POPULATION WGS: ACTIONABLE DATA

Dr Ng Oon Tek
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https://www.google.com.sg/search?q=genomics&source=lnms&tbm=isch&sa=X&ved=0ahUKEwjH3_OJuLTaAhUGR48KHdWUCw4Q_AUICigB&biw=1280&bih=659#imgrc=QMCBNU9uwPRPbM
Contents

• CaPES (CPE)

• Genomic tiers of transmission

• Transmission detection and linkage

• Novel reservoirs
Carbapenem-resistant Enterobacteriaceae (CRE) threat

Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis

Evelina Tacconelli, Elena Carrara*, Alessia Savoldi*, Stephan Harbarth, Marc Mendelson, Dominique L Monnet, Céline Pulcini, Gunnar Kahlmeter, Jan Kluytmans, Yehuda Carmeli, Marc Ouellette, Kevin Outterson, Jean Patel, Marco Cavaleri, Edward M Cox, Chris R Houchens, M Lindsay Grayson, Paul Hansen, Nalini Singh, Ursula Theuretzbacher, Nicola Magrini, and the WHO Pathogens Priority List Working Group†


- IMI-1 (California-retrospective in 1996)
- IMP (Japan- P. aeruginosa) 1996
- IMP (Singapore) 1996
- KPC (North Carolina) 1996
- VIM (Italy) 1997
- OXA-type (Turkey) 2001
- NDM-1 (Indian patient in Sweden) 2008
- NDM-1 (Singapore) 2010
- KPC (Singapore) 2011
- IMI-1 (Singapore) 2012
- OXA-type (Singapore) 2013
- VIM (Singapore) 2013
- MCR-1 (China/Mal) Feb 2016
- MCR-1 (Singapore) Aug 2016

- Imipenem-cilastatin approval 1/1/1985
- Meropenem approval 1/1/1995
- CaPES national Surveillance study
- UN declaration on AMR
- Dec 1 - Oct 10
- Sep 21

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Study population CaPES

- Time interval: **September 2010 to April 2015**
- Multi-centered cohort covering all 6 multi-disciplinary government hospitals
- Mandatory referral of CPE isolates to National Public Health Laboratory
- **80%** of inpatient care in Singapore
- Total patient-bed days (Sept 2010 to Apr 2015): **8,415,683**
- Age: 68 (IQR)
- Gender: 335 (42%) F
- Number of isolates sequenced: **1,324**
Clinical cultures/ Surveillance cultures
- **2010**: 2.9 per 100,000 patient-days/ 0.8 per 100,000 patient-days
- **2012**: 7.7 per 100,000 patient-days/ 16.0 per 100,000 patient-days
As the chromosome has approximately 5,000,000 base-pairs and plasmids have 50,000 to 250,000 base-pairs, the random chance of having genetically identical chromosomal or plasmid genomes is astronomically low.
No WGS (bacterial linkage)
With WGS (bacterial linkage)
Methods

• Genomically-linked transmission

EPIDEMIOLOGIC CONTACT

✓ Direct ward contact (same ward, **same time**)
✓ Indirect ward contact (same ward, **different time**)
✓ No ward contact
✓ Direct hospital-only contact
✓ Indirect hospital-only contact
✓ Residential contact (postcode)
✓ No known contact

Pending:

➢ Direct procedure contact
➢ Indirect procedure contact
### Genomically-linked bacterial transmission (n=263) [pilot]

<table>
<thead>
<tr>
<th>Possible Epidemiologic Transmission</th>
<th>Yes (%)</th>
<th>95% CI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct ward contact</td>
<td>73 (27.8)</td>
<td>22.7 to 33.4</td>
</tr>
<tr>
<td>Indirect ward contact</td>
<td>88 (33.4)</td>
<td>28.0 to 39.3</td>
</tr>
<tr>
<td>No ward contact</td>
<td>102 (38.7)</td>
<td>33.1 to 44.8</td>
</tr>
<tr>
<td>Direct hospital - Only contact</td>
<td>88 (33.5)</td>
<td>73.7 to 103.5</td>
</tr>
<tr>
<td>Indirect hospital - Only contact</td>
<td>21 (8.0)</td>
<td>13.7 to 31.4</td>
</tr>
<tr>
<td>Residential contact</td>
<td>13 (4.9)</td>
<td>2.8 to 8.4</td>
</tr>
<tr>
<td>No known contact</td>
<td>71 (26.9)</td>
<td>22.0 to 32.7</td>
</tr>
</tbody>
</table>
## Genomically-linked plasmid transmission (n=178) [pilot]

