New Mechanisms of Antimicrobial Resistance and Methods for Carbapenemase Detection

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Objectives

• At the conclusion of this presentation participants will be cognizant of:
  • Recently described mechanisms of antibiotic resistance
  • The diversity and classification of $\beta$-lactamases including carbapenemases
  • Evolving phenotypic methods for the laboratory detection and characterization of carbapenemases
β-Lactamases and the Genes Encoding Them among Gram-negatives

- Molecular class A (TEM, SHV, ESBLs, CTX-M, KPC)
- Molecular class B (metallo-β-lactamases (IMP, VIM, SPM, NDM))
- Molecular class C (AMP C: SPICE/SPACE bacteria)
- Molecular class D (OXA)

Suggested review:
Class C, Chromosomally or Plasmid Encoded Amp-C Enzymes

- Bush et. al. class 1, (molecular class C) AMP-C type β-lactamases
- May be plasmid mediated (relatively uncommon)
- Hydrolyze oxyiminocephalosporins (ceftriaxone, ceftazidime, cefotaxime, cefpodoxime) and 7-α- methoxy-cephalosporins (cefoxitin, cefotetan, moxalactam)
- Also hydrolyze carbapenems at very low rates
- They are not significantly inhibited by β-lactamase inhibitors
amp(C) β-lactamases among Gram-negative Bacilli

- Generally chromosomal and “non-transferable”
- Constant low level expression but inducible by cephamycins, clavulanate, and imipenem
- CMY-1 → 136, ACC-1 → 5, ACT-1 → 38, CFE-1, DHA-1 → 23, FOX-1 → 12*, LAT-1, MIR-1 → 18, MOX-1 → 11
- Stable derepression and hyper-production → high-level resistance (MICs ≥ 8 μg/mL) to:
  - penicillins and β-lactamase inhibitor combos
  - ALL cephalosporins and cephamycins (except cefepime and cefpirome)

*Queenan AM, Jenkins SG, Bush K. Cloning and biochemical characterization of FOX-5, an AmpC-type plasmid-encoded beta-lactamase from a New York City Klebsiella pneumoniae clinical isolate. AAC 45:3189-3194, 2001
Remember SPICE/SPACE for Organisms with Chromosomally-Encoded Type-1 / Amp-C β-lactamases

- S = Serratia
- P = Pseudomonas
- I = Indole positive Proteus spp. (Morganella morganii; Providencia spp.) / A = Acinetobacter or Aeromonas spp.
- C = Citrobacter
- E = Enterobacter group

- More recently E. coli

Positive AmpC by Disk Tests

Ceftazidime + clavulanic acid

Ceftazidime → Cefotaxime

Cefotaxime + clavulanic acid (CL)
AmpC Inducible by Clavulanic Acid

← Cefoxitin

← Cefotaxime (CT) versus cefotaxime + Clavulanic acid (CTL)

← Ceftazidime (TZ) versus Ceftazidime + Clavulanic acid (TZL)
Inducible AmpC Disk Test (D-Test)

- Cefotetan (left) Aztreonam (right)
- Cefotetan (left) Aztreonam (right)
For all *Enterobacter*, *Citrobacter*, and *Serratia* spp., *Cronobacter sakazakii*, *A. baumannii*, *Pluralibacter* spp., *P. aeruginosa*, *P. vulgaris*, *M. morganii*, and *Providencia* spp., the LIS automatically generates the following comment in the AST report:

“*Acinetobacter baumannii*, *Morganella morganii*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and all species of *Serratia*, *Citrobacter*, *Enterobacter* and *Providencia* may develop resistance during prolonged therapy with third-generation cephalosporins. Therefore, isolates that are initially susceptible may become resistant within three to four days after initiation of therapy. For life-threatening infections consider use of a carbapenem or fluoroquinolone if the organism is susceptible.”
Carbapenemases

- **Class A:** KPC, SME, IMI, NMC
  - serine residue at the active site
- **Class B:** IMP-1 → -53, VIM-1 → -46, GIM-1 and GIM-2, SPM, SIM, IND-1 → -15, NDM-1 → -16
  - Zn^{2+}-dependent metallo-enzyme
- **Class C:** N/A
- **Class D:** OXA family (OXA-1 → -498)
Class B Plasmid-Mediated Metallo-β-Lactamases

