Laboratory Practices and the New York City Experience with CRE

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Objectives

• At the conclusion of this presentation participants will be cognizant of:
  • The classification of β-lactamases including carbapenemases
  • The epidemiology of CRE in the greater NYC area
  • Methods being employed 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:
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, 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-β-Lactamases

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

- Now NDM-1 $\rightarrow$ 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

<table>
<thead>
<tr>
<th></th>
<th>KPC</th>
<th>NDM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-lactamase type</strong></td>
<td>Serine</td>
<td>Metallo-β-lactamase</td>
</tr>
<tr>
<td><strong>Ambler class</strong></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td><strong>Most commonly affected species</strong></td>
<td><em>K. pneumoniae</em></td>
<td><em>K. pneumoniae</em></td>
</tr>
<tr>
<td><strong>Other species commonly affected</strong></td>
<td><em>E. coli, E. cloacae</em></td>
<td><em>E. coli, E. cloacae</em></td>
</tr>
<tr>
<td><strong>Common MLST types</strong></td>
<td>ST258</td>
<td>Variable</td>
</tr>
<tr>
<td><strong>Geographic epicenter</strong></td>
<td>NE USA</td>
<td>India, Pakistan</td>
</tr>
<tr>
<td><strong>β-lactam antibiotics affected</strong></td>
<td>Penicillins, cephalosporins, carbapenems</td>
<td>Penicillins, cephalosporins, carbapenems</td>
</tr>
<tr>
<td><strong>Phenotypic Detection</strong></td>
<td>Modified Hodge Test (MHT) positive</td>
<td>Unknown (positive MHT likely)</td>
</tr>
<tr>
<td><strong>Inhibitors</strong></td>
<td>Boronic acid</td>
<td>EDTA</td>
</tr>
</tbody>
</table>

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

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
Case

- A 48 year old obese female was admitted for elective knee replacement surgery following an automobile accident
- Post-surgery she developed idiopathic heparin-induced thrombocytopenia
- Loss of perfusion to her intestines resulted in small bowel transplant
- Post-surgery #2 she developed ARDS and was placed on a ventilator
- The patient’s condition continued to deteriorate and she developed a nosocomial pneumonia
The antimicrobial susceptibility pattern of the isolate was as follows:

- Resistant to: ampicillin, piperacillin, amoxicillin-clavulanate, ampicillin-sulbactam, ticarcillin-clavulanate piperacillin-tazobactam, aztreonam, cefazolin, cefuroxime, cefotetan, ceftriaxone, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, ertapenem, gentamicin, tobramycin, levofloxacin, ciprofloxacin, chloramphenicol, and trimethoprim-sulfamethoxazole

- Intermediate susceptibility to: amikacin and tetracycline

- Susceptible to: tigecycline and polymyxin B
Case

- What gram-negative was recovered from BAL, an empyema collection, urine, and blood?
Klebsiella pneumoniae
Case

- Polymyxin B MIC = 2 \( \mu g/mL \) (Susceptible?) (ECV; WT/NWT)
- Patient treated with tigecycline and polymyxin B - responded
## Antibiotic Susceptibility Testing

### Subsequent Stool Isolate

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Klebsiella pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTIBIOTICS (µg/mL)</td>
<td>MIC</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;16       R</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&gt;16       R</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;32       R</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;16       R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;32       R</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>&gt;16       R</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;2        R</td>
</tr>
<tr>
<td>Cefepine</td>
<td>&gt;16       R</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>&gt;16       R</td>
</tr>
<tr>
<td>Amikacin</td>
<td>32        R</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;8        R</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;8        R</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>&gt;4        R</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>2         S (?)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8         R</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>&gt;4        R</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;8        R</td>
</tr>
<tr>
<td>Trimethoprim–Sulfamethox</td>
<td>&gt;2/38    R</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>&gt;8        R</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt;8        R</td>
</tr>
</tbody>
</table>
The Patient Developed a Second Pneumonia Related to:
Follow-up

Hyperinfestation with *Strongyloides stercoralis*
Follow-up

Treated using subcutaneous injections of a veterinarian preparation of ivermectin and recovered, only to develop a new pneumonia with:
Follow-up

- *Aspergillus fumigatus*
- Again responded to therapy (voriconazole), but developed bilateral CMV pneumonia
Follow-up

