Identifying CRE Infections and Interpreting CRE Lab Results

Infection Prevention Symposium:
Carbapenem-Resistant Enterobacteriaceae (CRE)
Philadelphia, PA

October 30, 2013

Alan T. Evangelista, PhD, D(ABMM)
Director of Microbiology, Virology, Molecular Diagnostics
St. Christopher’s Hospital for Children, Phila, PA
Drexel University College of Medicine, Phila, PA
Objectives

- Review mechanisms of multidrug and carbapenem resistance among Enterobacteriaceae
- Review molecular types of carbapenemases, global distribution, prominent types in US and PA
- Discuss appropriate diagnostic lab tests (advantages & limitations) for the detection of carbapenemases in clinical samples and in surveillance samples
  - Commercial MIC testing systems: automated & manual
  - Utilization of Modified Hodge Test
  - Phenotypic inhibiton tests for carbapenemases
  - Screening media: ertapenem broth screen, chromogenic agar
- Explain how to interpret and report CRE results
Question #1

What are the major mechanisms of antimicrobial resistance and specifically carbapenem resistance among Enterobacteriaceae?
Mechanisms of Antimicrobial Resistance Among Gram-Negative Pathogens

I. Reduced Drug Accumulation
• Down-reg outer membr porin prot (OmpF35 – carb-R w AmpC in K pneumoniae)\(^a\)
• Upreg of efflux pumps (AcrAB-ToI1C – cefurox-R in E coli)\(^b\)

II. Inactivating Enzymes
• Extended spectrum beta-lactamases (SHV-5 – 3g cephal-R in K pneumoniae)\(^c\)
• AmpC cephalosporinases (CMY-1 (pl) – 3g cephal-R in K pneumoniae & E coli)\(^c\)
• Carbapenemases (KPC-2 (plasmid) – pen, cephal, carb-R in K pneumoniae)\(^c\)
  (SME-3 (chromosomal) – S marcescens)\(^d\)

III. Alteration of Drug Targets
• Altered penicillin-binding protein (carb-R in P mirabilis)\(^e\)
• Modif PO\(_4\) grp of lipid A (colistin/polymyxin -R in E coli & P aeruginosa)\(^a,e,f\)

---

Gram-Negative Bacterial Cell Membranes

Outer Membrane

Porin Channels

β-Lactam

Penicillin Binding Protein

Inner Membrane

β-Lactamase

## Carbapenem Beta-Lactamases & Carb-R

<table>
<thead>
<tr>
<th>Class</th>
<th>Common β-lactamase type</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>β-lactamase w serine at active site (KPC 2, 3, 4) also TEM, SHV, CTX-M ESBLs + porin loss</td>
<td><em>Klebsiella, E coli, Enterobacter</em></td>
</tr>
<tr>
<td>B</td>
<td>metallo-β-lactamase (Zinc at active site)(VIM, IMP, NDM-1)</td>
<td><em>Klebsiella, Acinetobacter</em></td>
</tr>
<tr>
<td>C</td>
<td>AmpC-like + porin loss (plasmid: CMY, FOX, DHA)</td>
<td><em>Klebsiella, E coli, Enterobacter</em></td>
</tr>
<tr>
<td>D</td>
<td>Oxacillinase (OXA-48)</td>
<td><em>Klebsiella</em></td>
</tr>
</tbody>
</table>

Question #2

What is the global distribution of carbapenemase-producing Enterobacteriaceae (CRE) and which enzymes are found in isolates from the US and from Pennsylvania?
Geographic Locations of Carbapenemases

<table>
<thead>
<tr>
<th>Class: enzyme</th>
<th>Common Locations</th>
<th>Types identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: KPC</td>
<td>Israel, Greece, Europe, US (44 st), PR, S America, India, Eastern China</td>
<td>KPC 2-15</td>
</tr>
<tr>
<td>B: NDM</td>
<td>India, Pakistan, Europe, China, US (11 st)</td>
<td>NDM 1-10</td>
</tr>
<tr>
<td>B: VIM</td>
<td>Turkey, Greece, Europe, US (2 st)</td>
<td>VIM 1-39</td>
</tr>
<tr>
<td>B: IMP</td>
<td>Japan, Taiwan, Singapore, Australia, US (CA)</td>
<td>IMP 1-45</td>
</tr>
<tr>
<td>D: OXA</td>
<td>Turkey, W Europe, N Africa, India, US (5 st)</td>
<td>OXA 1-364</td>
</tr>
</tbody>
</table>

