Detection of Antibiotic Resistance in the Laboratory

Clinical and Public Health Laboratories

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Division of Healthcare Quality and Promotion
Centers for Disease Control and Prevention

Nothing to disclose
Antimicrobial Resistance (AR) Testing Overview

- Identify Issues
  - Critical Superbugs
  - Public Health Response
- Develop Lab Strategies
  - Critical-Common/Uncommon
  - Demonstrate strategies
- Discuss Resources
  - CDC, CLSI, APHL
Looking back through time

- **blaZ PENICILLIN**
  - 1947

- **mecA OXACILLIN**
  - 1997

- **mcr COLISTIN**
  - 2016

- **vanA VANCOMYCIN**
  - 1986

- **blaKPC B-LACTAMS**
  - 2001
2017 WHO “Superbug” List

- Priority 1: Critical
  1. *Acinetobacter baumannii*, carbapenem-resistant
  2. *Pseudomonas aeruginosa*, carbapenem-resistant
  3. Enterobacteriaceae, carbapenem-resistant and ESBL-producing

*These 12 superbugs pose the greatest threat to human health, WHO says*
2017 WHO List (continued)

- Priority 2: High
  4. *Enterococcus faecium*, vancomycin-resistant
  5. *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
  6. *Helicobacter pylori*, clarithromycin-resistant
  7. *Campylobacter* spp., fluoroquinolone-resistant
  8. *Salmonellae*, fluoroquinolone-resistant
  9. *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant
2017 WHO List (end)

- **Priority 3: Medium**
  10. *Streptococcus pneumoniae*, penicillin-non-susceptible
  11. *Haemophilus influenzae*, ampicillin-resistant
  12. *Shigella* spp., fluoroquinolone-resistant
2015 Geographical Distribution of KPC

*Klebsiella pneumoniae* Carbapenemase-Producers
2016 Geographical Distribution of NDM

New Delhi Metallo-β-lactamase Producers

This map was last updated on January, 2016
US Public Health Labs

- CDC ongoing response to AR threat
  - Outbreak support, surveillance
  - Emerging resistance detection
  - [https://www.cdc.gov/hai/settings/lab/lab_settings.html](https://www.cdc.gov/hai/settings/lab/lab_settings.html).

- CDC Core Action: Tracking and disease surveillance
  - 7 Antimicrobial Resistance Regional Network labs- ARLN
  - 55 State and Regional Public Health labs

- CDC Core Action: Support the development of new drugs and diagnostics
  - CDC-FDA AR Isolate Bank
ARLN - Antimicrobial Resistance Laboratory Network

Core Testing: CRE and CRPA CRE Colonization Testing

**WEST**
Washington State PHL
- Core testing
- *Candida* spp.
- *N. gonorrhoeae*

**MOUNTAIN**
Texas DSHS Lab
- Core testing
- *N. gonorrhoeae*
State and Regional Lab testing – CRE/CRPA

Network of participating clinical laboratories

Suspected CRE/CRPA isolates

K6 State testing

1. Species identification
2. Confirmatory AST
3. Phenotypic screening for carbapenemase production
4. Molecular detection of resistance determinants

Isolates with suspected novel resistance* or with discrepant results

4. CRE Colonization Testing
5. Acinetobacter/mcr testing

K7 Regional lab testing

1. Confirmatory AST
2. Confirmatory screening for carbapenemase production
3. Confirmatory detection of resistance determinants

*Positive for carbapenemase production by phenotypic methods and negative by PCR; Alert sent to state HAI coordinator and CDC within 1 day
# FDA-CDC AR Isolate Bank

## Antibiotic / Antimicrobial Resistance

### Overview
The AR Isolate Bank offers panels of resistant bacteria and species.

### Isolates Currently Available
Search through available isolate panels and review.

### Questions and Answers
Submit questions and find answers about the AR Isolate Bank.

### Requesting Isolates
Review isolate request procedures and request an isolate.

## Gram Negative Carbapenemase Detection Panel

This panel was assembled to challenge assays that detect carbapenemase production. Isolates in this collection represent a range of gram-negative bacteria, some producing a carbapenemase and others that demonstrate reduced susceptibility to carbapenems, but do not produce a carbapenemase. This collection was assembled to challenge assays that detect carbapenemase production.

