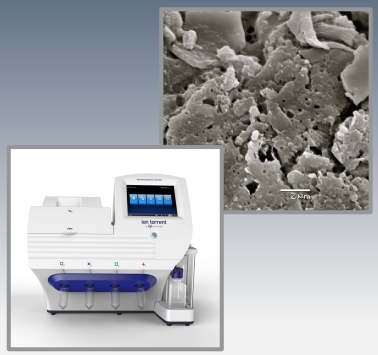
Genetic Analysis of Organisms Isolated From the ICU



A/Prof Slade Jensen
Infectious Diseases and Microbiology
School of Medicine
Western Sydney University

Hosted by Paul Webber
paul@webbertraining.com

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- Antibiotic Resistance and Mobile Elements Group

ARMEG

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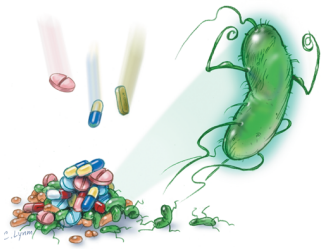
ESKAPE Pathogens

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



- 66% of hospital-acquired infections
- 'escaping' the action of antibiotics

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ARMEG 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*
  - *PLoS ONE*, 8(3): e59920.
- *Enterococcus faecium - vanA VRE*
  - *Infection Control & Hospital Epidemiology*, doi: 10.1017/ice.2018.29: 1-8.

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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

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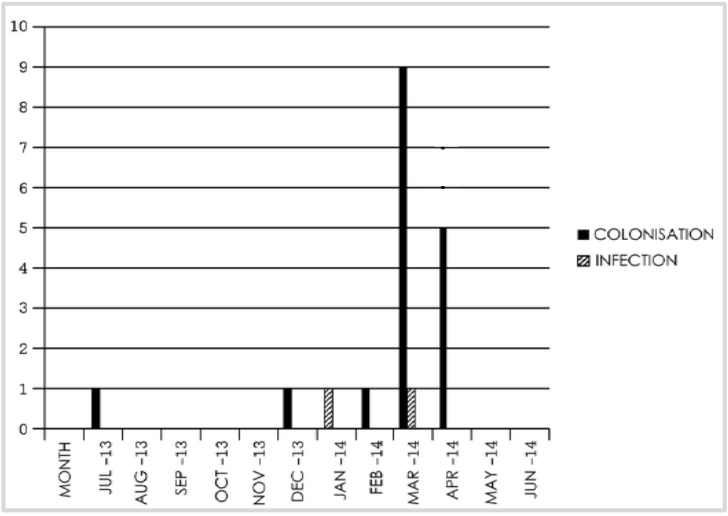
Incidence of Cases



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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. 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

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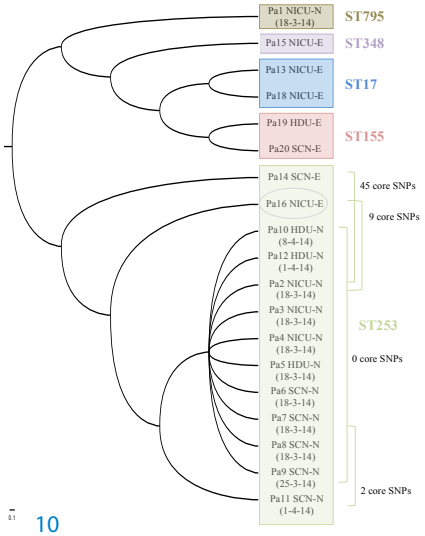
Phylogenetic Relationships



- 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

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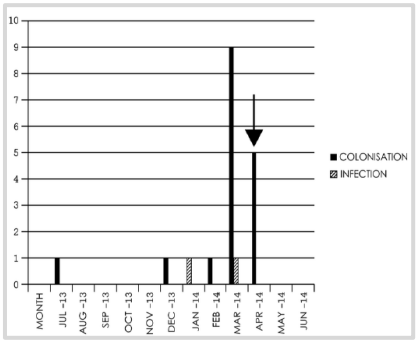
Control Measures