- Zinc containing β-lactamases: not inhibited by clavulanic acid, tazobactam, avibactam, relebactam, vaborbactam, or sulbactam
- Low rates of aztreonam hydrolysis
- Most common in *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacteriaceae* (outside of US)
- L1 Carbapenemase of *Stenotrophomonas maltophilia* (L2 is a serine cephalosporinase) – both harbored on same plasmid
Class B Plasmid-Mediated Metallo-β-Lactamases

- IMP-1: first identified and reported in *Pseudomonas aeruginosa* in 1991 and later in *Serratia marcescens*
- Variants (IMP-2 → -53) identified predominantly in *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* (worldwide)
Class B Plasmid-Mediated Metallo-\(\beta\)-Lactamases

- NDM-1: New Delhi metallo-\(\beta\)-lactamase
- First 3 \(\text{bla}_{NDM-1}\) isolates detected in US were in \textit{E. coli}, \textit{Enterobacter cloacae}, \textit{Klebsiella pneumoniae}
- NDM-1 has quickly spread among non-clonally related isolates: \textit{Citrobacter freundii}, \textit{Morganella morganii}, \textit{Providencia rettgeri}, \textit{Acinetobacter baumannii}, \textit{Providencia stuartii}
- Confers resistance to all \(\beta\)-lactams except aztreonam
- Plasmid also carries other \(\beta\)-lactamases and genes conferring resistance to other classes of antibiotics (3 isolates aztreonam-R due to other \(\beta\)-lactamases)
NDM β-lactamases

- Now NDM-1 → NDM-16
- Resistance reliably detected by standard susceptibility testing methods and some by MHT
- Recent NDM-1 blood culture isolate at NYP/WCMC in child from India; successfully treated polymyxin B / continuous infusion meropenem (MIC = 4 µg/mL) / gut decolonization with gentamicin
NDM-1-β-Lactamases

- “Laboratory ID of carbapenem-resistance mechanisms is not necessary to guide treatment or infection control practices but should be used for surveillance and epidemiologic purposes” - MMWR
- “Clinicians should be aware of the possibility of NDM-1 producing Enterobacteriaceae in patients who have received medical care in India and Pakistan and should specifically inquire about this risk factor when carbapenem-resistant enterics are reported”
- Isolates should be forwarded to CDC for confirmation (caveat)
## Comparison of NDM-1 and KPC

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<tr>
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<td><strong>β-lactamase type</strong></td>
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<td><strong>Ambler class</strong></td>
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<td>B</td>
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<td><strong>Most commonly affected species</strong></td>
<td><em>K. pneumoniae</em></td>
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<td><strong>Other species commonly affected</strong></td>
<td><em>E. coli, E. cloacae</em></td>
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<td><strong>Common MLST types</strong></td>
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<td><strong>Phenotypic Detection</strong></td>
<td>Modified Hodge Test (MHT) positive</td>
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<td><strong>Inhibitors</strong></td>
<td>Boronic acid</td>
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Verona Integron-Encoded Metallo-β-Lactamase (VIM)

- First report\(^1\) in the US (July 2010) of a VIM carbapenemase in *Klebsiella pneumoniae*
- Patient hospitalized in Greece (where endemic)
- Transferred to US where isolate was recovered from blood collected through a central venous catheter (placed in Greece)
- Nonsusceptible to all antibiotics usually used to treat *K. pneumoniae*
- Patient recovered and discharged after 26 days (line removed)
- Screened 22 other patients for colonization - negative
- Recent isolate confirmed in Indianapolis; no history of travel outside of Indiana

• KPC found in combination with VIM in 2 patients
• Deletions of 3 outer membrane porin genes affect carbapenem susceptibility (in combination with a host of β-lactamases)
  • ompF35
  • ompF36
  • ompF37
# Molecular Characterization of CRE

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[12 ESBL] 11 (396G) 11 (396T) 15 M 15
Multiple Reasons for Carbapenem Resistance

- Recent reports\(^1\) of blaOxa-232 (by whole genome sequencing) in Klebsiella spp.
- These were “negative” by CRE PCR (which included KPC, NDM, VIM, IMP, SME and Oxa-48-like targets)
- Imipenem MICs on the low end (1 - 4 µg/mL), but the meropenem and ertapenem MICs were high
- Carba-NP was also negative

\(^1\)R. Humphries; personal communication
Phantom zone between IP/IPI is indicative of MBL

Deformation of the IP or IPI ellipse is indicative of MBL (Keyhole)

MIC ratio of IP/IPI of ≥8 or ≥3 log₂ dilutions indicates MBL production.