<table>
<thead>
<tr>
<th>Bank #</th>
<th>Species</th>
<th>Carbapenem Susceptibility</th>
<th>Key Resistance Determinants</th>
<th>Sequence Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0032</td>
<td>Enterobacter cloaceae</td>
<td>Resistant</td>
<td>KPC, TEM-1</td>
<td>SAMN04014873</td>
</tr>
<tr>
<td>0033</td>
<td>Acinetobacter baumannii</td>
<td>Resistant</td>
<td>NDM-1</td>
<td>SAMN04014874</td>
</tr>
<tr>
<td>0034</td>
<td>Klebsiella pneumoniae</td>
<td>Reduced Susceptibility*</td>
<td>IMP</td>
<td>SAMN04014875</td>
</tr>
<tr>
<td>0035</td>
<td>Acinetobacter baumannii</td>
<td>Resistant</td>
<td>OXA-72</td>
<td>SAMN04014876</td>
</tr>
<tr>
<td>0036</td>
<td>Acinetobacter baumannii</td>
<td>Resistant</td>
<td>OXA-24, OXA-40</td>
<td>SAMN04014877</td>
</tr>
<tr>
<td>0037</td>
<td>Acinetobacter baumannii</td>
<td>Resistant</td>
<td>NDM-1</td>
<td>SAMN04014878</td>
</tr>
<tr>
<td>0038</td>
<td>Enterobacter cloaceae</td>
<td>Resistant</td>
<td>NDM</td>
<td>SAMN04014879</td>
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<tr>
<td>0039</td>
<td>Klebsiella pneumoniae</td>
<td>Resistant</td>
<td>OXA-181</td>
<td>SAMN04014880</td>
</tr>
<tr>
<td>0040</td>
<td>Klebsiella pneumoniae</td>
<td>Resistant</td>
<td>VIM</td>
<td>SAMN04014881</td>
</tr>
</tbody>
</table>
New Technology: Rapid and Gene Detection

Rapid Molecular Detection

- **Cepheid direct testing**
  - mecA, Xpert Carba-R

- **Verigene BC panels**
  - GP: Van A/B, mecA
  - GN: CTX-M, KPC, MBL, OXA

- **Biofire FilmArray BC panel**
  - Van A/B, mecA, KPC

Rapid Direct Susceptibility

- **Accelerate Diagnostics**
  - Recent FDA approval
  - FISH/Microscopy

- **Bacterioscan**
  - Laser light scatter technology

- **Geneweaver-vivoDX**
  - Gene based, smarticles

**CDC Core Activity**
Developing new drugs and diagnostics
Role of Clinical Microbiology Laboratory

- Patient treatment
  - Realtime AST
  - Report S, I, R, NS (Non-susceptible-rare), SDD (Susceptible dose dependent i.e. Cefepime)

- Antibiogram
  - Annual rates of resistance
    - CLSI M39 Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data

- Infection prevention and Antibiotic Stewardship
  - Resistance detection & notification
  - Testing and reporting with pharmacy, infectious disease specialist
Automated AST Systems

- FDA cleared for patient testing
  - MicroScan Walkaway, Beckman Coulter, Inc.
  - Phoenix™, Becton Dickinson
  - Vitek® 2, bioMérieux
  - “Sensititre™”, Thermo Scientific

High volume throughput, expert rules, limited offline testing required
CLSI Reference Methods

- **Broth Microdilution**
  - Minimal inhibitory concentration – MIC
  - Frozen -70°C
  - Custom made
  - Gold standard
  - Plus CLSI M07

- **Broth Macrodilution**
  - Rarely performed

Free M100 Performance standards

[http://em100.edaptivedocs.info/Login.aspx](http://em100.edaptivedocs.info/Login.aspx)
CLSI Reference Methods

- Disk Diffusion - Correlates to MIC
  - Mueller Hinton Agar + paper antibiotic disks
  - Applied to lawn of one strain
  - Read zone of inhibition in millimeters
  - No special equipment
  - Plus CLSI M02