Controlled with high-dose gancyclovir, but became septic with:
Multi-drug resistant strain of *Acinetobacter baumannii*

- $\beta$-lactam (including imipenem), aminoglycoside, and fluoroquinolone resistant
- Expired 13 months after initial surgery
KPC

- **Klebsiella pneumoniae carbapenemase**
- Mostly found in *K. pneumoniae*, but also in other enteric bacteria.
- $KPC_{bla}$ resides in plasmids.
- Hydrolyze all of the β-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.
## Susceptibility Testing

### Frequency of Very Major, Major, and Minor Errors

<table>
<thead>
<tr>
<th>Testing Method</th>
<th>Very Major</th>
<th>Major</th>
<th>Minor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2010 CLSI Meropenem Interpretive Criteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etest</td>
<td>1 (2.2)</td>
<td>0 (0)</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>11 (23.9)</td>
<td>0 (0)</td>
<td>18 (39.1)</td>
</tr>
<tr>
<td>Sensititre</td>
<td>3 (6.5)</td>
<td>0 (0)</td>
<td>12 (26.1)</td>
</tr>
<tr>
<td>Microscan</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td><strong>Pre-2010 Meropenem Interpretive Criteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etest</td>
<td>1 (2.2)</td>
<td>0 (0)</td>
<td>7 (15.2)</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>27 (58.7)</td>
<td>0 (0)</td>
<td>8 (17.4)</td>
</tr>
<tr>
<td>Sensititre</td>
<td>27 (58.7)</td>
<td>0 (0)</td>
<td>12 (26.1)</td>
</tr>
<tr>
<td>Microscan</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (4.3)</td>
</tr>
</tbody>
</table>

MSMC Microscan Results for Carbapenem Resistant *Klebsiella pneumoniae* (n = 531)

<table>
<thead>
<tr>
<th>Drug</th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ertapenem</td>
<td>0</td>
<td>0.6</td>
<td>99.4</td>
</tr>
<tr>
<td>Meropenem</td>
<td>10.5</td>
<td>7.5</td>
<td>82</td>
</tr>
<tr>
<td>Imipenem</td>
<td>11.9</td>
<td>19.2</td>
<td>68.9</td>
</tr>
<tr>
<td>Cefepime</td>
<td>3.8</td>
<td>12.4</td>
<td>83.8</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>79.1</td>
<td>9.8</td>
<td>11.1</td>
</tr>
<tr>
<td>Amikacin</td>
<td>25.6</td>
<td>46.3</td>
<td>28.1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>55.7</td>
<td>15.6</td>
<td>28.6</td>
</tr>
</tbody>
</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%
- 2014 rate at NYP/CPMC was 11% overall; 21% in the ICUs
- 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 carbapenems 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

*E. coli* ATCC® 25922

Inhibition of *E. coli* ATCC® 25922 by ertapenem

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.
MHT False Positive *Enterobacter cloacae*

Positive Control →

↑

*E. cloacae*
Positive KPC Test by Boronic Acid Rosco® Method

- Increase in zone of inhibition of $\geq 5$ mm for Meropenem (MR) + Boronic Acid (BO) disk, as compared to MR alone, AND

- There must also be a $\leq 3$ mm difference for MR versus MR + Clavulanic acid (CL) [AmpC test] and for MR versus MR + Dipicolinic acid (DP) [Metallo $\beta$-Lactamase (MBL) test]
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
- Will be described and included as an alternative to the MHT in the next iteration of 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
Modified Carbapenemase Inactivation Method (mCIM)

Published this year in CLSI M100

Method

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

2. **Vortex Suspension**
   - 10 - 15 seconds

3. **Add a 10µg Meropenem Disk**

4. **Incubate at 35°C**
   - 4 hours +/- 15 mins

5. **Remove Suspension Tubes With disks from the Incubator**

6. **Remove Disk Aseptically from Tube**
   - Place on Prepared Mueller Hinton Plate

7. **Prepare Muller-Hinton Agar**
   - (150mm McFarland Suspension)
   - *E. coli* (ATCC 25922)
   - Vortex 10-15 seconds
   - Lawn Streak (3 Directions)
   - 0.5 Allow to Dry (3-10 mins)