Geographic Distribution of KPC Carbapenemases

States Reporting KPC Carbapenemases

This map was last updated on September 9, 2013

www.cdc.gov
Regional Ceftazidime-Nonsusceptible Phenotypes of *E coli* and *K pneumoniae*

Ceftazidime-NS defined as an MIC of $\geq 16$ μg/mL; CLSI M100-S19, Jan 2009.
Question #3

What are the revised carbapenem MIC breakpoints (CLSI 2010) and how do the carbapenems differ in activity and by test method?
Determination of MIC Breakpoints

1. MIC Distribution of Isolates
   ▪ Large number (~500) clinically relevant isolates
   ▪ Varying suscep, geographically diverse areas

2. Pharmacokinetic & dynamic Criteria (PK/PD)
   ▪ Dosing regimens, achievable drug levels in vivo; percent target attainment in population studies
   ▪ Conc-dep killing: AUC/MIC ratio (FQs); 24h area under serum conc time curve to MIC ratio
   ▪ **Time-dep killing:** T>MIC (β-lactams); time that serum drug levels are above MIC

3. Clinical Outcome
   ▪ correlation of MIC to clinical success
   ▪ bacteriologic eradication in treated patients
Enterobacteriaceae: Revised CLSI Antimicrobial Breakpoints (MIC $\mu$g/mL)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susc</td>
<td>Int</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>$\leq 8$</td>
<td>16</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>$\leq 8$</td>
<td>16-32</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>$\leq 8$</td>
<td>16-32</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>$\leq 8$</td>
<td>16</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>$\leq 8$</td>
<td>16</td>
</tr>
<tr>
<td>Cefepime$^b$</td>
<td>$\leq 8$</td>
<td>16</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>$\leq 2$</td>
<td>4</td>
</tr>
<tr>
<td>Imipenem</td>
<td>$\leq 4$</td>
<td>8</td>
</tr>
<tr>
<td>Meropenem</td>
<td>$\leq 4$</td>
<td>8</td>
</tr>
<tr>
<td>Doripenem</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

$^a$: CLSI M100-S20. Table 2A. Jan 2010. 
$^b$: approved at CLSI AST Jun 2013, for M100-S24 Jan 2014; 
$^c$: FEP dosage previously based on 1g every 8h or 2g every 12h. 
$^c$: CLSI M100-S22. Jan 2012
Pharmacodynamic Properties of Beta-Lactams

<table>
<thead>
<tr>
<th>Antimicrobial Class</th>
<th>Cidal end pt&lt;sup&gt;a&lt;/sup&gt; (% of dosing interv)</th>
<th>hrs above MIC for q8h dosing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporins</td>
<td>60-70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6h</td>
</tr>
<tr>
<td>Penicillins</td>
<td>50-60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8h</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>35-40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2h</td>
</tr>
</tbody>
</table>

<sup>a</sup>: generally considered a 3-log reduction in colony forming units  
<sup>b</sup>: percent of dosing interval required for free drug concentration to be above MIC (%T>MIC)

Extended Infusion Regimens

Increase %T > MIC

Doripenem Concentration (mg/L)

Time Since Start of Infusion (h)

Dose
- 500 mg 1 h
- 500 mg 4 h

Question #4

How accurate are commercial antimicrobial susceptibility testing (AST) systems in detecting CRE?
Ability of commercial AST systems to infer carbapenemase production in isolates of Enterobacteriaceae with defined carbapenem-R mechanisms

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Number of Isolates</th>
<th>Phoenix</th>
<th>MicroScan NM36</th>
<th>Vitek 2a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>KPC (n=8)</td>
<td></td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>MBL (NDM, VIM) (n =16)</td>
<td></td>
<td>16</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>OXA-48 (n=11)</td>
<td></td>
<td>11</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>ESBL + porin loss (n=10)</td>
<td></td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>AmpC + porin loss (n=6)</td>
<td></td>
<td>6</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

a: updated Vitek 2 cards introduced in 2012 and 2013
Comparison of AST systems to detect meropenem resistance in 46 KPC-producing *K pneumoniae*

