *P. aeruginosa*
- Environmental swabbing of sink and patient areas
  - *P. aeruginosa* was isolated from seven sites

Incidence of Cases

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19 isolates were typed using WGS - 318™ Chip v2 and 400 bp kit

- 12 nasal isolates (babies)
- seven environmental isolates (different sources)
- Reads mapped to \( P. \text{aeruginosa} \) PA01 genome using CLC Genomics Workbench
  - 95% coverage of reference
  - average depth of 33-fold
  - MLST determined
- Core SNP tree constructed using kSNP
  - visualised using FigTree

Environmental isolates revealed several MLST
- large diverse bio-burden within the unit
- Nasal isolates predominantly ST253 – outbreak
- Two environmental isolates also ST253
- Pa16 isolated from sink
- likely ancestor of outbreak isolates
Control Measures

- Pa16 sink closed
- All other taps in unit
  - aerators replaced
  - bleached daily
- Weekly nasal swabs continued
  - no colonisation detected in following four months

Summary

- Demonstrated that there was a *P. aeruginosa* ST253 outbreak
  - not all colonised babies were part of the outbreak
  - diverse bio-burden within the unit
- Indicated that a sink (Pa16) was the likely source
  - directed infection control activities to focus on the sinks
  - clinically relevant time period
- Utility of WGS as an infection control tool
  - outbreak/cross infection investigation
  - defining resistome and genetic context
Klebsiella pneumoniae Outbreak

75yo M, returned traveller (Egypt)
- Presented to ED/ICU from Sydney Airport
- Multi-resistant *Klebsiella pneumoniae* (hip tissue, wounds, groin)

<table>
<thead>
<tr>
<th>Antibiotic susceptibilities</th>
<th>Vitek2 MICs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;=32</td>
</tr>
<tr>
<td>Augmentin</td>
<td>&gt;=32</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;=4</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;=64</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;=64</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>4</td>
</tr>
<tr>
<td>Timentin</td>
<td>&gt;=128</td>
</tr>
<tr>
<td>Tazocin</td>
<td>&gt;=128</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;=16</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt;=16</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt;=16</td>
</tr>
<tr>
<td>Amikacin</td>
<td>&gt;=64</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>1</td>
</tr>
<tr>
<td>Sulphamethoxazole</td>
<td>&lt;=20</td>
</tr>
</tbody>
</table>
Multi-resistant *K. pneumoniae* Outbreak

- 18 months later (different hospital)
- Patient undergoes a hernia repair
- Surgical mesh infection
- Multi-resistant *Klebsiella pneumoniae*

Same resistance profile

<table>
<thead>
<tr>
<th>Multi-resistant <em>K. pneumoniae</em> Outbreak</th>
<th>Directed PCRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP-4</td>
<td>negative</td>
</tr>
<tr>
<td>MBL multiplex</td>
<td>IMP, VIM, SPM, SIM, AIM, GIM negative</td>
</tr>
<tr>
<td>AmpC multiplex</td>
<td>DHA, CMY-2, ACC, MOX/CMY-1, MIR/ACT, FOX negative</td>
</tr>
<tr>
<td>Carbapenemase multiplex</td>
<td>KPC, GES negative</td>
</tr>
<tr>
<td></td>
<td>OXA-23 like, OXA-24 like, OXA-58 like not tested</td>
</tr>
<tr>
<td>ESBL multiplex</td>
<td>SHV-5/12, CTX-M Gp 1, VEB negative</td>
</tr>
<tr>
<td></td>
<td>CTX-M positive</td>
</tr>
</tbody>
</table>

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Multi-resistant *K. pneumoniae* Outbreak

WGS of Outbreak Isolates

- Six isolates were sequenced using WGS - 318™ Chip v2 and 400 bp kit
  - five ICU patient outbreak isolates
  - one patient isolate 18 months later
- Reads mapped to *K. pneumoniae* NTUH-K2044 genome
  - 93% coverage of reference
  - average depth of 50-fold
- Variant analysis of isolates using CLC Genomics Workbench

Multi-resistant *K. pneumoniae* Outbreak

Variant Analysis of Isolates

- Mapped reads to NTUH-K2044
  - non-MR-KP strain
- Quality-based algorithm
  - 80% frequency cut-off
  - 10 read minimum coverage
- Variants associated with homopolymers excluded
- Variants present in all isolates excluded

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Multi-resistant *K. pneumoniae* Outbreak

- Initial outbreak isolates identical
- Carriage isolate (18 months later) differed by 11 SNPs
  - Associated with genes involved in tolerance/resistance to antibiotics, metals or organic solvents, and transcriptional regulation.
  - Collectively, these SNPs are likely to be associated with changes in virulence (at least to some extent) that have refined the *in vivo* colonization capacity of the original outbreak isolate.