- Agar Dilution - MIC
  - Agar plates with antibiotic
  - Up to 36 strains applied to plate
  - Antibiotic stock solutions, media and replicator
Gradient diffusion-MIC

- Read MIC where ellipse intersects strips
- Sources
  - bioMérieux Etest®
  - Liofilchem MIC Test Strip
    - limited availability in US
  - Oxoid –M.I.C.Evaluator Strips
    - Europe only
## Interpretation: Breakpoints vs. Epidemiological Cutoff Value

<table>
<thead>
<tr>
<th>Categories</th>
<th>Meaning</th>
<th>Purpose</th>
</tr>
</thead>
</table>
| Clinical BP                     | **Categories**:  
  - Susceptible S  
  - Intermediate I  
  - Resistant R  
  - Non-susceptible NS  
  - Susceptible dose dependent SDD  
|                                | **Meaning**:  
  - Patient Treatment or Surveillance  
  - Based on MIC distributions  
  - PK/PD(pharmokinetic/pharmodynamics)  
  - Clinical outcomes  | **Purpose**:  
  - Predictor of likely clinical outcome |
| DD & MIC equivalent             | **Wild type**  
|                                | **Non wild type**  
|                                | **Meaning**:  
  - Species specific  
  - Surveillance  
  - Based on MIC distribution  | **Purpose**:  
  - Separates microbial population with/without acquired or mutational resistance |
| ECV                             | **Wild type**  
|                                | **Non wild type**  
|                                | **Meaning**:  
  - Species specific  
  - Surveillance  
  - Based on MIC distribution  | **Purpose**:  
  - Separates microbial population with/without acquired or mutational resistance |
AR News and Resources

- clsi.org
  - CLSI Outreach Working group
  - Clinical Laboratory Newsletter
    - Education on new methods
    - Info on emerging resistance
    - Templates, i.e. verification
- APHL.org – webinars
- eucast.org (“European CLSI”)
CLSI Verification template: Example

- Verify Breakpoints
- Test 30 S/R
- Compare Category
- Determine agreement
- Errors
  - Very Major R /S
  - Major S/R
  - Minor S/I
Staphylococcus Mechanisms of Resistance
Predominantly S. aureus, but applies to Coag Neg Staph too!

- Vancomycin
  - VRSA acquired on a plasmid
  - VISA chromosomal mediated cell wall mutations

- Methicillin MRSA
  - acquired Staph cassette, chromosomal

- Penicillin Resistance
  - acquired β-lactamase, chromosomal
**Staphylococcus Newer antimicrobials**

- **Daptomycin**: rare non-susceptible
- **Linezolid**: rare resistance, clusters
- **Ceftaroline**: new anti-MRSA, very rare resistance

When resistance encountered, confirm id and purity, repeat testing, refer to CLSI M100 Appendix A. Suggestions for confirmation of R, I, NS Results and Organisms identification.
Staphylococcus / Vancomycin

• **VRSA:** Glycopeptide resistant *S. aureus*
  – Vancomycin MIC $\geq 16 \mu g/ml$, plasmid mediated, refer

• **VISA:** Glycopeptide intermediate *S. aureus*
  – Vancomycin MIC 4 – 8 µg/ml, cell wall mediated
    • **hVISA:** Heterogeneous glycopeptide susceptible *S. aureus*
      – Majority of population MICs $\leq 2$, Minority populations MIC $> 2$

• **CoNS:**
  intermediate MIC 8 -16µg/ml; resistant $\geq 32$, refer

www.cdc.gov/HAI/settings/lab/visa_vrsa_algorithm.html
VISA Case: Patient is 35 yo female, umbilical hernia repair 2 weeks prior to first positive culture-wound

- *Staphylococcus aureus* culture positive

<table>
<thead>
<tr>
<th>Date</th>
<th>Specimen</th>
<th>VA MIC µg/ml</th>
<th>BP</th>
<th>Etest</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>08/17</td>
<td>Wound</td>
<td>0.5</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>08/31</td>
<td>Blood</td>
<td>1</td>
<td>S</td>
<td>2</td>
<td>S</td>
</tr>
<tr>
<td>09/05</td>
<td>Blood</td>
<td>2</td>
<td>S</td>
<td>3</td>
<td>I</td>
</tr>
<tr>
<td>09/06</td>
<td>Blood</td>
<td>4</td>
<td>I</td>
<td>3</td>
<td>I</td>
</tr>
</tbody>
</table>
Etest® Vancomycin  MIC = 3µg/ml