<table>
<thead>
<tr>
<th>Testing Method</th>
<th>Very Major (false S)</th>
<th>Major (false R)</th>
<th>Minor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etest</td>
<td>1 (2.2)</td>
<td>0</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>Vitek 2 (GN28)</td>
<td>11 (23.9)</td>
<td>0</td>
<td>18 (39.1)</td>
</tr>
<tr>
<td>(discontinued)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitek 2 (GN142)</td>
<td>0</td>
<td>0</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>Sensititre</td>
<td>3 (6.5)</td>
<td>0</td>
<td>12 (26.1)</td>
</tr>
<tr>
<td>MicroScan</td>
<td>0</td>
<td>0</td>
<td>1 (2.2)</td>
</tr>
</tbody>
</table>

Question #5

How are ESBL- and AmpC-producers, and CRE detected by phenotypic testing?
Detection of ESBL-Producing Enterobacteriaceae

ESBLs (TEM, SHV, CTX-M)

- Inhibited by clavulanate
  - (cefotax + clav = MIC 8-fold lower than cefotax alone)
  - (also ceftaz + clav = MIC 8-fold lower than ceftaz alone)
  - Disk test w & w/o clavulanate ≥5mm inh, Double disk syn test
  - Etest TZ and TZ + clav (MIC reduction ≥ 8 fold; RUO in US)
  - Detected by Vitek 2, MicroScan, Phoenix, & Sensititre

- Usually R to cefotaxime/ceftriaxone & ceftazidime
- S to cefoxitin & cefotetan (cephamycins) (AmpC are cefox-R)
- CTX-M are cefotaxime/ceftriaxone-R and ceftaz-S
- Not inhibited by boronic acid in disk (KPC inh)
- Not inhibited by EDTA in disk (MBL inh)
- Not inhibited by cloxacillin when testing carbapenems for AmpC (AmpC inh)
Double Disk Test for ESBL with amox/clav disk in center

Klebsiella pneumoniae
Etest for ESBL with clavulanate combo

Ceftazidime + clavulanate
Confirmatory ESBL test using the ceftazidime and ceftazidime/clavulanic disks

Detection of AmpC-Producing Enterobacteriaceae

**AmpC** (plasmid-mediated: CMY, FOX, DHA)
- Inhibited by boronic acid
  - cefotetan+ boronic acid $\geq$5mm inh by disk compared to cefotetan alone
- Inhibited by cloxacillin when testing carbapenems
  - mero + clox $\geq$5mm inh by disk compared to meropenem alone
  - Etest MP and MP + clox (MIC reduction $\geq$ 8 fold; RUO in US)
- R to cefotax/ceftriax, ceftazidime, & cefox & cefotetan
- R to cefoxitin & cefotetan (cephamycins)
- Not inhibited by EDTA in disk (MBL inh)
- Not inhibited by clavulanate in disk (ESBL inh)
- May be present w ESBL and mask ESBL clav disk test
Detection of KPC-Producing Enterobacteriaceae

**KPC** (KPC-2, KPC-3)

- Strains have a mean of 3.5 other beta-lactamases
  - Usually R to cefotax/ceftriax, ceftazidime, & if carrying AmpC also R to cefox & cefotetan
- **Positive by modified Hodge Test (MHT)** (carbapenem inactivation test)
- **CLSI**: MHT not necessary when all carbapenems are I or R with new breakpts: test for Inf Prev & epidemiological purposes
- **Inhibited by boronic acid**
  - mem + boronic acid ≥5mm inh by disk compared to mem alone
- Not inhibited by cloxacillin when testing carbapenems
- Not inhibited by EDTA in disk (MBL inh)
- Slightly inhibited by clavulanate in disk (ESBL inh)
- **KPC-3**: common MLST – ST258
KPC-Producing *K pneumoniae*: Modified Hodge Test

**Inhibition of *E coli* ATCC 25922 by ertapenem disk**

1. Prepare 1:10 dil of 0.5 McFarland suspension of *E coli* ATCC 25922.

2. Swab onto MHA as for disk diff and place ERT or MEM disk (preferred) on lawn (for isolates w MIC 2-4μg/mL)

3. Streak test isolates (#1-3) from edge of disk outward using sterile loop.

4. Incubate overnight.

5. Look for growth of *E coli* around test streak isolate – indicates KPC-producing strain.

---

**Enhanced growth** of test *K pneumoniae* strain due to production of KPC enzyme inhibiting the activity of ertapenem from disk

