Detecting CRE
(Carbapenem-resistant Enterobacteriaceae)
what does one need to do?

Dr Nizam Damani
Associate Medical Director
Infection Prevention and Control
Southern Health & Social Care Trust, Portadown, UK
Senior Lecturer, Queen’s University, Belfast, UK
Evolution of microbes vs human beings
Evolution of *Gram negative* bacteria

Evolution of human being

- **Apes**
- **Homo erectus**
- **Homo sapiens**
- **McDonald Man**
Novel Antimicrobial Development

8 years & $800,000,000 to develop a novel drug

No novel anti-GNB agent developed since 1960s

‘Blockbuster’ drugs/billion dollar sales

Finch R & Hunter PA. JAC, 2006; 58 (Suppl. S1): i3-i22.
‘The development of new antibiotics without having mechanisms to insure their appropriate use is much like supplying your alcoholic patients with a finer brandy’.

Dennis Maki 1998
Multi-resistant Gram-negative

Extended-spectrum β-lactamases (ESBL)

Resistant to all β lactam & cephalosporin antibiotics ± others
Often treated with Carbapenem (meropenem, ertapenem etc.)

Carbapenem-resistant Enterobacteriaceae (CRE)

Resistant to Carbapenems and all other groups of antibiotics
Require treatment with IV Colistin
Definition of CRE

- **Enterobacteriaceae** that produce any β-lactamase (carbapenemase) that hydrolyses carbapenems (*any or all of ertapenem, doripenem, imipenem and meropenem*) and are *resistant* to all of the following **third-generation cephalosporins** i.e. ceftriaxone, cefotaxime, and ceftazidime.

- **CPE**: Carbapenem-producing **Enterobacteriaceae**

- **CRE**: Carbapenem-resistant **Enterobacteriaceae**

**CDC Tool kit. Guidance for Control of Carbapenem-resistant Enterobacteriaceae. Atlanta: CC, 2012.**

**UK Health Protection Agency. Laboratory detection and reporting of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases). London: Health Protection Agency, 2013.**
# Carbapenemases

<table>
<thead>
<tr>
<th>Classification</th>
<th>Enzyme</th>
<th>Most Common Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class A</td>
<td>KPC, SME, IMI, NMC-A, GES, PER, SFO, IBC</td>
<td>Enterobacteriaceae (rare reports in <em>P. aeruginosa</em> USA, Greece, Italy, Israel and China, and are increasingly encountered elsewhere, including the UK)</td>
</tr>
<tr>
<td>Class B</td>
<td>NDM, VIM, IMP, GIM, SIM, and SPM</td>
<td><em>P. aeruginosa</em> Enterobacteriaceae Acinetobacter spp.</td>
</tr>
<tr>
<td>Class D</td>
<td>OXA, PSE</td>
<td>Acinetobacter spp. Enterobacteriaceae (OXA-48) <em>Pseudomonas spp.</em> (OXA-198)</td>
</tr>
</tbody>
</table>

*Int J Anti Ag* 2010; 36: 205-210
The ‘Big 5’ Carbapenemases

KPC  VIM  IMP  NDM  OXA-48

Courtesy: © Crown copyright (Public Health England)
### Multi-resistant gram-negative bacteria

#### Carbapenem-resistant Enterobacteriaceae (CRE)

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Geographic distribution</th>
<th>Molecular epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NDM</strong> (New Delhi metallo beta lactum)</td>
<td>Widespread in Enterobacteriaceae (esp. K. pneumoniae and E. coli)</td>
<td>Indian sub-continent</td>
</tr>
<tr>
<td><strong>VIM</strong></td>
<td>Mostly <em>K. pneumoniae</em></td>
<td>Greece</td>
</tr>
<tr>
<td><strong>IMP</strong></td>
<td>Scattered worldwide; no clear associations</td>
<td></td>
</tr>
<tr>
<td><strong>KPC</strong></td>
<td>K. pneumoniae, occasionally other Enterobacteriaceae</td>
<td>USA since 1999. Israel and Greece; outbreaks elsewhere in Europe</td>
</tr>
<tr>
<td><strong>OXA 48</strong></td>
<td>Widespread <em>K. pneumoniae</em></td>
<td>Turkey, Mid-East and N. Africa</td>
</tr>
</tbody>
</table>

