Laboratory testing for carbapenems resistant Enterobacteriaceae (CRE)

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Layout

• Introduction to carbapenemases producing Enterobacteriaceae
• Referral to Antimicrobial Resistance Reference Laboratory (AMRRL)
• Discussion on diagnostics and screening methods
Carbapenemases in Enterobacteriaceae are mostly plasmid encoded, which largely explains their common association with other resistance markers and their multidrug resistance patterns.

The type of CREs depend on the country, and might be associated with historical/cultural relationships and exchange of populations with other countries of high prevalence. Cross border transfer of patients, travel, medical tourism and refugees might also play an important role.
## Main Carbapenemases: distribution and molecular epidemiology

<table>
<thead>
<tr>
<th>Type of carbapenemases</th>
<th>Geographical spread</th>
<th>Molecular epidemiology</th>
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</thead>
<tbody>
<tr>
<td>NDM</td>
<td><em>K. pneumoniae</em> and <em>E. coli</em> in India Imported to UK firstly and other countries via travel (hospitalization) in India. There have been a cases of cross-infections. In South Africa since 2011.</td>
<td>Widespread in Enterobacteriaceae. Diverse strain type in UK. Plasmid spread among strains and species is more important than clonal spread among patients.</td>
</tr>
<tr>
<td>VIM</td>
<td>Scattered globally, endemic in south Europe (Greece)</td>
<td>Plasmid spread among strains is more important than clonal spread of producer strains.</td>
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<tr>
<td>IMP</td>
<td>Scattered worldwide; no clear associations</td>
<td>Mostly plasmid spread.</td>
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<tr>
<td>GES</td>
<td>GES enzymes have been identified worldwide, with reports from Greece, France, Portugal, South Africa, French Guiana, Brazil, Argentina, Korea, and Japan</td>
<td>The genes encoding the GES family of enzymes are located in integrons on plasmids and were initially classified as extended-spectrum -lactamases. Their hydrolysis spectrum was expanded in 2001 to include imipenem, with the report of GES-2 in a clinical isolate of <em>P. aeruginosa</em> from South Africa.</td>
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</table>
European situation regarding carbapenemase-producing Enterobacteriaceae
NDM-1, new emerging

• NDM-1 enzyme is highly potent to degrade carbapenem antibiotics.
• NDM-1 belongs to the Metallo-β-lactamase (MBL, class B) family containing Zn$^{2+}$ and other divalent cations as cofactors.
• It inactivates almost all classes of β-lactams antibiotics including carbapenems by catalyzing the hydrolytic cleavage of the substrate amide bond.
• On the basis of the protein sequence similarities, three different lineages, named as subclass B1, B2 and B3 have been characterized.
Figure 1. An overview of the blaNDM-1 encoding plasmid, pNDM-HK.

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0017989
Figure 4. Complex models comparison between NDM-1(A), VIM-2(B) and FEZ-1(C).

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0023606
Carbapenemase producers: AMRRL referrals from public and private laboratories

Enterobacteriaceae growth from any specimen

- Interpretation of susceptibility by CLSI
- Susceptibility testing by 10 µg disk: ertapenem, meropenem and imipenem > 23 mm zone diameter
- Susceptibility Testing by MIC (µg/mL): ertapenem ≥1, meropenem and imipenem ≥4
- MBL-Etest or double disk synergy or the Modified Hodge Test (MHT) test positive
- If organism is resistant consider presumptive carbapenemase producers
- Consultation with an infectious disease physician or clinical microbiologist is strongly recommended before initiating treatment

1. Complete Case Report Form (CRF)
2. Advise AMRRRL/NICD
3. Send to AMRRRL/NICD

NICD
Multiplex RT-PCR for KPC, OXA and NDM1
### Case Report Form for CRE referral to NICD