CLSI M100-S22. Jan 2012
Detection of MBL-Producing Enterobacteriaceae

**MBL (NDM, VIM, IMP)**

- Inhibited by EDTA
  - mem + EDTA ≥ 5mm inh by disk compared to mem alone
  - Etest MP and MP + EDTA (MIC reduction ≥ 8 fold; RUO in US)
- Inhibited by dipicolinic acid (DPA)
  - mem + DPA ≥ 5mm inh by disk compared to mem alone
- Strains have other beta-lactamases
  - Usually R to cefotax/ceftriax, ceftaz, & if carrying AmpC also R to cefox & cefotetan; aztreonam (AT)-S but usually AT-R due to beta-lactamases other than MBL
- Some positive by modified Hodge Test (carbapenem inactivation test; low sensitivity NDM strains, false neg)
- Not Inhibited by boronic acid (KPC inh)
- Not inhibited by cloxacillin when testing carbapenems
- Not inhibited by clavulanate in disk (ESBL inh)
Detection of MBL: EDTA disk synergy test

Etest for MBL (NDM, VIM, IMP) with meropenem + EDTA and meropenem

Meropenem + EDTA
(> 8-fold reduction in MIC compared to MP alone)
Question #6

What type of screening agars are available for ESBL, AmpC, and CRE surveillance samples?
Surveillance Testing for MDRO: MRSA, VRE, MRD Gram Neg Pathogens

Surveillance Samples
- Nasal, axilla, and rectal swabs (pediatric patients)
  - 37% more pos screen samples w 3 swabs than nasal alone (St Christopher’s Hosp for Children, data on file)

MRSA Screen
- MRSA Chromogenic agar (BD, Remel/Oxoid, Hardy, CHROM)
- CNA (colistin nalidixic acid) agar -> Staph col -> AST
- Molecular for mecA -> several MRSA PCR & NAA tests

VRE Screen
- VRE Chromogenic agar
- CNA (colistin nalidixic acid) agar -> enterococcus col -> vanco screen agar (6µg/mL vanco)
- Molecular: Film Array PCR (BioFire) BCID for vanA/B
Surveillance: MRD Gram Neg Pathogens

**MRD-GN Screening agar (for ESBL & AmpC)**
- Vacc agar (Remel/Oxoid): vanco + amphoB + ceftaz + clinda
- MAC + ceftaz + cloxacillin (Remel/Oxoid)
- ESBL Isolation agar (Remel/Oxoid): cefpodoxime

**Chromogenic Media for MRD-GN Screening**
- CHROMagar ESBL (CHROMagar)
- chromID ESBL (bioMerieux)
- Brilliance ESBL (Remel/Oxoid)
Surveillance: CRE Screening Media

Screening media for CRE
- CDC TSB + ertapenem disk: inc overnight -> MAC
- CDC TSB + meropenem disk: inc overnight -> MAC
- MAC + meropenem

Screening media for ESBL

Chromogenic Media for CRE Screening
- chromID CARBA (bioMerieux)
- Brilliance CRE (Oxoid)
- CHROMagar KPC (CHROMagar)
- SUPERCARBA medium (bioMerieux)

(Gerlich et al. *DMID* 2013; higher sens 96.5% for SUPERCARBA including OXA-48 compared to Brilliance CRE and CHROMagar)
CHROMagar

CHROMagar™ KPC
Typical Appearance of Microorganisms

E. coli CarbapenemR
Dark pink to reddish

Klebsiella, Enterobacter, Citrobacter CarbapenemR
Metallic blue

CHROMagar, Paris, France
## Comparison of Selective Media for CRE

<table>
<thead>
<tr>
<th>Carba-penemase</th>
<th>n</th>
<th>Brilliance CRE (Oxoid)</th>
<th>chromID CARBA (bioMer)</th>
<th>chromID ESBL (bioMer)</th>
<th>TSB + erta</th>
<th>TSB + meropenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC</td>
<td>12</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>NDM</td>
<td>88</td>
<td>77</td>
<td>85</td>
<td>87</td>
<td>87</td>
<td>85</td>
</tr>
<tr>
<td>IMP</td>
<td>9</td>
<td>1</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>VIM</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>OXA</td>
<td>15</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>130</td>
<td>106</td>
<td>125</td>
<td>126</td>
<td>129</td>
<td>126</td>
</tr>
</tbody>
</table>