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

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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

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Klebsiella pneumoniae Outbreak

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Multi-resistant *K. pneumoniae*

Antibiotic susceptibilities Vitek2 MICs	
Ampicillin	>=32
Augmentin	>=32
Ciprofloxacin	>=4
Ceftriaxone	>=64
Cefepime	>=64
Ceftazidime	4
Timentin	>=128
Tazocin	>=128
Meropenem	>=16
Gentamicin	>=16
Tobramycin	>=16
Amikacin	>=64
Trimethoprim	1
Sulphamethoxazole	<=20

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

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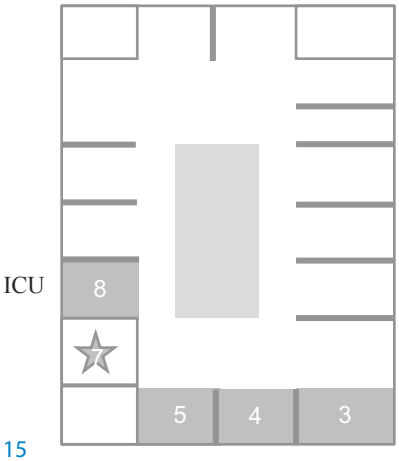
Multi-resistant *K. pneumoniae* Outbreak



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

→ Same resistance profile

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Multi-resistant *K. pneumoniae* Outbreak

Directed PCRs

IMP-4	negative
MBL multiplex	IMP, VIM, SPM, SIM, AIM, GIM negative
AmpC multiplex	DHA, CMY-2, ACC, MOX/CMY-1, MIR/ACT, FOX negative
Carbapenemase multiplex	KPC, GES negative OXA-23 like, OXA-24 like, OXA-58 like not tested
ESBL multiplex	SHV-5/12, CTX-M Gp 1, VEB negative CTX-M positive

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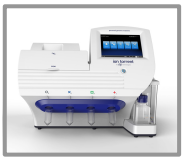
Multi-resistant *K. pneumoniae* Outbreak

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



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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

Variant Analysis of Isolates

- 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.

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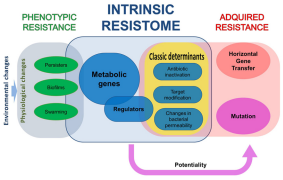
Multi-resistant *K. pneumoniae* Outbreak

Defining the Resistome

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

Antibiotic	MIC (μg mL^{-1})
Nalidixic acid	≥ 32
Ciprofloxacin	≥ 4
Norfloxacin	≥ 16



- β-lactams, aminoglycosides?

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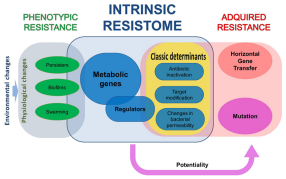
Multi-resistant *K. pneumoniae* Outbreak

Defining the Resistome

- *De novo* assembly of unmapped reads
  - Query in-house resistance database
- β-lactamase resistance genes
  - bla_{SHV-1}, $bla_{CTX-M-14}$, bla_{OXA-9}, bla_{OXA-48}
- 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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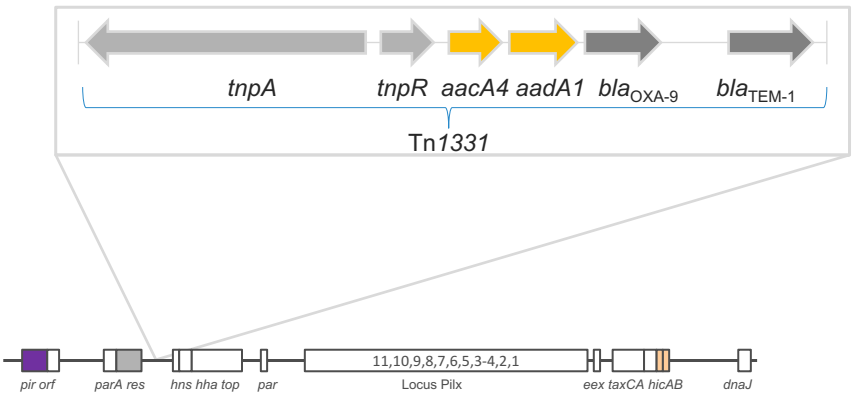
Multi-resistant *K. pneumoniae* Outbreak