8. **Incubate at 35°C**
   - 18-24 hours
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 (1-µl loop, TSB, 4 hours)
  - Positive 6 - 15 mm (protocol 6-10 mm)
  - Indeterminate 16 - 18 (protocol ≤ 19 mm but ≥ 11 mm)
  - Negative ≥ 19 mm (protocol ≥ 20mm)

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

**AND/OR**

- Additional Studies and modify the method in one year
  - Increased inoculum *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex (2 X 10 µl)
  - Increased incubation time for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (6 hours)
  - *Acinetobacter baumannii* and *Pseudomonas aeruginosa* – mixed genes; other mechanisms for carbapenemase negative isolates
  - Pilot study data presented at January 2017 meeting – above parameters, all sites, for June meeting
Examples
• 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

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

• Potentially important from both an epidemiologic and therapeutic perspective
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:
imCIM 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

- imCIM Carbapenemase activity not inhibited by EDTA
+ imCIM Carbapenemase activity inhibited by EDTA

Incubate for at least 18 hours at 35°C
Read presence or absence of inhibition zone at 18 and 24 hours of incubation
Results: QC testing

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

The images show petri dishes with bacterial cultures labeled with different concentrations of EDTA.
Positive MBL Tests by Etest®
Positive MBL Test by Dipicolinic Acid Rosco® Method

- Increase in zone of inhibition of ≥5 mm for MR + DP, versus MP alone, **AND**

- There must also be a ≤ 3 mm difference for MR alone versus MR+BO (KPC) and MR alone versus MR+CL (AmpC)
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.
## Enterobacteriaceae Carbapenem Breakpoints

### Revised CLSI and FDA MIC Breakpoints (µg/mL)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doripenem</td>
<td>≤ 1 (NA)</td>
<td>2 (NA)</td>
<td>≥4 (NA)</td>
</tr>
<tr>
<td>Meropenem*</td>
<td>≤ 1 (4)</td>
<td>2 (8)</td>
<td>≥4 (16)</td>
</tr>
<tr>
<td>Imipenem*</td>
<td>≤ 1 (4)</td>
<td>2 (8)</td>
<td>≥4 (16)</td>
</tr>
<tr>
<td>Ertapenem*</td>
<td>≤0.5 (2)</td>
<td>1 (4)</td>
<td>≥2 (8)</td>
</tr>
</tbody>
</table>

### Revised CLSI and FDA Disc Breakpoints (mm) and FDA

<table>
<thead>
<tr>
<th>Drug</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>≥23</td>
<td>20-22</td>
<td>≤19</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≥23</td>
<td>20-22</td>
<td>≤19</td>
</tr>
<tr>
<td>Doripenem</td>
<td>≥22</td>
<td>20-21</td>
<td>≤19</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≥22</td>
<td>19-21</td>
<td>≤18</td>
</tr>
</tbody>
</table>

Previous breakpoints are shown in parentheses.
CARBAPENEMS

- 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.
Screening Cultures for CRKP

- All ICU patients screened weekly and upon admission (rectal swabs)
- Specimens inoculated to 5 mL tube of tryptic soy broth containing 10 μg imipenem disk and incubated overnight at 37º C
- Broth subcultured onto MacConkey agar; imipenem disk placed in the area of heavy inoculum
- Incubated overnight at 37º C
- Suspicious colonies identified and \textit{in vitro} susceptibility to ertapenem, imipenem, and meropenem determined by broth microdilution using Microscan® system; resistant isolates confirmed by E-testing
- 27% of KPC-positive patients detected by screening subsequently became bacteremic with the organism

Mechanisms of Carbapenem Resistance

- In U.S., Harboring KPC enzyme most frequent etiology
- Cross-resistance with fluoroquinolones and aminoglycosides
- Hyper-production of AmpC or CTX-M β-lactamases along with an outer membrane porin mutation\(^8,9\); OMP K37?
- 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