From: UK Health Protection Agency
Carbapenem Resistant Enterobacteriaceae (CRE)

- Many acquired carbapenemases are **plasmid-mediated** (especially when found in Enterobacteriaceae), giving potential for **spread** between strains, species and genera.
- β lactamase producer with 2nd mechanism (e.g. **impermeability due to reduced porin expression**); **not readily transferable**

Loss of/ altered porin = no carbapenem entry to organism
# Mechanisms of Carbapenem Resistance

<table>
<thead>
<tr>
<th>Family</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>Cephalosporinase + Porin loss</td>
</tr>
<tr>
<td></td>
<td>Carbapenemase</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Porin loss</td>
</tr>
<tr>
<td></td>
<td>Up-regulated efflux</td>
</tr>
<tr>
<td></td>
<td>Carbapenemase</td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp.</td>
<td>Cephalosporinase + Porin loss</td>
</tr>
<tr>
<td></td>
<td>Carbapenemase</td>
</tr>
</tbody>
</table>
Carbapenem-resistance in Gram negative bacteria

- Carbapenemases are intrinsic (found naturally) in a few clinical bacteria, such as Stenotrophomonas maltophilia, Aeromonas spp., and ‘chryseobacteria’, including Elizabethkingia meningoseptica.
- Non-susceptibility or resistance to specific carbapenems is an intrinsic characteristic of some Gram negative bacteria: most non-fermenters are naturally resistant to ertapenem (but not to other carbapenems); Serratia spp. and Proteeeae have intrinsically poor susceptibility or low-level resistance to imipenem.
Role of Laboratory in detection of CRE
Laboratory in detection of CRE
Laboratory Protocol for Detection of Carbapenem-Resistant or Carbapenemase-Producing, *Klebsiella* spp. and *E. coli* from Rectal Swabs

**Purpose**
To identify patients colonized with carbapenem-resistant or carbapenemase-producing Enterobacteriaceae in the intestinal tract. Patients who grow these organisms should be placed on Contact Precautions (5) to prevent transmission of the resistant bacteria. The procedure described below is a modification of the procedure described by Landman et al. (4). See the procedural notes for steps in the procedure which can be modified.

**Background**
Carbapenem-resistant Enterobacteriaceae (CRE) are usually resistant to all β-lactam agents as well as most other classes of antimicrobial agents. The treatment options for patients infected with CRE are very limited. Healthcare-associated outbreaks of CRE have been reported. Patients colonized with CRE are

http://www.cdc.gov/hai/pdfs/labsettings/klebsiella_or_ecoli.pdf

**Review**
Detection, treatment, and prevention of carbapenemase-producing *Enterobacteriaceae*: Recommendations from and International Working Group

Gabriel Levy Hara¹, Ian Gould², Andrea Endimiani³, Pilar Ramón Pardo⁴, George Daikos⁵, Po-Ren Hsueh⁶, Shaheen Mehta⁷, George Petrikos⁸, José María Casellas⁹, Lucía Daciuk¹⁰, Daniela Paciel¹¹, Andrea Novelli¹², Raphael Saginur¹³, Daniel Pryluba¹⁴, Julio Medina¹⁵, Eduardo Savio¹⁶

*Journal of Chemotherapy* 2013

DOI 10.1179/1973947812Y.0000000062
Screening specimen for CRE

- *Enterobacteriaceae* are normal GIT flora
- **Screening swab**: Stool or rectal (perinal swab) sample
Infections caused by multidrug-resistant gram negative organisms

- **Blood stream infections**
  (esp. immunocompromised patients)

- **GIT infections**
  Post surgical infections
  Intra abdominal sepsis

- **Skin colonization**

- **Additional specimens** are required if patient is clinically unwell:
  Catheter specimen of urine, wound swab, blood culture etc.