(MIC IP/IPI= 16/<1 = >16)

Positive MBL Tests by Etest®
Class D, Chromosomally Encoded Carbapenem Hydrolyzing Enzymes

- OXA-enzymes (oxacillinases) mostly identified in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*
- Usually chromosomally located
- OXA-23: shown to be plasmid-mediated
- OXA-48 and OXA-48-like emerging as major resistance determinants worldwide
Gram-Negative Resistance Mechanism Phenotypic Testing

- **KPC:**
  - Inhibited by boronic acid
  - Not inhibited by EDTA
  - Slightly inhibited by clavulanic acid
  - Not inhibited by cloxacillin when testing carbapenems

- **Metallo-:**
  - Inhibited by EDTA
  - Not inhibited by boronic acid
  - Inhibited by dipicolinic acid
  - Inhibited by phenanthroline chelators
  - Inhibited by 2-mercaptopropionic acid
Gram-Negative Resistance Mechanism Phenotypic Testing

• amp C (with or without outer membrane porin loss)
  • Not inhibited by EDTA
  • Inhibited by boronic acid
  • Not inhibited by clavulanate
  • Inhibited by cloxacillin when testing carbapenems
  • Some inhibited by dipicolinic acid

• ESBLs:
  • Not inhibited by EDTA
  • Not inhibited by boronic acid
  • Inhibited by clavulanate
Gram-Negative Resistance Mechanism
Phenotypic Testing

- **OXA-48:**
  - Not inhibited by dipicolinic acid
  - Not inhibited by boronic acid (rare and slight)
  - Not inhibited by cloxacillin

- **CTX-M (i.e., CTX-M-15):**
  - Ceftazidime-susceptible
  - Cefotaxime/ceftriaxone-resistant

- **K1: Type of ESBL in *Klebsiella oxytoca***
  - Cefoxitin-susceptible
  - Inhibited by clavulanic acid
  - Aztreonam-resistant; ceftazidime-susceptible
  - Ceftriaxone-resistant; cefotaxime-susceptible
KPC

- **Klebsiella pneumoniae carbapenemase**
- Mostly found in *K. pneumoniae*, but also in other enteric bacteria.
- \( KPC_{bla} \) resides in plasmids.
- Hydrolyze all of the \( \beta \)-lactam antibiotics including cephalosporins and monobactams (as well as the carbapenems) → Very few therapeutic options
- Endemic in NYC; spreading across nation / world
Class A, KPC Carbapenem-hydrolyzing Enzymes

- KPC-1; *Klebsiella pneumoniae* - North Carolina
- Now KPC-1 → KPC-24
- KPC-positive isolates often possess additional beta-lactamases (average=3.5)
Reporting Cases of Klebsiella spp. Infection or Colonization

   a. Clusters of cases of Klebsiella spp. infection or colonization; and/or
   b. Single cases of carbapenem-resistant Klebsiella spp. infection or colonization.

2. The DOH 4018 form should be faxed to the Regional Epidemiology Program at 518-408-1745. Local health departments can be notified by telephone (a confidential case report does not need to be completed).
Elderly Canadians who spend their winters in Florida face and pose the most serious risk because they are more likely to find themselves in United States hospitals, in which carbapenem-resistant *Klebsiella pneumoniae* is rampant.
<table>
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<th>Antibiotic</th>
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<th>I</th>
<th>R</th>
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<tr>
<td>Ertapenem</td>
<td>0</td>
<td>0.6</td>
<td>99.4</td>
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<tr>
<td>Meropenem</td>
<td>10.5</td>
<td>7.5</td>
<td>82</td>
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<tr>
<td>Imipenem</td>
<td>11.9</td>
<td>19.2</td>
<td>68.9</td>
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<td>Cefepime</td>
<td>3.8</td>
<td>12.4</td>
<td>83.8</td>
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<tr>
<td>Tetracycline</td>
<td>79.1</td>
<td>9.8</td>
<td>11.1</td>
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<tr>
<td>Amikacin</td>
<td>25.6</td>
<td>46.3</td>
<td>28.1</td>
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<tr>
<td>Gentamicin</td>
<td>55.7</td>
<td>15.6</td>
<td>28.6</td>
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</table>
Carbapenem Resistance in *Klebsiella pneumoniae* in NYC