### Other β-lactamases

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ESBL</strong></td>
<td>49</td>
<td>19</td>
<td>11</td>
<td>46</td>
<td>44</td>
</tr>
<tr>
<td><strong>AmpC</strong></td>
<td>21</td>
<td>9</td>
<td>6</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>70</td>
<td>28</td>
<td>17</td>
<td>66</td>
<td>63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sens (%)</th>
<th>Spec (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ESBL</strong></td>
<td>82</td>
<td>76</td>
</tr>
<tr>
<td><strong>AmpC</strong></td>
<td>60</td>
<td>10</td>
</tr>
</tbody>
</table>

Surveillance: CRE Screening Molecular

Molecular Methods (require growth of colonies on agar)

- **Commercial PCR:** FDA-cleared
  - Film Array BCID (blood culture ID) (BioFire) for KPC

- **Commercial PCR:** RUO
  - BD MAX PCR (BD) for KPC, NDM, OXA-48
  - Check-MDR Carba (Check-Points, The Netherlands) for KPC, NDM, VIM, IMP, OXA-48
  - equipt req’d: DNA extraction method (e.g. EasyMag), thermocycler, PCR plate spinner

- **PCR, Home Brew:** RUO for $bla_{KPC}$ PCR

- **Mass Spectrometry (RUO):** (growth on agar)
  - MALDI (matrix-assisted laser desorption ionization)
  - Vitek MS: detect peak shift in substrate (spt) by MS after growth in carbapenem solution (3h growth)
Question #7

What are the recognizable MDR-GN phenotypes and what are some suggestions for reporting CRE results?
Positive for ESBL

- Phenotype: R to 3rd gen ceph, S to cefoxitin, inh by clavulanate
- “The isolate produces an extended spectrum beta lactamase (ESBL) and is resistant to all penicillins, cephalosporins, and aztreonam.” (CLSI M100-S23, Jan 2013)

Positive for AmpC (w or w/o porin loss or efflux)

- Phenotype: R to 3rd gen ceph, R to cefoxitin, inh by cloxacillin
- “Testing for ESBL not indicated due to other mechanisms of high level 3rd generation cephalosporin resistance. The isolate is resistant to all penicillins, cephalosporins, and aztreonam.”

Positive for KPC Carbapenemase

- Phenotype: R to 3rd gen ceph, usu R to cefoxitin, pos by MHT
- “The isolate demonstrates KPC carbapenemase production, which confers resistance to all penicillins, cephalosporins, aztreonam, and carbapenems. The clinical efficacy of carbapenems to treat infections due to these isolates has not been established.”
Positive for MBL Carbapenemase (NDM, VIM, IMP – less frequently seen in US than KPC)

- Phenotype: R to 3\(^{rd}\) gen ceph, can be pos by MHT, inhibited by EDTA
- aztreonam (AT)-S but usually AT-R due to beta-lactamases other than MBL (MIC results determine susceptibility to AT)
- “The isolate demonstrates MBL carbapenemase production, which confers resistance to all penicillins, cephalosporins, and carbapenems. “

Positive for OXA Carbapenemase (less frequently seen in US than KPC or NDM among Enterobacteriaceae)

- Phenotype: R to carbapenems (MIC results determine susceptibility to 3\(^{rd}\) gen ceph and aztreonam)
1. The imipenem disk performs poorly as a screen for carbapenemases in the MHT. Use ertapenem or meropenem disks.

2. The screening and confirmatory test recommendations (MHT) were largely derived following testing of US isolates of Enterobacteriaceae and provide high sensitivity (>90%) and specificity (>90%) in detecting KPC-type carbapenemases in these isolates.

3. Not all carbapenemase-producing isolates of Enterobacteriaceae are MHT positive (NDM-producing isolates have low sensitivity).

4. *Proteus* spp, *Providencia* spp, and *Morganella* spp. may have elevated MICs to imipenem by mechanisms other than production of carbapenemases (*Omp loss*); thus the usefulness of the imipenem MIC screen test for the detection of carbapenemases is not established for these 3 genera.

5. MHT-positive results (false pos) may be encountered in isolates with carbapenem resistance mechanisms other than carbapenemase production.