- 09/05 BD CX: Report MIC and comment;
  The vancomycin MIC and Etest are within one doubling dilution. Since this BMD is the reference method, this isolate is susceptible to vancomycin. The elevated BMD and Etest results suggest the isolate is developing reduced susceptibility
- Strategy: VA Etest, available, test all isolates
**Staphylococcus Oxacillin and mecA detection**

- Strategy: Supplement automated instruments with FOX 30µg & OX 1µg disk testing

<table>
<thead>
<tr>
<th></th>
<th>Cefoxitin</th>
<th>Oxacillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disk</td>
<td>BMD</td>
<td>Disk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BMD</td>
</tr>
<tr>
<td>S. aureus</td>
<td>$S \geq 22$</td>
<td>$S \leq 4$</td>
</tr>
<tr>
<td></td>
<td>$R \leq 21$</td>
<td>$R \geq 8$</td>
</tr>
<tr>
<td>CoNeg Staph</td>
<td>$S \geq 25$</td>
<td>Do not use</td>
</tr>
<tr>
<td></td>
<td>$R &lt; 24$</td>
<td></td>
</tr>
<tr>
<td>S. Pseudo-</td>
<td>Do not use</td>
<td>Do not use</td>
</tr>
<tr>
<td>intermedius</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Staphylococcus *mecC*
homologue *mecALGA251*

- Why routine AST still matters
  - OX susceptible or reduced susceptibility
  - FOX usually resistant
  - For serious infections, do *mecA* or PBP2a
    - If negative, use cefoxitin result to report oxacillin

García-Álvarez et al., 2011 11:595-603 Lancet Infect Di
Staph B-lactamase testing

- Penicillin susceptible *Staphylococcus*
  - Nitrocefin for \(\beta\)-lactamase: *S. aureus* and *CoNS*
  - *For S. aureus/lugdunensis*--If negative, do the PEN Zone Edge

PEN 10µg disk

![Image](image_url)

- Sharp or "cliff"
- Fuzzy or "beach"

Report PEN R

Report PEN S
Enterococcus

• Vancomycin
  – ID important in serious infections
• High level Gentamicin/Streptomycin
  – Endocarditis, other sterile sites
• Linezolid-rare resistance
• Daptomycin- rare non-susceptible

Hollenbeck, BL & Rice LB, 2012 Virulence 3:5, 421-433
Enterococcus - Vancomycin

- *vanA* plasmid mediated, MIC > 256 $\mu$g/ml
  - *E. faecium*, Ampicillin/teicoplanin R
  - *E. faecalis*, Ampicillin/teicoplanin S

Recent Report: [NEWVancomycin-variableEnterococcus](#)

- *vanB* plasmid mediated, MIC 16-64 $\mu$g/ml
  - *E. faecalis*, Ampicillin/teicoplanin S

- *vanC*, chromosomal MIC 8-16 $\mu$g/ml,
  - *E. gallinarium/cassiaflavus*, teicoplanin S
Pathology Question of the Day: Vancomycin (VA)

- Which of the following susceptibility profiles would be typical of an *Enterococcus faecalis* harboring *vanB*?

- VA MIC 2, susceptible to teicoplanin
- VA MIC 8, susceptible to teicoplanin
- VA MIC 32, susceptible to teicoplanin
- VA MIC 256, resistant to teicoplanin

- Pathology Question of the Day
- https://pathquestions.com
Detection of CRE is a challenge. Why?

- Entire family of organisms!
  - Many genera & species
  - *E. coli, Klebsiella, Enterobacter* are most common
  - Definition updated in 2015: Resistant to any carbapenem

- Numerous mechanisms for resistance
  - Carbapenemase production – Largest public health threat
  - ESBL + porin mutations
  - AmpC + porin mutations
  - Intrinsic resistance
    - Imipenem Not-susceptible: *Proteus, Providencia, Morganella*
Detection of CRPA and CR-Acinetobacter is a challenge. Why?