- **Urinary tract infections**
  (colonization/infections)
Role of Laboratory in detecting CRE

- Advise on collection of appropriate specimens
- Provide appropriate *sterile* containers to prevent specimen been contaminated from environmental gram negative bacteria
Stool specimen wrapped in toilet tissue paper!
Stool specimen without any container!
Stool specimen in Tesco plastic bag!
Role of Laboratory in detecting CRE

• Advise on accurate filling of form
  – Clinical details: Clinical vs screening specimen
  – Current Antibiotic therapy
  – Transfer from other hospital and/or country etc…
<table>
<thead>
<tr>
<th>Field</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital &amp; Ward/Health Centre</td>
<td></td>
</tr>
<tr>
<td>G.P./Consultant</td>
<td></td>
</tr>
<tr>
<td>G.P./Cytology</td>
<td></td>
</tr>
<tr>
<td>Type of Specimen</td>
<td>Faeces</td>
</tr>
<tr>
<td>Site of Specimen</td>
<td>Arse</td>
</tr>
<tr>
<td>Request</td>
<td>O.S. Meak</td>
</tr>
<tr>
<td>Symptoms/Clinical Details</td>
<td>Morbid Stool 18hrs</td>
</tr>
<tr>
<td></td>
<td>Menaeces 6hrs No Fever</td>
</tr>
<tr>
<td>Antibiotic Therapy</td>
<td></td>
</tr>
<tr>
<td>Date of Specimen</td>
<td>6/6/01</td>
</tr>
<tr>
<td>Time</td>
<td>AM</td>
</tr>
<tr>
<td>Date received</td>
<td></td>
</tr>
<tr>
<td>Lab. Ref. No.</td>
<td></td>
</tr>
</tbody>
</table>

Date of Birth: 1/1
Hospital Unit No.: 1/1
Sex: F

2001-1 THU 07-06-01

FC
Specimen Swabs & Containers
Role of Laboratory in detecting CRE

**DAY 1**
Screen patient
Set up in lab in broth

**DAY 2**
Plate & Re-incubate

**DAY 3**
Susceptibility testing results
? CRE
Confirmatory tests

**DAY 4**
Further MICs available
Send to reference laboratory
Direct plating (+ enrichment in broth) onto MacConkey with carbapenem disks

**Chromogenic agars**

- Brilliance CRE
- CHROMagar KPC
- ChromID Carba
- ChromID OXA-48
- ChromID Carba Smart*
- Colorex chromogenic KPC
- Expensive
- Not all are suitable for all of ‘big five’ carbapenemases
  - May need two agars for maximum sensitivity
- There will be problematic strains

Courtesy: Neil Woodford, © Crown copyright (Public Health England)
Problem with spotting the carbapenemase producers

Enterobacteriaceae with ESBL or AmpC enzymes may lose outer membrane porins (through mutations or other disruptions in chromosomal genes), reducing carbapenem uptake.

- Human experts, subjective: computer algorithms, poor specificity
- ‘relative ease’: \( E. coli > Klebsiella \) spp. \( >> \) \( Enterobacter \) spp.
- High index of suspicion; supplemental tests, locally or in Ref. Lab.