- Remains endemic, particularly in Brooklyn, although rates have declined
- During peak (2009) at MSMC, 36% of 1163 isolates tested carbapenem-resistant
- The rate held steady (≈16%) at NYP/WCMC for past several years until 2016 when dropped to 6%
- For 2008 – 2012, the US rate was 4.7% (N = 5467; SENTRY)¹

Not necessary to test isolates for a carbapenemase by modified Hodge test (carbapenem inactivation test) when all of the carbapenem that are reported by a laboratory test either intermediate or resistant (i.e., these carbapenem susceptibility results should be reported as tested)

However, modified Hodge test may still be useful in such cases for infection control and epidemiologic purposes
The MHT performed on a small MHA plate.
(1) *K. pneumoniae D-05*, positive result;
(2) *K. pneumoniae* 6179, negative result; and
(3) a clinical isolate, positive result

**Modified Hodge Test**
*(Carbapenem Inactivation Test)*

Enhanced growth of *E. coli ATCC® 25922*. Carbapenemase produced by *K. pneumoniae D-05* destroyed ertapenem that diffused into the media. Thus, there is no longer sufficient ertapenem to inhibit *E. coli ATCC® 25922* and an indentation of the zone is noted.

*E. coli ATCC® 25922*

Inhibition of *E. coli ATCC® 25922* by ertapenem
MHT False Positive *Enterobacter cloacae*

Positive Control →

↑

*E. cloacae*
Carba NP Test for Detection of Carbapenemase Production in Enterobacteriaceae and *P. aeruginosa*

- Detects hydrolysis of imipenem
- Isolate suspended in TRIS-HCl lysis buffer, vortexed, incubated for 30 minutes, and centrifuged
- Was described and included as an alternative to the MHT in CLSI M-100

Carba NP Test for Detection of Carbapenemase Production in Enterobacteriaceae and *P. aeruginosa*

- Supernatant transferred to 4 wells of a microtiter plate respectively containing:
  - Dilute phenol red solution with ZnSO4
  - Dilute phenol red solution with ZnSO4 and imipenem
  - Dilute phenol red solution containing ZnSO4, imipenem, and tazobactam
  - Dilute phenol red solution containing imipenem and EDTA
A

<table>
<thead>
<tr>
<th></th>
<th>No antibiotic</th>
<th>Imipenem</th>
<th>Imipenem + Zn(^{2+})</th>
<th>Imipenem + EDTA</th>
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<td><strong>No carbapenemase</strong></td>
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<tr>
<td><strong>Ambler class A carbapenemase</strong></td>
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<tr>
<td><strong>Ambler class B carbapenemase</strong></td>
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<tr>
<td><strong>Ambler class D carbapenemase</strong></td>
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<tr>
<td><strong>Not interpretable</strong></td>
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B

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<tr>
<th>Strain</th>
<th>Treatment</th>
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<tr>
<td><strong>K. pneumoniae A28006 (KPC-2)</strong></td>
<td>No antibiotic</td>
</tr>
<tr>
<td><strong>E. coli LIL-1 (KPC-2)</strong></td>
<td>Imipenem + Zn(^{2+})</td>
</tr>
<tr>
<td><strong>E. coli JAP (IMP-1)</strong></td>
<td>Imipenem + Zn(^{2+}) + Tazobactam</td>
</tr>
<tr>
<td><strong>P. aeruginosa 12870 (IMP-1)</strong></td>
<td>Imipenem + EDTA</td>
</tr>
<tr>
<td><strong>E. coli MAD (VIM-1)</strong></td>
<td>Imipenem + EDTA</td>
</tr>
<tr>
<td><strong>P. aeruginosa KA-209 (VIM-2)</strong></td>
<td>Imipenem + EDTA</td>
</tr>
<tr>
<td><strong>E. coli 271 (NDM-1)</strong></td>
<td>Imipenem + EDTA</td>
</tr>
<tr>
<td><strong>P. aeruginosa 73-5674 (GIM-1)</strong></td>
<td>Imipenem + EDTA</td>
</tr>
<tr>
<td><strong>P. rettgeri RAP (OXA-181)</strong></td>
<td>Imipenem + EDTA</td>
</tr>
<tr>
<td><strong>K. pneumoniae BIC (OXA-48)</strong></td>
<td>Imipenem + EDTA</td>
</tr>
</tbody>
</table>