- Numerous mechanisms of resistance!
  - Chromosomal:
    - Poor outer membrane permeability
    - Multidrug efflux pumps
    - Inducible β-lactamase (AmpC)
  - Integron/plasmid mediated:
    - Carbapenemases; IMP and VIM variants have been detected in the U.S. since 2001
    - OXA genes with weak carbapenemases OXA-72
## Acquired 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</td>
<td><em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td></td>
<td>SME</td>
<td><em>Serratia marcescens</em></td>
</tr>
<tr>
<td></td>
<td>IMI, NMC</td>
<td><em>Enterobacter cloacae</em></td>
</tr>
<tr>
<td>Class B</td>
<td>NDM</td>
<td><em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td>(metallo-β-lactamase)</td>
<td>IMP, VIM</td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td>Class D</td>
<td>OXA-48-like</td>
<td><em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td></td>
<td>OXA-23, -24/40, -58</td>
<td><em>Acinetobacter spp.</em></td>
</tr>
</tbody>
</table>
1. *Klebsiella pneumoniae* carbapenemase (KPC)
   - Most common carbapenemase in the United States
   - Confers resistance to ALL β-lactam agents

2. New Delhi Metallo-β-lactamase (NDM)
   - First detected in 2009; rapid global spread, primarily among Enterobacteriaceae
   - Previously associated with travel, now with domestic transmission

3. OXA-48- like enzymes
   - Inefficient carbapenemases, don’t hydrolyze cephalosporins well
   - Commonly travel with other β-lactamases

4. Verona Integron-encoded Metallo-β-lactamase (VIM)
   - Relatively slow global spread; mostly in *P. aeruginosa*

5. Imipenem Metallo-β-lactamase (IMP)
   - Relatively slow global spread; mostly in *P. aeruginosa*
## CRE and CRPA

<table>
<thead>
<tr>
<th>Lab detection</th>
<th>State/Local Labs</th>
<th>Regional Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• ATIs (Vitek, MicroScan, Phoenix etc.)</td>
<td>• ATIs (Vitek, MicroScan, Phoenix etc.)</td>
</tr>
<tr>
<td>2. Antimicrobial</td>
<td>• Broth Microdilution</td>
<td>• Broth Microdilution</td>
</tr>
<tr>
<td>Susceptibility Testing</td>
<td>• Disk Diffusion</td>
<td>• (Disk Diffusion)</td>
</tr>
<tr>
<td></td>
<td>• Etest</td>
<td></td>
</tr>
<tr>
<td>3. Carbapenemase</td>
<td>• mCIM</td>
<td>• mCIM</td>
</tr>
<tr>
<td>screening</td>
<td>• Carba NP</td>
<td>• Carba NP</td>
</tr>
<tr>
<td>4. Molecular Detection of</td>
<td>• Real-time PCR</td>
<td>• Real-time PCR</td>
</tr>
<tr>
<td>AR Determinants</td>
<td><strong>CRE</strong>: KPC, NDM, OXA-48-like (IMP, VIM, mcr optional)</td>
<td><strong>CRE</strong>: KPC, NDM, OXA-48, VIM, IMP, mcr</td>
</tr>
<tr>
<td></td>
<td><strong>CRPA</strong>: KPC, NDM, VIM (VIM, mcr optional)</td>
<td><strong>CRPA</strong>: KPC, NDM, VIM, IMP, mcr</td>
</tr>
</tbody>
</table>
# CR-Acinetobacter spp. and ESBL producers

<table>
<thead>
<tr>
<th>Lab detection</th>
<th>CR-Acinetobacter spp.</th>
<th>ESBL-prod <em>E. coli</em> and <em>Klebsiella spp.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Species ID</strong></td>
<td>• MALDITof Mass Spec. • ATIs (Vitek, MicroScan, Phoenix etc.)</td>
<td>• MALDITof Mass Spec. • ATIs (Vitek, MicroScan, Phoenix etc.)</td>
</tr>
<tr>
<td><strong>2. Antimicrobial Susceptibility Testing</strong></td>
<td>• Broth Microdilution</td>
<td>• Broth Microdilution • ESBL</td>
</tr>
<tr>
<td><strong>3. Carbapenemase screening</strong></td>
<td>• Carba NP</td>
<td>• Not Applicable</td>
</tr>
<tr>
<td><strong>4. Molecular Detection of AR Determinants</strong></td>
<td>• Real-time PCR <strong>Most common: KPC and NDM Optional: OXA-48, IMP, VIM, mcr</strong></td>
<td>• Real-time PCR <strong>mcr-1</strong></td>
</tr>
</tbody>
</table>
1. Species Identification