<table>
<thead>
<tr>
<th>Possible Epidemiologic Transmission</th>
<th>Yes (%)</th>
<th>95% CI count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct ward contact</td>
<td>145 (81.5)</td>
<td>75.1 to 86.5</td>
</tr>
<tr>
<td>Indirect ward contact</td>
<td>30 (16.8)</td>
<td>12.02 to 23.08</td>
</tr>
<tr>
<td>No ward contact</td>
<td>3 (1.7)</td>
<td>0.35 to 5.0</td>
</tr>
<tr>
<td>Direct hospital - Only contact</td>
<td>33 (18.5)</td>
<td>13.5 to 24.9</td>
</tr>
<tr>
<td>Indirect hospital - Only contact</td>
<td>4 (2.2)</td>
<td>0.7 to 5.8</td>
</tr>
<tr>
<td>Residential contact</td>
<td>11 (6.1)</td>
<td>3.4 to 10.8</td>
</tr>
<tr>
<td>No known contact</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Summary

Adding whole-genome sequencing to surveillance information:

• Much improved transmission linkage

• Determine reservoirs of transmission e.g. ward, hospital, procedures
  – Role of plasmid-mediated transmission added to bacterial transmission?

• Could plasmids persist longer in other environments?

• Plasmid transmission different from bacterial transmission?
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Rama Narayana Deepak (KTPH)
Asok Kurup (Mount Elizabeth Hospital)
Raymond Fong (CGH)
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Tan Thean Yen (CGH)
Koh Tse Hsien (SGH)
Partha Pratim De (TTSH)
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Koh Cheng Thoon (KKWCH)
Rick Ong Twee Hee (NUS School of Public Health)

Raymond Tzer Pin Lin (NPHL)
Hsu Li Yang (TTSH)
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Victor Lee (TTSH)
Liang De (TTSH)
Amanda Chua (TTSH)

(Note: Hospitals and academic institution noted in brackets.)
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Teikyo University School of Medicine
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University of Belgrade
Dr Milan Kojic, Dr Branko Jovcic

University of Calgary
Dr Johann Pitout, Dr Gisele Peirano
High-level Meeting on Antimicrobial Resistance

September 21 @ 10:00 am - 6:00 pm

On 21 September 2016, the President of the UN General Assembly convenes an one-day high-level meeting at the UN Headquarters in New York on “Antimicrobial Resistance”, with the participation of Member States, non-governmental organizations, civil society, the private sector and academic institutions, in order to provide input.

The primary objective of the meeting is to summon and maintain strong national, regional and international political commitment in addressing antimicrobial resistance comprehensively and multi-sectorally, and to increase and improve awareness of antimicrobial resistance.

The meeting emphasizes the important role and the responsibilities of governments, as well as the role of relevant inter-governmental organizations, particularly the World Health Organization within its mandate and in coordination with FAO and OIE, as appropriate, in responding to the challenges of antimicrobial resistance, and the essential need for multi-sectorial and cross-sectorial efforts and engagement of all relevant sectors of society, such as human and veterinary medicine, agriculture, finance, environment and consumers, to generate an effective response, including towards a one-health approach.

It further recalls the World Health Assembly Resolution WHA 68.7 entitled “Global Action Plan on antimicrobial resistance” which reflects a global consensus that antimicrobial resistance poses a significant public health challenge, and emphasizing the paramount significance of achieving the five strategic objectives of the WHA Global Action Plan.

http://www.un.org/pga/71/event-latest/high-level-meeting-on-antimicrobial-resistance/
Carbapenem-resistant
Enterobacteriaceae (CRE) threat

Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis

Evelina Tacconelli, Elena Carrara*, Alessia Savoldi*, Stephan Harbarth, Marc Mendelson, Dominique L Monnet, Céline Pulcini, Gunnar Kahlmeter, Jan Kluytmans, Yehuda Carmeli, Marc Ouellette, Kevin Outterson, Jean Patel, Marco Cavaleri, Edward M Cox, Chris R Houchens, M Lindsay Grayson, Paul Hansen, Nalini Singh, Ursula Theuretzbacher, Nicola Magrini, and the WHO Pathogens Priority List Working Group†

what can we do?

... Especially when there is no cure
The good news...