Defining the Resistome pJEG012



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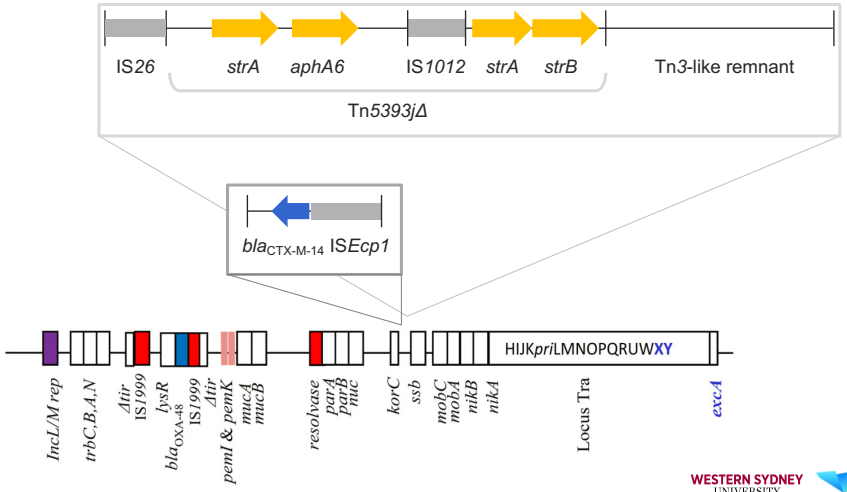
Multi-resistant *K. pneumoniae* Outbreak

Defining the Resistome pJEG011



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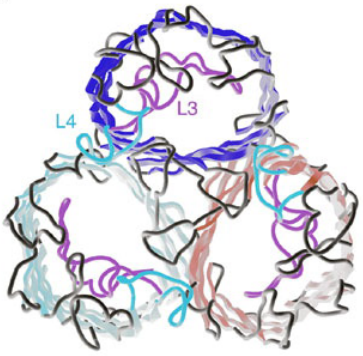
Porins

- OmpK36
  - Loop 3: 2aa ins
  - Loop 4: 3aa Δ
  - Loop 7: 1aa ins
- OmpK35
  - Partial Δ
- OmpK37/PhoE = wt



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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

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Enterococcus faecium – vanA VRE

Role of the Environment (2 studies)

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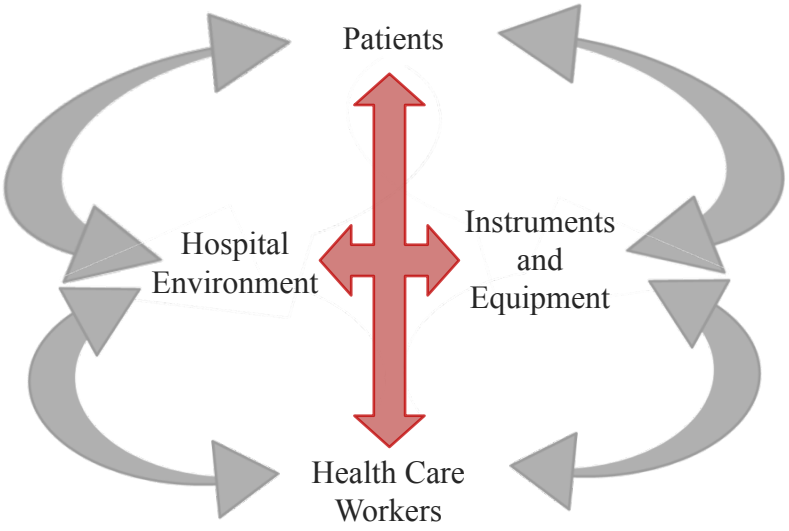
Hospital Acquired Infections