- MALDI-TOF instruments are commonly used for bacterial identification in clinical microbiology labs
  - Very rapid, accurate, inexpensive bacterial ID
- Lots of interest in using these systems for other purposes
  - Strain typing
  - AR detection (e.g. carbapenemase detection)
2. AST–CRE and CRPA

Table 1. Drugs used to confirm and further characterize carbapenem-resistant Enterobacteriaceae (CRE) and carbapenem-resistant *P. aeruginosa* (CRPA)

<table>
<thead>
<tr>
<th>Drug class</th>
<th>CRE</th>
<th>CRPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbapenems</td>
<td>2 carbapenems (ertapenem and either imipenem, doripenem or meropenem)</td>
<td>2 carbapenems (selected from imipenem, doripenem and meropenem)</td>
</tr>
<tr>
<td>Cepheims</td>
<td>ceftazidime, ceftriaxone&lt;sup&gt;a&lt;/sup&gt;, and cefepime</td>
<td>ceftazidime and cefepime</td>
</tr>
<tr>
<td>B-lactam/B-lactamase inhibitor combinations</td>
<td>NA</td>
<td>piperacillin-tazobactam</td>
</tr>
<tr>
<td>Monobactams</td>
<td>aztreonam</td>
<td>aztreonam</td>
</tr>
<tr>
<td>Polymyxins</td>
<td>colistin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>colistin&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Other third-generation cephalosporin(s) may be substituted for ceftriaxone

<sup>b</sup>Broth microdilution only
## 2. AST-Acinetobacter and ESBL-producers

Table 1. Drugs used to confirm carbapenem-resistant *Acinetobacter* and third-generation-resistant *E. coli* and *Klebsiella* spp.

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Carbapenem-resistant <em>Acinetobacter</em></th>
<th>Third-generation cephalosporin-resistant <em>E. coli</em> and <em>Klebsiella</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbapenems</td>
<td>2 carbapenems (imipenem, doripenem, or meropenem)</td>
<td>2 carbapenems (ertapenem and either imipenem, doripenem, or meropenem)</td>
</tr>
<tr>
<td>Cephems</td>
<td>ceftazidime and cefepime</td>
<td>cefotaxime, ceftriaxone, ceftazidime, and cefepime</td>
</tr>
<tr>
<td>B-lactam/B-lactamase inhibitor combinations</td>
<td>piperacillin-tazobactam</td>
<td>ceftazidime- and cefotaxime-clavulanate</td>
</tr>
<tr>
<td>Monobactams</td>
<td>aztreonam</td>
<td>aztreonam</td>
</tr>
<tr>
<td>Polymyxins</td>
<td>colistin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>colistin&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> BMD only
## SENSITITRE™ GRAM NEGATIVE PLATE FORMAT