\(^8\) Reviews on Medical Micro. 2004;15:63-72
Mount Sinai Experience

- 721 patients colonized/infected with carbapenem-resistant *K. pneumoniae* from 1/04 to 4/08
- 97 patients colonized/infected with carbapenem-resistant Enterobacteriaceae other than *Klebsiella pneumoniae* since 2006: (*Enterobacter* spp. – 73; *Providencia stuartii* – 1; *Morganella morganii* – 1; *Serratia marcescens* – 1; *Klebsiella oxytoca* – 6; *E. coli* – 11; *Citrobacter freundii* – 4)
- 29 in patients concomitantly infected with KPC-producing *K. pneumoniae* (confirmed as KPCs by isoelectric focusing and PCR)
Mount Sinai Hospital: Patients with Carbapenem-Resistant *K. Pneumoniae* by Year

- 1998 – 2
- 1999 – 1
- 2000 – 1
- 2001 – 7
- 2002 – 2
- 2003 – 13
- 2004 – 40
- 2005 – 167
- 2006 – 219
- 2007 – 225
- 2008 – 44 (through 03/21)
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** – 77; **Citrobacter freundii** – 10; **Citrobacter koseri** – 1; **Serratia marcescens** – 6; **Enterobacter cloacae** - 82; **Enterobacter aerogenes** – 16; **Enterobacter asburiae** – 2; **Pluralibacter gergoviae** – 1; **Pantoea** spp. – 2; **Providencia rettgeri** – 2; **Providencia stuartii** – 1; **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)
<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 99)</th>
<th>Controls (n = 99)</th>
<th>OR [95%CI] P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior Antibiotics</td>
<td>98</td>
<td>55</td>
<td>78.40 [12.41-3197] p &lt;0.0001</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>63</td>
<td>31</td>
<td>3.84 [2.04-7.25] p &lt;0.00001</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>36</td>
<td>23</td>
<td>1.89 [0.97-3.7] p = 0.04</td>
</tr>
<tr>
<td>β-lactam/β-lactamase inhibitor</td>
<td>54</td>
<td>33</td>
<td>2.40 [1.3-4.45] p = 0.0024</td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>14</td>
<td>3</td>
<td>5.27 [1.39-29.35] p = 0.0093</td>
</tr>
<tr>
<td>Monobactam</td>
<td>6</td>
<td>1</td>
<td>6.32 [0.73-142] p = 0.054</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>54</td>
<td>6</td>
<td>18.6 [7.01-52.16] p &lt;0.00001</td>
</tr>
</tbody>
</table>
## Receipt of “Effective” Therapy

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>OR [95%CI]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective Antibiotics</td>
<td>67</td>
<td>95</td>
<td>0.09 [0.03 - 0.28]</td>
<td>p &lt;0.00001</td>
</tr>
<tr>
<td>Time to therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.12</td>
<td>0.76</td>
<td></td>
<td>p &lt;0.001</td>
</tr>
<tr>
<td>Median</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0 - 12</td>
<td>0 - 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjunct Therapy</td>
<td>73</td>
<td>58</td>
<td>1.98 [1.04 - 3.78]</td>
<td>p = 0.024</td>
</tr>
</tbody>
</table>

Effective therapy: Antibiotic to which the isolate is susceptible *in vitro*
Overall Mortality (%)

Mortality:
- Controls: 3.72 [1.9 - 7.34] p <0.0001
- Cases: 4.5 [2.07 - 10] p <0.0001

Attributable mortality:
- Controls: 20
- Cases: 48

- Controls: 12
- Cases: 38
## Predictors of Mortality in Cohort (n = 198)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Expired (n = 68)</th>
<th>Survived (n = 130)</th>
<th>OR [95% CI] p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effective antibiotics</strong></td>
<td>53(78%)</td>
<td>109(84%)</td>
<td>0.68 [0.31-1.52] p = 0.31</td>
</tr>
<tr>
<td><strong>Delay to Antibiotics</strong></td>
<td>2.34</td>
<td>1.45</td>
<td>1 [0.81-1.24] p = 0.9891</td>
</tr>
<tr>
<td><strong>Adjunct Therapy</strong></td>
<td>35(51%)</td>
<td>53(41%)</td>
<td>0.30 [0.12 - 0.75] p = 0.0095</td>
</tr>
</tbody>
</table>
Clearance of CRKP Bacteriuria

Pesst! Hey kid! Wanna be a Superbug...?
Stick some of this into your genome...
Even won't be able to harm you...!

It was on a short-cut through the Surgical ICU that Albert was first approached by a member of the Antibiotic Resistance.
Thoughts?

Questions?