**1. PATIENT DETAILS**

1.1 Surname: ___________ Name/s: ___________

1.2 Hospital Number: ______________________________________

1.3 Occupation: ______________________________________

1.4 Date of birth [ ] Male □ Female □

1.5 Gender: Male □ Female □

| Age: _________ | Both DOB/age unknown: □ |

**2. DETAILS OF CURRENT CONSULTATION/AIDSMISSION**

2.1 Name of clinician: ____________________________

2.2 Healthcare facility name: ____________________________

2.3 Province: ____________________________

2.4 If hospitalised: □ Adult Ward □ Paediatric Ward □ ICU □ Other □ Specify: ____________________________

2.5 Date of admission: [ ] Male □ Female □

| Date of admission | Date started | Duration (days) of treatment |

2.6 Current working diagnosis: ____________________________

2.7 Antibiotics prescribed during this admission? □ Yes □ No □ Unknown □

| Antibiotic Route (po/IV/IM) | Date started | Duration (days) of treatment |

**3. PAST MEDICAL AND TRAVEL HISTORY**

3.1 Underlying illness: □ Yes □ No □ Unknown □

3.2 Travel outside of South Africa in year prior to this admission/consultation: □ Yes □ No □ Unknown □

| Date of travel and return (dd/mm/yyyy) | Country where medical care received: |

3.3 Received medical care in a foreign country during the year prior to this admission/consultation? □ Yes □ No □ Unknown □

3.4 Received medical care in South Africa during the year prior to this admission/consultation? □ Yes □ No □ Unknown □

| Healthcare facility | Reason for admission | Date of admission | Duration of admission |

**4. OUTCOME**

4.1 Outcome known: □ Yes □ No □

| Outcomes: | Discharged □ | Deceased □ |

4.2 CRE categorisation known: □ Yes □ No □

| Details if available: |

4.3 Antibiotic management of CRE known: □ Yes □ No □

| If yes: was CRE treated with antibiotics: □ Yes □ No □ |

| If yes, which antibiotic/s? |

**5. ADDITIONAL COMMENTS**
Genotypic Methods

• DNA Extraction
  – Crude

• Screening of antimicrobial resistance genes by real time PCR
  – The LightCycler 480 (Roche Applied Science, Germany) instrument for the real-time polymerase chain reaction (PCR).
# Multiplex real-time PCR used by AMRRL

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primers and probes</th>
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<tbody>
<tr>
<td><strong>KPC</strong></td>
<td>Forward 5’- GGC CGC CGT GCA ATA C -3’</td>
</tr>
<tr>
<td></td>
<td>Reverse 5’- GCC GCC CAA CTC CTT CA -3’</td>
</tr>
<tr>
<td></td>
<td>KPC Probe 5’- YAK- TGA TAA CGC CGC GCG CAA TTT GT -BBQ -3’</td>
</tr>
<tr>
<td><strong>NDM</strong></td>
<td>Forward 5’- GAC CGC CCA GAT CCT CAA -3’</td>
</tr>
<tr>
<td></td>
<td>Reverse 5’- CGC GAC CGG CAG GTT -3’</td>
</tr>
<tr>
<td></td>
<td>Probe 5’- FAM- TGG ATC AAG CA+GGA+GAT -BBQ -3’</td>
</tr>
<tr>
<td><strong>OXA-48 and its variants</strong></td>
<td>OXA-48 Forward 5’T-TTCGGCCACGGAGCAAATCAG-3</td>
</tr>
<tr>
<td></td>
<td>Reverse 5’T-GATGTGGGCCATATCCATATTCCATCGCA-3’</td>
</tr>
<tr>
<td></td>
<td>Forward variant 5’– gcTggTTAaggATgAACAC-3</td>
</tr>
<tr>
<td></td>
<td>S variant 5’T- CATYTcggCAATgTAGACAg-3</td>
</tr>
<tr>
<td></td>
<td>Probe ’-FAM-CTGGCTGCCTCCGATACGTTAAGG-ATTATTG-BBQ-3’</td>
</tr>
<tr>
<td></td>
<td>Probe (all) 5’-CY5 - CATTggCTTCCgTCAGCATggCT—BBQ-3’</td>
</tr>
<tr>
<td><strong>IMP</strong></td>
<td>IMPgenF1 5’T- GAATAG(A/G)(A/G)TGGCTTAA(C/T)TCTC -3’</td>
</tr>
<tr>
<td></td>
<td>IMPgenR1 5’T- CCAAAC(C/T)ACTA(G/C)GTATC -3’</td>
</tr>
</tbody>
</table>