*Laboratory Detection and Reporting of Bacteria with Carbapenem-Hydrolysing \( \beta \)-lactamases (Carbapenemases). London: Public Health England, 2014*
Problem with spotting the carbapenemase producers

- **When seeking carbapenemases**, clinical laboratories should have a high index of suspicion and **be alert to two confounders**:
  - *Not all carbapenem-resistant isolates produce a carbapenemase* (resistance can be mediated by other mechanisms, such as the combination of ESBL/AmpC plus impermeability)
  - *Not all carbapenemase producers are resistant to carbapenems*

Problem with spotting the carbapenemase producers

• The ideal indicator carbapenem is one to which all carbapenemases confer resistance, even when production is scanty.
• No single carbapenem satisfies this criterion for all host species (Enterobacteriaceae and non-fermenters)
• As a general principle, frontline diagnostic methods must have high sensitivity (ability to detect carbapenem resistance), even at the expense of specificity (ability to distinguish true carbapenemase producers)

Problem with spotting the carbapenemase producers (Enterobacteriaceae)

• Test a carbapenem against all clinically-significant isolates
• Do carbapenemase confirmatory tests on isolates found resistant to the indicator carbapenem
• Identification to genus/species level is highly desirable for the interpretation of resistance patterns.
• Identify all isolates found resistant to the indicator carbapenem

Problem with spotting the carbapenemase producers (Non-fermenters)

- Acquired carbapenemases are also encountered in *Acinetobacter* sp, *Pseudomonas* spp. (most commonly, though not exclusively in *P. aeruginosa*) and in other non-fermenters
- Test *imipenem, meropenem or doripenem* against all clinically-significant isolates. *Do not use ertapenem* because these species are intrinsically resistant to this carbapenem
Antibiotic susceptibility testing

• Qualitative methods (S/I/R)
  – Disc diffusion
  – Agar incorporation breakpoint method

• Quantitative methods (MIC)
  – Agar or broth dilution
  – Gradient e.g. E-test

• Automated methods (Vitek, Phoenix, Microscan)
  – Automated systems should flag non-susceptibility to any carbapenem, irrespective of the expert interpretation
CLSI and EUCAST criteria for interpretation of susceptibility testing of carbapenems in Enterobacteriaceae

<table>
<thead>
<tr>
<th>Carbapenem</th>
<th>Criteria</th>
<th>MIC (mg/l)</th>
<th>Disk diffusion (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Imipenem</td>
<td>CLSI-2012</td>
<td>≤1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>EUCAST-2012</td>
<td>≤2</td>
<td>4–8</td>
</tr>
<tr>
<td>Meropenem</td>
<td>CLSI-2012</td>
<td>≤1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>EUCAST-2012</td>
<td>≤2</td>
<td>4–8</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>CLSI-2012</td>
<td>≤0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>EUCAST-2012</td>
<td>≤0.5</td>
<td>1</td>
</tr>
<tr>
<td>Doripenem</td>
<td>CLSI-2012</td>
<td>≤1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>EUCAST-2012</td>
<td>≤1</td>
<td>2–4</td>
</tr>
</tbody>
</table>

Note: S, susceptible; I, intermediate; R, resistant.

a CLSI document M100, S22 2012; EUCAST document 2.0–2012.
Proposed EUCAST screening cut-off values for possible Carbapenemase-producing Enterobacteriaceae*

<table>
<thead>
<tr>
<th>Carbapenem</th>
<th>MIC (mg/L)</th>
<th>Zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>&gt;0.125</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;1</td>
<td>&lt;23</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>&gt;0.125</td>
<td>&lt;25</td>
</tr>
</tbody>
</table>

Confirmatory Test: Modified Hodge Test

Modified Hodge Test for Carbapenemase Detection in *Enterobacteriaceae*

**Background**
The Modified Hodge Test (MHT) detects carbapenemase production in isolates of *Enterobacteriaceae*. In the United States, the most common carbapenemase found in *Enterobacteriaceae* is the *Klebsiella pneumoniae* carbapenemase (KPC). Other carbapenemase, like the metallo β lactamase (MBL) and the SME-1 in *Serratia marcescens*, can also produce a positive MHT, but are found infrequently in the United States.

**Purpose**
Carbapenemase production is detected by the MHT when the test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (E.coli ATCC 25922) towards a carbapenem disk. The result is a characteristic cloverleaf-like indentation. See Figure 1.