Multi-resistant *K. pneumoniae* Outbreak

- Chromosomal: Quinolones ONLY!
  - Mutations in *gyrA* (DNA Gyrase) and *parC* (Topoisomerase IV)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg mL⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>Nalidixic acid</td>
<td>≥ 32</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≥ 4</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>≥ 16</td>
</tr>
</tbody>
</table>

- β-lactams, aminoglycosides?

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Multi-resistant *K. pneumoniae* Outbreak

**Defining the Resistome**

- *De novo* assembly of unmapped reads
  - Query in-house resistance database
- β-lactamase resistance genes
  - *bla*<sub>SHV-1</sub>, *bla*<sub>CTX-M-14</sub>, *bla*<sub>OXA-9</sub>, *bla*<sub>OXA-48</sub>
- Aminoglycoside resistance genes
  - *aphA6*, *strAB*, *aaC2*, *aacA4*, *aadA1*
- BLASTn analysis of contigs
- Further mapping of reads
  - Identified two multiresistance plasmids
  - Closed gaps

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Multi-resistant *K. pneumoniae* Outbreak

**Defining the Resistome**: pJEG012

- *tnpA*  *tnpR*  *aacA4*  *aadA1*  *bla*<sub>OXA-9</sub>  *bla*<sub>TEM-1</sub>  *Tn1331*

*px orf*  *parA res*  *hhs hha tsp*  *par*  *Locus P*<sub>px</sub>  *sir tarCA HicAB*  *dhuJ*

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Multi-resistant *K. pneumoniae* Outbreak  

Defining the Resistome  
PJEG011

- **IS26**  
- **strA**  
- **aphA6**  
- **IS1012**  
- **strB**  
- **Tn3-like remnant**

- **Tn5393Δ**

- **blaCTX-M-14**  
- **ISEcp1**

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**Porins**

- **OmpK36**
  - Loop 3: 2aa ins
  - Loop 4: 3aa Δ
  - Loop 7: 1aa ins

- **OmpK35**
  - Partial Δ

- **OmpK37/PhoE = wt**
Summary

- WGS used to confirm multi-resistant *K. pneumoniae* isolates were outbreak associated
- Defined the resistome; determinants mostly plasmid-borne
  - pJEG011 (*bla*OXA-48, *bla*CTX-M-14) & pJEG012
  - Silent spread of resistance genes (plasmid spread)
- Inform isolation and infection control policies
- Facilitate understanding of resistance gene transmission

**Enterococcus faecium – vanA VRE**

Role of the Environment (2 studies)
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Hospital Acquired Infections

- Patients
- Instruments and Equipment
- Health Care Workers
- Hospital Environment

Previous Work

- Gosbell *et al.* (unpublished data)
  - Samples collected from different wards of a NSW hospital
  - MRSA isolated when MRSA patients in ward
    - 93% Environmental isolates indistinguishable from patient isolates

  - Destructive sampling of decommissioned NSW hospital ICU following terminal cleaning
    - Biofilm found on blind cord, curtain, wall paint, door, etc.
    - Culture positive for MDROs

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(Study 1) ICU Sampling

- ATP bioluminescence readings used as an indicator for high touch surfaces
  - ATP presence = presence of organic matter
  - High ATP presence = greater probability of microbial contamination
  - Sites with high readings sampled for microbial contamination

- Gauze moistened in 0.9% saline solution used to swab surfaces
  - 18-24hr 37°C enrichment
  - Plated on HBA, MRSA, VRE, ESBL selective agar
    - Preliminary MDROs confirmed using MALDI-TOF and VITEK-2 antibiotic sensitivity testing

(Study 1) ICU Sampling

- 85% of MDROs found were in the clinical station (mainly VRE)
  - HTO (chairs, clipboards and keyboards)

<table>
<thead>
<tr>
<th>Site Sampled</th>
<th>MDRO</th>
</tr>
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<tbody>
<tr>
<td>Patient folder</td>
<td>MRSA</td>
</tr>
<tr>
<td>Patient bed railing</td>
<td>MRSA</td>
</tr>
<tr>
<td>Bed pan room - storage boxes</td>
<td>VRE</td>
</tr>
<tr>
<td>Clinical station - keyboard</td>
<td>VRE</td>
</tr>
<tr>
<td>Clinical station - crash cart clipboard</td>
<td>VRE, MRSA, ESBL producer</td>
</tr>
<tr>
<td>clinical station - office chairs</td>
<td>VRE, ESBL producer</td>
</tr>
</tbody>
</table>
VRE environmental isolates compared to clinical isolates obtained during same time period (as environmental sampling)