Reference:
Multiplex Real-Time PCR Detection of Klebsiella pneumoniae Carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM-1)
Testing for CREs from public and private referral laboratories

Number of positive CREs from total of 191 isolates received from 2011-2013

- NDM: 37 positive, 152 negatives, 191 total
- KPC: 5 positive, 184 negatives, 190 total
- IMP: 6 positive, 65 negatives, 71 total
- OXA-48: 8 positive, 106 negatives, 114 total
- OXA-48 like: 6 positive, 56 negatives, 62 total
Number of isolates tested at AMRRL

Total of 191 isolates received from 2011-2013 and distributed per month:

- November 2011: 2
- December 2011: 3
- January 2012: 7
- February 2012: 5
- March 2012: 3
- April 2012: 1
- May 2012: 4
- June 2012: 1
- July 2012: 1
- August 2012: 4
- September 2012: 21
- October 2012: 7
- November 2012: 12
- December 2012: 6
- January 2013: 33
- February 2013: 3
- March 2013: 78

Note: 0 isolates in October 2013.
Evaluation of PCR-based testing for surveillance of CRE

- Culture-based and molecular-based processing of 755 rectal swabs for CRE detection
How To Detect NDM-1 Producers

Patrice Nordmann,1* Laurent Poirel,1 Amélie Carré,1 Mark A. Toleman,2 and Timothy R. Walsh2

Detection of infecting strains

Screening of carriers

Susceptibility testing (disk diffusion, Vitek 2 and other automated systems)

ChromID ESBL (CHROMagar KPC+)

IMP/IMP + EDTA (E-test MBL++)

Molecular identification of carbapenemase genes

Stools or rectal swabs (with or without enrichment in the presence of a carbapenem) should be plated on selective media. The problem is that the level of resistance to carbapenems displayed by carbapenemase producers varies significantly, making their detection difficult unless they show high-level carbapenem resistance [38,39].

FIG. 1. Strategy for identification of NDM-1 producers as a source of clinical infections and for detecting carriers of NDM-1 producers.* This culture medium can be used for surveillance of outbreaks of infections with NDM-1 producers after validation of its detection sensitivity for the specific strain responsible for an outbreak. ** Etest MBL is reliable when the MIC of imipenem is not too low.
Screening of colonized patients

- The first marketed screening medium was the CHROM agar KPC medium, which contains a carbapenem (CHROMagar, Paris France)
  - It detects carbapenem-resistant bacteria only if they exhibit high-level resistance to carbapenems. Its main disadvantage is lack of sensitivity; it does not detect a low level of carbapenem resistance, as observed for several MBL or OXA-48 producers.

- The second screening medium also contains a carbapenem (CRE Brilliance, Thermo Fisher Scientific, UK)
  - It detects KPC and MBL producers well, and most but not all OXA-48 producers.

- Finally, one of the most recently developed screening media (SUPERCARBA) contains cloxacillin, zinc and ertapenem
  - It shows excellent sensitivity and specificity for detection of any kind of carbapenemase producer (not only high-level carbapenem-resistant isolates).
  - Compared with the two other media, it shows improved sensitivity and specificity for detecting all types of carbapenemase producers (including the OXA-48 producers) when present in low amounts in stools. Once carbapenem-resistant isolates are selected on SUPERCARBA medium, we recommend use of the Carba NP test for detecting carbapenemase activity. If needed, molecular identification of the carbapenemase genes may be performed.
Non-molecular tests for carbapenemases production

• Some have good sensitivity and specificity but none 100%,
  – Modified Hodge Test-low sensitivity and specificity and time consuming,
  – MALDI-TOF – detection of CREs,
  – Carba NP test-in vitro hydrolysis of the carbapenems and change the pH value of the indicator.
Conclusions

• Standardized methods for screening and identifications are needed in South Africa.
• Evidence based methods should be introduced.
• Evaluation of new methods or technique should be performed before developing diagnostic algorithms.
Thank you for your attention!