Modified Hodge test

Method

• Inoculate MH agar with a 1:10 dilution of a 0.5 McFarland suspension of *E. coli* ATCC 25922 and streak for confluent growth using a swab.

• **Place 10-μg ertapenem or meropenem (best) disk in centre**

• Streak each test isolate from disk to edge of plate

• **Isolate A** is a KPC producer and positive by the modified Hodge test.

Modified Hodge test

• The modified Hodge test (MHT) is a generic phenotypic test that can be useful to demonstrate the production of carbapenemase enzymes

• Limitations:
  – Time consuming
  – Lack specificity (e.g. false positive strains when ESBL or pAmpC are associated to porin loss)
  – Lack sensitivity (e.g. weak detection of NDM and VIM production)
## Rosco Diagnostica

Kit for detection of the carbapenemases; KPC, MBL and OXA-48 in Enterobacteriaceae

<table>
<thead>
<tr>
<th></th>
<th>Meropenem + Phenylboronic MRPBO</th>
<th>Meropenem + DPA MRPDP</th>
<th>Meropenem + Cloxacillin MRPCX</th>
<th>Temocilin 30 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmpC + porin loss</td>
<td>Meropenem 10 µg MRP10</td>
<td>≥ 4mm and</td>
<td>≤ 3 mm</td>
<td>≥ 5mm</td>
</tr>
<tr>
<td>ESBL + porin loss (a)</td>
<td>Meropenem 10 µg MRP10</td>
<td>≤ 3 mm</td>
<td>≤ 3 mm</td>
<td>≤ 3 mm</td>
</tr>
<tr>
<td>KPC</td>
<td>Meropenem 10 µg MRP10</td>
<td>≥ 4mm</td>
<td>≤ 3 mm</td>
<td>≤ 3 mm</td>
</tr>
<tr>
<td></td>
<td>Meropenem + Cloxacillin (MRPCX)</td>
<td>≥ 4mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MβL</td>
<td>Meropenem 10 µg MRP10</td>
<td>&lt; 4mm</td>
<td>≥ 5mm</td>
<td>≤ 3 mm</td>
</tr>
<tr>
<td>OXA-48 and similars</td>
<td>Meropenem 10 ug MRP10</td>
<td>≤ 3 mm</td>
<td>≤ 3 mm</td>
<td>≤ 3 mm</td>
</tr>
<tr>
<td>OXA-48 + ESBL (a)</td>
<td>Meropenem 10 ug MRP10</td>
<td>≤ 3 mm</td>
<td>≤ 3 mm</td>
<td>≤ 3 mm</td>
</tr>
</tbody>
</table>

(a) : synergism CAZ / Clavulanate.
Laboratory Detection of Carbapenemases

- **Molecular method**
  - Block-based or real-time PCR assays
  - *Only reliable means* of detecting production of multiple carbapenemases by an isolate

- **Limitations**
  - Expensive
  - Required trained personnel
  - Inability to detect any novel carbapenemase gene
Send strain to Reference Laboratory for confirmation

- If isolate is Intermediate or Resistant to carbapenem (imipenem, meropenem, ertapenem, doripenem)
- Resistant to 3rd generation cephalosporin
- Modified Hodge Test (or Rosco disc test): Positive
- Send to Reference Lab for confirmation and enzyme detection using molecular methods
Conclusions

• Laboratory must have written Standard Operating Procedure for screening and detection of CRE
• Accurate identification of bacteria to genus or species level is important
• Perform disk testing first and perform MIC against carbapenem on all suspected strains
• Depending on the method (e.g. CLSI, EUCAST), use recommended ATCC or NTCC Quality Control strain of recommended bacteria. Use both negative and positive control
• Perform Quality Control of media if prepared in your Lab. Buy media from good supplier
• Send CRE strain to local Reference Laboratory for confirmation, if available; otherwise consider sending strain to the Reference Laboratory in other county
Thank you

I think about CRE therefore I worry!