Modified Carbapenemase Inactivation Method (mCIM)

Published last year in CLSI M100

Inoculum
2ml - TSB Tube
1µl - Fermenters
10µl – Non-fermenters

Vortex Suspension
10 - 15 second

Add a 10µg Meropenem Disk

Incubate at 35°C
4 hours +/- 15 mins

Remove Suspension Tubes With disks from the Incubator

Remove Disk Aseptically from Tube Place on Prepared Mueller Hinton Plate

Method

Incubate at 35°C
18-24 hours

Prepare Muller-Hinton Agar
(150mmMcFarland Suspension
E. coli (ATCC 25922)
Vortex 10-15 seconds
Lawn Streak (3 Directions)
)
0.5 Allow to Dry (3-10 mins)
Interpretation of Results

Read for the presence or absence of a zone of inhibition

- **Carbapenemase-positive**: If the isolate being tested produces a carbapenemase, the meropenem in the disk will be inactivated allowing uninhibited growth (zones diameter 6 - 10 mm) of the meropenem-susceptible *E. coli* strain (ATCC 25922)

- **Carbapenemase-negative**: If the gram-negative rod being tested does not produce a carbapenemase, the meropenem in the disk will not be inactivated resulting in inhibited growth (zone diameter ≥ 20 mm) of the meropenem-susceptible *E. coli* strain (ATCC 25922)

- **Indeterminate**: A zone of inhibition (≤19 mm but ≥11 mm) is an indeterminate result. The presence or absence of a carbapenemase cannot be confirmed. PCR for carbapenemase genes is recommended.

- **Carbapenemase-positive**: When small colonies are observed growing in the zone of inhibition around the disk the results are classified as carbapenemase positive. Record as positive and keep note of growth within zone of inhibition
Recommendations

• Enterobacteriaceae (Enterobacteriales): 1-µl loop, TSB, 4 hours
  • Positive 6 - 15 mm (or presence of pinpoint colonies with a 16 – 18 mm zone of inhibition)
  • Indeterminate 16 - 18
  • Negative ≥ 19 mm

• *Pseudomonas aeruginosa*: zone size ≤10mm 100% positive predictive value (10-µl loop, TSB, 4 hours)

• Additional multi-center study demonstrated that the method not perform well for *Acinetobacter baumannii*
  • *Acinetobacter baumannii*: mixed genes (including a number of OXA enzymes); other mechanisms for carbapenemase negative isolates
Examples
imCIM (eCIM)

- The modified Carbapenem Inactivation Method (mCIM) is a simple phenotypic test that detects carbapenemase production in *Enterobacteriaceae*, but cannot distinguish between serine-based carbapenemases and MBLs.

- eCIM employs ethylenediaminetetraacetic acid (EDTA), in conjunction with the mCIM assay to differentiate serine from metallo-carbapenemases (MBLs).
eCIM

- Potentially important from both an epidemiologic and therapeutic perspective
- Published in CLSI M100 28th addition
Methods:
modified CIM (w/o EDTA)

Suspend 1 μl loopful of bacteria in TSB
Add 10 microgram of meropenem disc
Incubate for 4 hours at 35°C
Place on Mueller Hinton agar inoculated with E. coli ATCC 25922

Incubate for at least 18 hours at 35°C

Read presence or absence of inhibition zone at 18 and 24 hours of incubation

+ mCIM Carbapenemase activity present

- mCIM Carbapenemase activity absent
Methods: eCIM with EDTA

Suspend 1 μl loopful of bacteria in TSB + 0.1 mM EDTA

Add 10 microgram of meropenem disc

Incubate for 4 hours at 35°C

Place on Mueller Hinton agar inoculated with *E. coli* ATCC 25922

Incubate for at least 18 hours at 35°C

Read presence or absence of inhibition zone at 18 and 24 hours of incubation

- imCIM Carbapenemase activity not inhibited by EDTA

+ imCIM Carbapenemase activity inhibited by EDTA
Results: QC testing

- *K. pneumoniae* ATCC 1705 (KPC+)
- *K. pneumoniae* ATCC 1706 (KPC -)
- *K. pneumoniae* ATCC 2146 (NDM+)

**No EDTA**

**EDTA**
Using a 20G venting needle, inoculate 4 drops of broth from positive blood culture into 2 mL of TSB.

Add meropenem disc, incubate 4 h at 35°C in ambient air without shaking.

