Carbapenemases in Enterobacteriaceae

September 2010
Has the Era of Untreatable Infections Arrived?

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Gram positives

• Therapeutic options available
  – Daptomycin, linezolid, tigecycline

Gram negatives

• ESBLs
• AmpC cephalosporinases
• Quinolone resistance
• Carbapenemases
  – No /limited option
Carbapenems:

- Broadest antibacterial spectrum
- Stability against most Class A, C and D enzymes
- Affinity for PBPs:
  - both Gram positives / Gram negative organisms
- Ability to act as beta-lactamase inhibitors
  - very slow hydrolysis by beta-lactamases
Class A Carbapenemases

- Chromosomal
  - < 40 isolates (in past 20 years)
  - *S. marcescens* (SME-1/2), *Enterobacter spp* (IMI-1, NMC-A)
- Integron / Plasmid encoded
  - Rapidly growing group
  - KPC, GES and OXA type enzymes
KPC enzymes

• Confer high level resistance to carbapenems

• Difficult to detect in the laboratory

• First described in *K. pneumoniae*
  – 45% homology with SME-1 chromosomal enzyme of *Serratia marcescens*
KPC enzymes

- **KPC-1**
  - Inhibited by BLI but susceptibility tests do not demonstrate this
    - MICs ↑ for amox/clav and pip/tazo

- **KPC-2**
  - will show positive ESBL confirmatory tests

- **KPC-3**
  - associated with reduced outer membrane permeability (OmpK35) and with aminoglycoside AAC(6’) and quinolone resistance
  - high mortality rate
  - tygecycline or colistin + rifampin
KPC enzymes

- **Worldwide spread**
  - Seven different subtypes (KPC 2-8)
  - Widespread in China, Israel, Greece, South America, USA

- **Hospital acquired infections**
  - Rapid transfer of resistance (similar to ESBLs)
  - Interspecies transfer within institutions (transposon)

- **Susceptibility breakpoints may not detect**
  - Need to rely on decreased susceptibility
    - Ertapenem screen

- **Often multi/pan resistant**
  - Other beta-lactamases,
  - Plasmid mediated quinolone / aminoglycoside resistance
GES enzymes

- First classified as ESBLs
- Hydrolyze imipenem
- Many GES variants
  - (GES-2, GES-4, GES-5, GES-6- carbapenemase activity
- Initially in Pseudomonas but now Enterobacteriaceae
- Single occurrence but also small outbreaks
- Inhibited by clavulanic acid
OXA enzymes

Class D Beta-Lactamases

- Penicillinases that hydrolyze cloxacillin
- Poorly inhibited by clavulanic acid and EDTA
- > 100 different sequences
  - > 10 - ESBL
  - > 35 - carbapenemases
OXA carbapenemases

- First described in *Acinetobacter spp*

- Hydrolyze imipenem slowly
  - Depends if chromosomal or plasmid
  - Contribution by efflux and porin mutations

- Some OXA enzymes may not hydrolyze 3\textsuperscript{rd}/4\textsuperscript{th} generation cephalosporins

- Still rare in Enterobacteriaceae
Metalloenzymes

- Zinc based hydrolytic mechanism
- Not inhibited by clavulanic acid/tazobactam
- Not always detected by nitrocefin
Metalloenzymes

Ubiquitous in several species

- *Aeromonas, Stenotrophomonas, Bacillus spp*
  - Chromosomal
  - often found in association with another serine beta-lactamase
    - both enzymes inducible after exposure to BL antibiotics
  - not easily transferrable
Metalloenzymes

Dramatic increase in acquired / transferrable metalloenzymes

- VIM, IMP, GIM, SIM, SPM, NDM-1
  - Resistant to penicillins, cephalosporins, carbapenems
  - Susceptible to aztreonam
  - Inhibited by EDTA
  - Not inhibited by clavulanic acid
- **IMP**
  - 1st detected in *P. aeruginosa* and *B. fragilis*
  - Integron – *S. marcescens* and other Enterobacteriaceae
- **VIM**
  - most common
  - Class 1 integron
- **SPM**
  - not integron
  - transposable structure with recombinase and promoter sequences (integrions embedded in transposons)
- **GIM**
  - homology 30% VIM, 43% IMP, 29% SPM
- **SIM**
  - *Pseudomonas spp* and *Acinetobacter spp*
- **NDM-1**
  - Plasmid mediated, highly transferrable
  - India/Pakistan/Bangladesh
  - Pan resistance
IMP and VIM:
- worldwide spread
- 1st case in USA (Klebsiella) - September 2010
  - hospitalized in Greece

SPM, GIM, SIM:
- not spread beyond country of origin

NDM-1:
- potential for worldwide spread
  - Australia/ France / Japan / Kenya / North America / Singapore / Taiwan / UK
  - UK - National Resistance Alert 3
  - Issues around medical tourism
  - Several cases Canada / USA
- pan resistance - no treatment
Carbapenemases

Detection
- Modified Hodge Test
- Molecular Diagnosis

- KPC enzymes
  - inhibited by clavulanate
  - imipenem and meropenem (10µg) discs 10, 15, 20 mm from amox/clac (30µg) disc

- OXA enzymes
  - inhibited by NaCl (25%)
  - not clinically applicable yet??