- Multi-modal strategy (structural factors)
  - Hand hygiene
  - Surveillance
  - Contact precautions
  - Patient isolation
  - Environmental cleaning
- Surveillance cultures of the environment
- Monitoring, audit and feedback

### Sequencing and Genomic Workflow Outline

**Species Count Percent**

<table>
<thead>
<tr>
<th>Species</th>
<th>Count</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>556</td>
<td>44.4%</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>385</td>
<td>30.8%</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>180</td>
<td>14.4%</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>67</td>
<td>5.4%</td>
</tr>
<tr>
<td><em>Morganella morganii</em></td>
<td>4</td>
<td>0.3%</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>16</td>
<td>1.3%</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca (R)</em></td>
<td>15</td>
<td>1.2%</td>
</tr>
<tr>
<td><em>Citrobacter rodentium</em></td>
<td>2</td>
<td>0.2%</td>
</tr>
<tr>
<td><em>Citrobacter amalonaticus</em></td>
<td>10</td>
<td>0.8%</td>
</tr>
<tr>
<td><em>Citrobacter koseri</em></td>
<td>8</td>
<td>0.6%</td>
</tr>
<tr>
<td><em>Citrobacter farmer</em></td>
<td>2</td>
<td>0.2%</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>5</td>
<td>0.4%</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>1</td>
<td>0.1%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1251</strong></td>
<td>100%</td>
</tr>
</tbody>
</table>
Main questions

What are the sources of ongoing transmission of CPE in Singapore?

What are the sources of CPE bacterial transmission in Singapore?

What are the sources of CPE plasmid transmission in Singapore?
Pilot analysis (n=33)

<table>
<thead>
<tr>
<th>Genetically-Linked Bacterial Transmission</th>
<th>Number of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetically Distinct</td>
<td>18</td>
</tr>
<tr>
<td>Genetically linked to previous cases</td>
<td>15</td>
</tr>
<tr>
<td>- Hospital contact</td>
<td>6</td>
</tr>
<tr>
<td>- Ward contact</td>
<td>4</td>
</tr>
<tr>
<td>Genetically linked but no known ward or hospital contact</td>
<td>5</td>
</tr>
</tbody>
</table>

- Of 15 genetically-linked bacterial transmissions, 5 (33%) had no apparent ward or hospital contact
- 2 predominant plasmids found (pEC-S01 and pNDM-Sg1)

CaPES WGS transmission study

• Determine the sources of CPE transmission at a population level

• Sub-groups:
  ➢ Determine the sources of genomically-linked *bacterial transmission* at a population level
  ➢ Determine the sources of genomically-linked *plasmid transmission* at a population level
Further analysis

- Compare odds ratio of **genomically-linked bacterial transmission** compared with **genomically-unlinked bacteria** for correlation with various epidemiologic contact scenarios.

- Compare odds ratio of **genomically-linked plasmid transmission** compared with **genomically-unlinked plasmid** for correlation with various epidemiologic contact scenarios.

- **Trend data** of **genomically-linked bacterial transmission** compared with genomically-unlinked bacteria (with subgroup analysis)

- **Trend data** of **genomically-linked plasmid transmission** compared with genomically-unlinked bacteria (with subgroup analysis)
International NDM study

AIMS:

1) Determine the main genomic pathways of NDM spread across borders.

2) Will NDM mimic the successful global genomic spread of the previous Extended-Spectrum-beta-lactamases? e.g. CTX-M-15 – E. coli ST 131 pairing?
Geographic distribution of study isolates

Case = 622 (year 2007 – 2015)

\textit{bla}_{\text{NDM}}\textit{positive Enterobacteriaceae}

Control = 108 (year 2007 – 2015)

\textit{bla}_{\text{NDM}}\textit{negative, ESBL-positive Enterobacteriaceae from same year, same region}
Geographic distribution of NDM-positive Enterobacteriaceae species

- Southeast Asia
- East Asia
- South Asia
- Middle East
- North America
- Europe
K. pneumoniae phylogeny (n=298)

blaCTX-M-15 positive: 222/298 (74.4%)

aac(6’)Ib-cr positive: 234/298 (78.5%)

Limited instances of clonal bacterial transmission:
International clonal transmission clusters
(SNP cutoff ≤ 18):
4 clusters, 22 isolates
**E. coli** phylogeny (n=262)

Main NDM variants:
- **NDM-1** 151/262 (57.6%)
- **NDM-5** 39/262 (14.9%)
- **NDM-7** 10/262 (3.8%)

blaCTX ESBLs:
- **CTX-M-15** 106/262 (40.4%) [clustered in ST131]
- **CTX-M-14** 10/262 (3.8%)
- **CTX-M-27** 16/262 (6.1%)

aac(6')Ib-cr positive:
- **126/262 (48.9%)**

Limited instances of clonal bacterial transmission:
International clonal transmission clusters (SNP cutoff ≤ 19):
- **3 clusters, 31 isolates**
## Genomic arrangements of NDM cassette groups (n=622)