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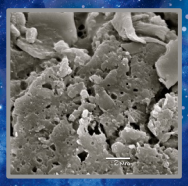
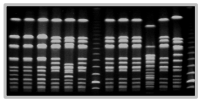
Previous Work



- 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



- Vickery *et al.* (2012). *Journal of Hospital Infection*, 80: 52-55
  - 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
  - Whiteley *et al.* (2015). *American Journal of Infection Control*, 43: 1270-5
- 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

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(Study 1) ICU Sampling

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

Site Sampled	MDRO
Patient folder	MRSA
Patient bed railing	MRSA
Bed pan room - storage boxes	VRE
Clinical station - keyboard	VRE
Clinical station - crash cart clipboard	VRE, MRSA, ESBL producer
clinical station - office chairs	VRE, ESBL producer

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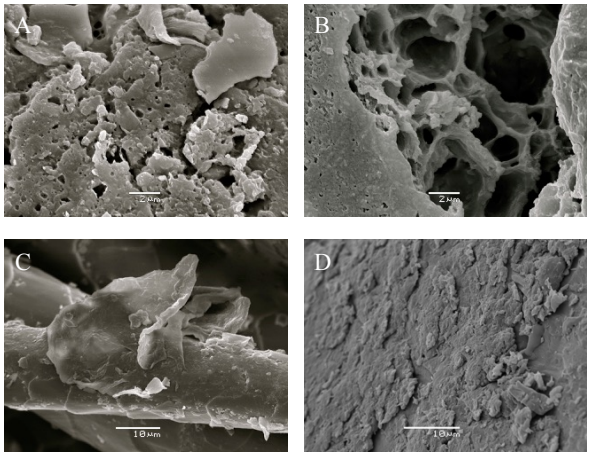
(Study 1) ICU Sampling - SEM



A, C = Chair
B = Keyboard
D = Clipboard

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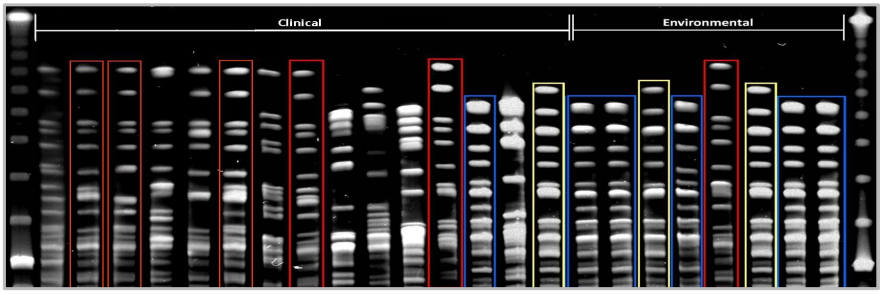
(Study 1) ICU Sampling - PFGE



- 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

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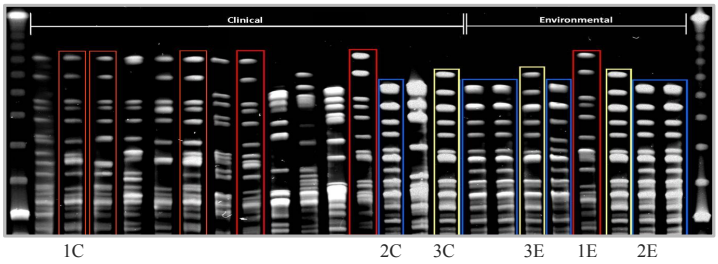
(Study 1) WGS of ICU Isolates