Place disc on MHA plate inoculated with lawn of *E. coli* 25922. Incubate 18-24 h.

**CPE Negative** (zone of growth inhibition)

**CPE Positive** (no zone of growth inhibition)
Bacterial Test Strains

• Test isolates: 61 *Enterobacteriaceae* from the Antimicrobial Resistance Bank (ARB), CDC (10 genera, 14 species):
  - *Citrobacter freundii*
  - *Citrobacter* species
  - *Enterobacter aerogenes*
  - *Enterobacter cloacae*
  - *E. coli*
  - *Klebsiella oxytoca*
  - *Klebsiella ozaenae*
  - *Klebsiella pneumoniae*
  - *Morganella morganii*
  - *Proteus mirabilis*
  - *Providencia rettgeri*
  - *Raoultella ornithinolytica*
  - *Salmonella Senftenberg*
  - *Serratia marcescens*
Bacterial Test Strains

- Test isolates: 61 *Enterobacteriaceae* (*Enterobacteriales*) from the ARB
- 27/61 (44.3%) CPE and 34/61 (55.7%) non-CPE
- Resistance mechanisms (number of isolates):
  - AmpC
  - ESBL
  - IMI (1)
  - IMP (1)
  - KPC (8)
  - NDM (7)
  - OXA: OXA-48 (2), OXA-181 (2), and OXA-232 (2)
  - SME (2)
  - VIM (2)
  - Porin mutants
Quality Control/Reporter Strains and Result Interpretation

- **Quality control (QC) strains:**
  - *K. pneumoniae* ATCC 700603, carbapenemase negative
  - *K. pneumoniae* ATCC BAA-1705, carbapenemase (KPC) positive

- **Reporter strain:** *E. coli* ATCC 25922

- **Interpretations:**
  - CPE negative; zone size $\geq 19$ mm
  - CPE positive; zone size 6-15 mm
  - Indeterminate; zone size 16 – 18 mm
Study Design

• Isolates subcultured from -80°C freezer stocks (x 2)
• Each strain suspended in inoculum saline (Beckman Coulter) to a concentration of ~1.5 x 10^3 CFU/mL
• ~ 1.5 x 10^3 CFU of each test and QC strain inoculated into negative patient blood cultures (mix of aerobic, anaerobic, and pediatric bottles), and loaded onto BACTEC FX (Becton, Dickinson and Company)
• Once positive, blood culture broth sub-cultured (purity plate) and mCIM set up (4 drops broth into 2 mL TSB)
• QC set up each day of testing, had to pass to accept test isolate results
Diagnostic Performance

- Time taken for test strains to signal positive post-inoculation: range, 6.7 to 17.58 h (mean, 9.04 h)
- Time taken to set up mCIM for test strains once culture signaled positive: range, 0 to 12.25 h (mean, 7.81 h)
- Time taken for QC strains to signal positive post inoculation: range, 6.7 to 11 h (mean, 8.87 h)
- Time taken to set up mCIM for QC strains once culture signaled positive: range, 0.48 to 11.3 h (mean, 8.09 h)
- Sensitivity and specificity, 100%
# Reproducibility of Quality Control

<table>
<thead>
<tr>
<th>Mean Zone Size (mm)</th>
<th>Standard Deviation$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>700603$^b$</td>
<td>22</td>
</tr>
<tr>
<td>1705$^b$</td>
<td>6$^c$</td>
</tr>
</tbody>
</table>

$^a$ Standard deviation of the mean  
$^b$ 17 independent replicates for each strain  
$^c$ No zone of growth inhibition
Summary

- mCIM can be employed for the detection of CPE directly from blood culture broths
- Inexpensive: cost and labor
- Results at least a day earlier than conventional mCIM
- However, only assessed in one laboratory
- Non-fermentative gram-negatives not assessed yet
- Additional characterization required: multi-center study
In the setting of limited treatment options, clinicians managing infections due to these isolates may wish to consider maximum approved dosage regimens and/or prolonged intravenous infusions of carbapenems as described in the medical literature.