<table>
<thead>
<tr>
<th>CG</th>
<th>No. of isolates (%)</th>
<th>NDM contig core structure</th>
<th>Reference plasmids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>280 (45%)</td>
<td><img src="link" alt="Diagram 1" /></td>
<td>• pNDM-ECS01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• p0801-IMP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• pV323-a</td>
</tr>
<tr>
<td>2</td>
<td>147 (24%)</td>
<td><img src="link" alt="Diagram 2" /></td>
<td>• pNDM-Ec1GN574</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• pNDM1_SZ2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• pNDM-US-2</td>
</tr>
<tr>
<td>3</td>
<td>45 (7%)</td>
<td><img src="link" alt="Diagram 3" /></td>
<td>• pNDM5_WCHEC0215</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• pNDM-WS2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• pAR_0068</td>
</tr>
<tr>
<td>4</td>
<td>33 (5%)</td>
<td><img src="link" alt="Diagram 4" /></td>
<td>• pM218A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• pMC-NDM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• pCC1410-1</td>
</tr>
<tr>
<td>5</td>
<td>49 (8%)</td>
<td><img src="link" alt="Diagram 5" /></td>
<td>• pNDM-HK</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• pNDM-OM</td>
</tr>
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<td></td>
<td></td>
<td>• pM218A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• pEC2-NDM-3</td>
</tr>
</tbody>
</table>

* 68 NDM-positive isolates not assigned
## Distribution of NDM cassette groups (n=622)

<table>
<thead>
<tr>
<th>CG</th>
<th>Species composition</th>
<th>ST coverage</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  (n=280)</td>
<td>E. coli</td>
<td>E. coli</td>
<td>Singapore 278, USA 2</td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae</td>
<td>K. pneumoniae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. cloacae</td>
<td>E. cloacae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other species</td>
<td>Other species</td>
<td></td>
</tr>
<tr>
<td></td>
<td>115</td>
<td>34</td>
<td></td>
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<tr>
<td></td>
<td>84</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>12</td>
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<tr>
<td>2  (n=147)</td>
<td>E. coli</td>
<td>E. coli</td>
<td>Singapore 88, Nepal 31, Vietnam 6, Canada 5, Saudi Arabia 5, China 4, USA 4, UK 2, India 1, Japan 1</td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae</td>
<td>K. pneumoniae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. cloacae</td>
<td>E. cloacae</td>
<td></td>
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<tr>
<td></td>
<td>Other species</td>
<td>Other species</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>111</td>
<td>20</td>
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</tr>
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<td></td>
<td>10</td>
<td>9</td>
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</tr>
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<td></td>
<td>3</td>
<td>3</td>
<td></td>
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<tr>
<td>3  (n=45)</td>
<td>E. coli</td>
<td>E. coli</td>
<td>Singapore 30, USA 7, Saudi Arabia 4, Malaysia 2, Canada 1, UK 1</td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae</td>
<td>K. pneumoniae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. cloacae</td>
<td>E. cloacae</td>
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<tr>
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<td>Other species</td>
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<td>6</td>
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<tr>
<td>4  (n=33)</td>
<td>E. coli</td>
<td>E. coli</td>
<td>Singapore 14, Malaysia 13, USA 2, India 1, Nepal 1, Saudi Arabia 1, UK 1</td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae</td>
<td>K. pneumoniae</td>
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<tr>
<td></td>
<td>E. cloacae</td>
<td>E. cloacae</td>
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<td>Other species</td>
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<td>4</td>
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<td>0</td>
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<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5  (n=49)</td>
<td>E. coli</td>
<td>E. coli</td>
<td>Singapore 43, Nepal 3, Ireland 1, UK 1, USA 1</td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae</td>
<td>K. pneumoniae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. cloacae</td>
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<tr>
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<td>Other species</td>
<td>Other species</td>
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<td></td>
<td>10</td>
<td>7</td>
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<td>7</td>
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<td>4</td>
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</tbody>
</table>

In ST131 C1: NDM introduced also between 2009 to 2011 into CTX-M-27 positive ST131 clone. Does not seem to be as successful in transmission/replication.
Other ongoing studies...

- Outbreak genomics (Singapore, East Malaysia)
- Community household CPE transmission
- Long-term care facility CPE transmission
- Hospital environmental microbiome
- Bench studies (Type VI secretion system, Glycosylated cationic β-peptide block copolymers)
Summary (Epidemiologic)

• Genomically-linked plasmid transmission accounts for a significant amount of CP gene transmission.

• Features of genomically-linked bacterial transmission:
  – Significant proportion no known epidemiologic contact (30%).
  – Close to a third have direct ward contact.
  – A further third have indirect ward contact (suggesting ongoing sources in wards e.g. sinks/ inanimate objects as a source).

• Genomically-linked plasmid transmission different:
  – Majority have direct ward contact.

• WGS can help uncover ongoing transmission routes and help focus/ augment infection prevention efforts.