- Metalloenzymes
  - inhibited by EDTA
  - 4µL of 0.5M EDTA to imipenem and ceftazidime disc
  - IMI/IM Etest – not as reliable for Enterobacteriaceae
Klebsiella pneumoniae
2nd isolate
Cefoxitin R, ESBL -
Cefotetan +/- boronic acid

Klebsiella pneumoniae

2nd isolate

Amp C +
Ertapenem/Imipenem/Meropenem R

Klebsiella pneumoniae

2nd isolate
## Carbapenem breakpoints

<table>
<thead>
<tr>
<th></th>
<th>CLSI</th>
<th>EUCAST</th>
<th>ECOFF&lt;sup&gt;≤&lt;/sup&gt;*</th>
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<tbody>
<tr>
<td></td>
<td>S ≤ R&gt;</td>
<td>S</td>
<td>R&gt;</td>
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<tr>
<td>Imipenem</td>
<td>4</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Meropenem</td>
<td>4</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>2</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Doripenem</td>
<td>ND</td>
<td>1</td>
<td>4</td>
</tr>
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Meropenem MIC: KPC- and MBL-isolates

CLSI 4 / 8
EUCAST 2 / 8

WT

No isolates

MIC

0.032 0.064 0.125 0.5 1 2 4 8 16 >=32

MP KPC

MP MBL
### Carbapenem breakpoints

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<tbody>
<tr>
<td>Imipenem</td>
<td>S ≤ 4</td>
<td>S ≤ 2</td>
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<tr>
<td></td>
<td>R &gt; 8</td>
<td>R &gt; 8</td>
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<tr>
<td>Meropenem</td>
<td>S ≤ 4</td>
<td>S ≤ 2</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td>R &gt; 8</td>
<td>R &gt; 8</td>
<td></td>
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<tr>
<td>Ertapenem</td>
<td>S ≤ 2</td>
<td>S ≤ 0.5</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>R &gt; 4</td>
<td>R &gt; 1</td>
<td></td>
</tr>
<tr>
<td>Doripenem</td>
<td>ND</td>
<td>ND</td>
<td>0.125</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th></th>
<th>CLSI 2010 (?)</th>
<th>EUCAST (review 2009)</th>
<th>ECOFF ≤*</th>
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<tbody>
<tr>
<td>Imipenem</td>
<td>1 ≤</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1 ≤</td>
<td>2</td>
<td>0.125</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0.25 ≤</td>
<td>0.5</td>
<td>0.06</td>
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<tr>
<td>Doripenem</td>
<td>1 ≤</td>
<td>2</td>
<td>0.125</td>
</tr>
</tbody>
</table>
Detection of Beta Lactam Resistance based on Ertapenem Resistance

ETP

\[ \leq 1 \mu g/mL \]
- Interpret:
  - ETP = S
  - MEM = S
  - IPM = S
- Report as per Susceptibility Reporting Chart

\[ \geq 2 \mu g/mL \]
- IPM Etest
- MEM Etest
- Modified Hodge test
  - Modified Hodge test
  - Consult microbiologist

Probable Carbapenemase
Comment 13

Send to Reference Laboratory

ESBL Present

\[ \text{ESBL + impermeability (Chromosomal or plasmid)} \]
- Amp C present
  - Amp C + impermeability
  - Undetermined mechanism. Consult microbiologist
  - Comment 15

ESBL + impermeability
Comment 15

Amp C present

Impermeability to Ertapenem*

Consult microbiologist

IPM \geq 2 \mu g/mL or MEM \geq 1 \mu g/mL

* Permeability mutations are more common in Klebsiella spp. and Enterobacter spp. In Klebsiella spp., ETP and MEM are affected more than IPM whereas in Enterobacter spp. ETP and IPM are affected more than MEM. Additionally, rare strains of chromosomal carbapenemases (SME, IMI-1, and NMC-A) have been found in Enterobacter spp. and Serratia spp., where carbapenemases are resistant but 3rd generation cephalosporins test susceptible. Serratia fonticola may possess an inducible chromosomal carbapenemase resulting in resistance to carbapenems and aztreonam, but not 3rd generation cephalosporins (especially ceftazidime). Note: For all carbapenemase producers, cephalosporins should be considered resistant.
Managing the Impact of Carbapenemases

Antimicrobial Stewardship
- Minimizing use of carbapenems
- Optimizing use of carbapenems
  - Correct dose and duration
- Prospective audits and feedback

Preventing Infection

Controlling Environmental Spread
- Surveillance
- Role of screening??
  - Imipenem/ertapenem discs on MaConkey
- Laboratory reporting
- Prioritizing ARO management