Each laboratory should develop a mechanism for informing clinicians about such circumstances in a timely manner. This might include a telephone call and/or a comment appended to the laboratory report. Consultation with an Infectious Disease specialist is recommended.
Resistance to Polymyxins

- Transferable plasmid-encoded mcr-1 has been detected worldwide in *K. pneumoniae*, *E. coli*, and other Enterobacteriaceae.
- At times co-localized with other resistance genes such as those for ESBLs and carbapenemases.
- Co-localization of mobile genetic elements could lead to horizontal transfer of multi-drug resistance to other nosocomial pathogens and increase the difficulty of antibiotic treatment.
Polymyxin Resistance

- When colistin resistance is genetically linked to resistance to other antibiotics on mobile genetic elements, such resistance can be co-selected by other antibiotics and spread in the hospital environment, even in the absence of colistin use (as was the case in a recent hospital outbreak in China)
- Retrospectively NYP/WCMC identified a KPC-positive *E. coli* from a patient at LMH in 2014 that harbored mcr-1 (prior to first reports in literature in 2015)
Reporting of Other Antimicrobial Agents

- Do not routinely report tigecycline on urine or blood isolates (possibly for combination therapy)
- Do not report polymyxin B (or colistin) on isolates from pulmonary specimens (for aerosol on request)
- When reporting polymyxins, add a disclaimer indicating that interpretive criteria do not exist for Enterobacteriaceae but if *Acinetobacter* breakpoints were employed the isolate would be considered…..
- Only test polymyxins by BMD (not disk or Etest)
- Test and report minocycline as an alternative to tigecycline as achievable levels in urine and blood are higher
NOTE: Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (e.g., MICs in the intermediate and at the breakpoint of resistance) than those with meropenem or doripenem MICs. These isolates can be imipenem resistant by mechanisms other than production of carbapenemases.
Mechanisms of Carbapenem Resistance

- In U.S., harboring KPC enzyme most frequent etiology
- Alternate mechanism: hyper-production of ampC or CTX-M β-lactamases along with an outer membrane porin mutation (OMP K37 and others)
- Recent multi-center study examined strains of *E. coli* and *K. pneumoniae* resistant to piperacillin-tazobactam, amoxicillin-clavulanate, and ampicillin-sulbactam, but fully susceptible to cephalosporins – mutants with porin deletions and hyperproduction of TEM
Cornell Experience
(Carbapenem-resistant *Enterobacteriaceae* (843) since 2006)

- **Klebsiella pneumoniae** – 668 patients
  - 2007 – 61
  - 2008 – 77
  - 2009 – 64
  - 2010 – 79
  - 2011 – 64
  - 2012 – 75
  - 2013 – 120
  - 2014 – 59
  - 2015 – 14 (through 4/30/15)

- **Klebsiella oxytoca** – 8; **E. coli** – 78; **Citrobacter freundii** – 10; **Citrobacter koseri** – 1; **Serratia marcescens** – 7; **Enterobacter cloacae** - 91; **Enterobacter aerogenes** – 17; **Enterobacter asburiae** – 2; **Pluralibacter gergoviae** – 1; **Pantoea** spp. – 2; **Providencia rettgeri** – 2; **Providencia stuartii** – 2; **Proteus mirabilis** – 1; **Morganella morganii** - 2
10 Patients: Both *K. pneumoniae* and Another Enteric Bacterial Species

- 6 of 10 patients’ pairs possess KPC genes confirmed by PCR (patient 1, 4, 5, 6, 8 and 9)
Clearance of CRKP Bacteriuria

New Drugs and/or in Development

- β-lactamase inhibitors
  - Avicaz™ (Avibactam (NXL104) combined with ceftazidime)
    - A non-β-lactam inhibitor of β-lactamase
    - Active against class A and class C enzymes, variable activity against class D enzymes
    - Not active against class B enzymes (metallo-β-lactamases)
  - Imipenem-relebactam (very similar spectrum as ceftazidime avibactam) (in clinical trials)
  - Vabomere™ (meropenem-vaborbactam)
    - RPX7009, a boron-containing beta lactamase inhibitor, and meropenem

- Novel aminoglycosides ("neoglycosides")
  - Plazomycin (ACHN-490)
    - Resistance has been associated with genes encoding 16S rRNA methylase
Thoughts?

Questions?