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

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(Study 1) WGS of ICU Isolates

- 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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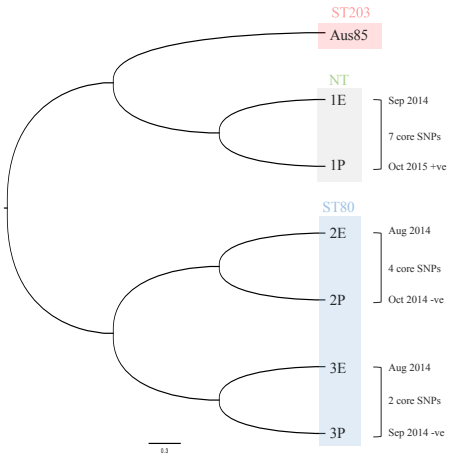
(Study 1) Phylogenetic Relationships



- 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

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(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

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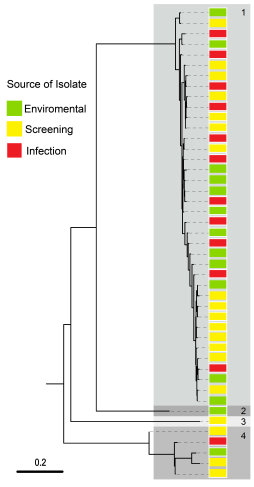
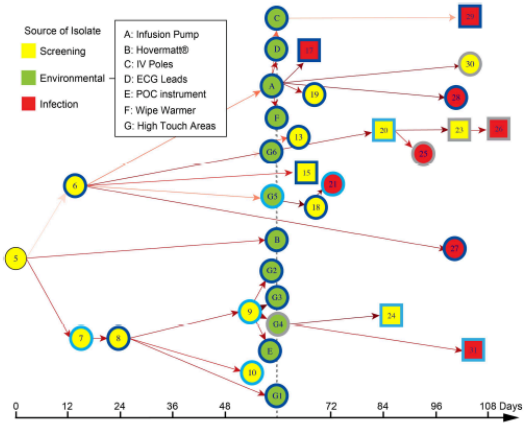
(Study 2) Isolate Relationships





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(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**

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Acknowledgements

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Rebecca Davis
Andie Lee

MACQUARIE University

Karen Vickery
Khalid Johani

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Genetic Analysis of Organisms Isolated From the ICU
Prof. Slade Jensen, Western Sidney University, Australia
A Webber Training Teleclass

www.webbertraining.com/schedulep1.php	
April 19, 2018	TOPICAL ANTIBIOTICS TO PREVENT POST-OPERATIVE SURGICAL INFECTION ... IS THE PARADIGM CHANGING? Speaker: **Dr. Hilary Humphreys**, The Royal College of Surgeons in Ireland
May 3, 2018	*(FREE … WHO Teleclass - Europe)* SPECIAL LECTURE FOR 5 MAY Speaker: **Prof. Didier Pittet**, University of Geneva Hospitals
May 10, 2018	*(FREE CBIC Teleclass)* HOW THE CERTIFICATION BOARD OF INFECTION CONTROL (CBIC) WORKS FOR YOU Speaker: **Ivan W. Gowe**, CBIC Director, and **Lita Jo Henman**, CBIC Past President
May 17, 2018	THE SILENT TSUNAMI OF AZOLE-RESISTANCE IN THE OPPORTUNISTIC FUNGUS *ASPERGILLUS FUMIGATUS* Speaker: **Prof. Paul E. Verweij**, Radboud University Center of Expertise in Mycology, The Netherlands
May 28, 2018	*(FREE Teleclass – Broadcast live from the IPAC Canada conference)* TREKKING SAFELY THROUGH THE STORM – MANAGING COMPLEX IPAC ISSUES Speaker: **Dr. Mark Joffe**, Alberta Health Services **Live broadcast sponsored by GOJO Canada (www.gojocanada.ca)**

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