A number of environmental and patient isolates have closely related/indistinguishable PFGE banding patterns
(Study 1) WGS of ICU Isolates

Selected 3 different clinical/environmental isolate pairs, representing different PFGE patterns, for WGS (6 isolates in total)

- 6 isolates were subjected to WGS - 318™ Chip v2 and 400 bp kit
  - 3 different VRE clinical/environmental isolate pairs
- Reads mapped to *E. faecium* Aus0085 genome using CLC Genomics Workbench
  - 90% coverage of reference
  - average depth of 53-fold
  - MLST determined
- Core SNP tree constructed using kSNP
  - visualised using FigTree
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(Study 1) Phylogenetic Relationships

- ST80 and NT vanA VRE identified
- associated with vanA emergence in Australia
- Selected PFGE pairs
- minimal core SNP differences
- Environmental isolates pre-date patient isolates
- ICU environment (clinical workstation) likely source of ST80 patient isolates

(Study 1) Summary

- ATP meters may be a useful tool in guiding environmental sampling for MDROs
- Majority of MDROs found in clinical station (mainly VRE)
  - Biofilm associated with sample sites (keyboards, chairs)
  - VRE environmental isolates similar/same PFGE pattern as patient isolates
  - WGS indicated that the ICU environment was the source of patient colonisation isolates

Cleaning needs to account for MDRO living in dry-surface biofilms?

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(Study 2) VRE Transmission Dynamics

- Retrospective study – patients admitted to an ICU over an 11 month period
- Patient and environmental vanA VRE isolates collected and sequenced
  - Patient isolates: screening (19), urine (4), bloodstream (3), skin/wound (3), intra-abdominal (2)
  - Environmental isolates: bed spaces, equipment and waste rooms (14)
- Genomic analysis
  - Core SNPs determined using kSNP and a maximum likelihood phylogeny generated
  - Links between isolates analyzed using the R package “outbreaker” software

(Study 2) Isolate Relationships
(Study 2) Summary

- Sequencing confirmed a predominantly clonal outbreak
- Environmental reservoir (shared equipment) played a key role in VRE spread
- Supports use of multifaceted strategies for successful VRE control
  - Emphasis on measures that reduce environmental burden

Acknowledgements

WESTERN SYDNEY UNIVERSITY
Iain Gosbell
Björn Espedido
Jessica Knight

Whiteley Corporation
Greg Whiteley
Darran Leyden
Trevor Glasbey

Health South Western Sydney Local Health District
Sebastiaan van Hal
Raymond Chan
Rebecca Davis
Andie Lee

MACQUARIE University
Karen Vickery
Khalid Johani
<table>
<thead>
<tr>
<th>Date</th>
<th>Topic</th>
<th>Speaker/Details</th>
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<tbody>
<tr>
<td>April 19, 2018</td>
<td><strong>TOPICAL ANTIBIOTICS TO PREVENT POST-OPERATIVE SURGICAL INFECTION... IS THE PARADIGM CHANGING?</strong>&lt;br&gt;Speaker: Dr. Hillary Humphreys, The Royal College of Surgeons in Ireland</td>
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<tr>
<td>May 3, 2018</td>
<td><strong>FREE... WHO Teleclass - Europe</strong>&lt;br&gt;SPECIAL LECTURE FOR 5 MAY&lt;br&gt;Speaker: Prof. Diclor Pittet, University of Geneva Hospitals</td>
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<tr>
<td>May 10, 2018</td>
<td><strong>FREE CBIC Teleclass</strong>&lt;br&gt;HOW THE CERTIFICATION BOARD OF INFECTION CONTROL (CBIC) WORKS FOR YOU&lt;br&gt;Speaker: Ivan W. Gowe, CBIC Director, and Lita Jo Henman, CBIC Past President</td>
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<tr>
<td>May 17, 2018</td>
<td><strong>THE SILENT TSUNAMI OF AZOLE-RESISTANCE IN THE OPPORTUNISTIC FUNGUS ASPERGILLUS FUMIGATUS</strong>&lt;br&gt;Speaker: Prof. Paul E. Verweij, Radboud University Center of Expertise in Mycology, The Netherlands</td>
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<tr>
<td>May 28, 2018</td>
<td><strong>FREE Teleclass – Broadcast live from the IPAC Canada conference</strong>&lt;br&gt;TREKKING SAFELY THROUGH THE STORM – MANAGING COMPLEX IPAC ISSUES&lt;br&gt;Speaker: Dr. Mark Joffe, Alberta Health Services</td>
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