Plate Code: GNX2F

<p>| | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>AMI</td>
<td>AZT</td>
<td>SXT</td>
<td>FEP</td>
<td>LEVO</td>
<td>CIP</td>
<td>MERO</td>
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<td>AZT</td>
<td>SXT</td>
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<td>PT14</td>
<td>GEN</td>
<td>TCB</td>
<td>DOX</td>
<td>MIN</td>
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<tr>
<td>16/2</td>
<td>8/4</td>
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<td>1</td>
<td>2</td>
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<td>2</td>
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<td>8</td>
<td>16</td>
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<tr>
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<td>PT14</td>
<td>GEN</td>
<td>TCB</td>
<td>DOX</td>
<td>MIN</td>
<td>TGC</td>
<td>TGC</td>
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<td>32/2</td>
<td>16/4</td>
<td>2</td>
<td>4</td>
<td>4</td>
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<td>0.5</td>
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<td>2</td>
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<tr>
<td>G</td>
<td>TIM2</td>
<td>PT14</td>
<td>GEN</td>
<td>TCB</td>
<td>DOX</td>
<td>MIN</td>
<td>ETP</td>
<td>ETP</td>
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</tr>
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<td>0.5</td>
<td>1</td>
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<tr>
<td>H</td>
<td>TIM2</td>
<td>PT14</td>
<td>GEN</td>
<td>TCB</td>
<td>DOX</td>
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<td>IMI</td>
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<tr>
<td>128/2</td>
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<td>8</td>
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<td>16</td>
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<td>2</td>
<td>4</td>
<td>8</td>
<td>POS</td>
<td></td>
</tr>
</tbody>
</table>

**ANTIMICROBICS**

- **AMI**: Amikacin
- **TIM2**: Tioconazole / sivulanid acid constant 2
- **AZT**: Aztreonam
- **PT14**: Pipemidic / tazobactam constant 4
- **SXT**: Trimethoprim / sulfamethoxazole
- **GEN**: Gentamycin
- **FEP**: Cefepime
- **TOB**: Tobramycin
- **LEVO**: Levofloxacin
- **DOX**: Doxycycline
- **CIP**: Ciprofloxacin
- **MIN**: Minocycline
- **MERO**: Meropenem
- **FOT**: Cotrimoxazole
- **TGC**: Ticarcillin
- **ETP**: Eriyepenim
- **IMI**: Imipenem
- **DOR**: Doripenem
- **COL**: Colistin
- **POL**: Polymyxin B
- **TAZ**: Cefazolin
- **POS**: Positive Control
A few words about colistin susceptibility testing...

- Associated with methodological issues and technical challenges
  - Adherence of colistin to surfaces used for MIC trays/panels (e.g. polystyrene)
  - Disk diffusion does not work because of poor diffusion of the colistin molecule
  - Warning against Etest issued during 2016 – underestimates MIC by 1-2 doubling dilutions
    - Reports of up to 32% of VMEs (false susceptibility) compared to BMD
    - CDC is currently evaluating BMD vs Etest
Colistin Susceptibility Testing - Recommendations

- Test and report according to CLSI guidance (CLSI M100-S27)

<table>
<thead>
<tr>
<th>Organism Group</th>
<th>MIC µg/ml</th>
<th>Interpretation</th>
<th>MIC µg/ml</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae*</td>
<td>2</td>
<td>ECV = WT Suggests no acquired/mutational resistance</td>
<td>4</td>
<td>ECV = NWT Suggests acquired/mutational resistance</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>2</td>
<td>BP = Susceptible</td>
<td>4</td>
<td>BP = Resistant</td>
</tr>
<tr>
<td>Ac. baumannii</td>
<td>2</td>
<td>BP = Susceptible</td>
<td>4</td>
<td>BP = Resistant</td>
</tr>
</tbody>
</table>

*Appendix G: E. coli, E. aerogenes, E. cloacae, K. pneumoniae and R. ornithinolytica
Colistin resistance; PCR-screening for *mcr* genes

- *mcr*; first described in China 2015; plasmid-mediated gene conferring colistin resistance
- Multiple *mcr-positive* isolates have been confirmed in the United States; (*E. coli, Salmonella enterica* ser. Enteritidis, Typhimurium and Corvallis)
- An *mcr* real-time PCR assay has been developed and is available from CDC

**Screening Recommendations:**
- Enterobacteriaceae isolates displaying a COL MIC of ≥4 µg/ml
- *If other risk factors exist* (e.g. recent travel outside the United States) - test *Enterobacter* species with COL MIC of ≥2 µg/ml

HAN June 2016: [https://emergency.cdc.gov/han/han00390.asp](https://emergency.cdc.gov/han/han00390.asp)
3. Phenotypic screening for carbapenemase production

- Used to determine whether isolate is producing a carbapenemase, but does not determine which carbapenemase is present
  - Modified Carbapenem Inactivation Method (mCIM) 2017
    - CLSI approved (Enterobacteriaceae, *P. aeruginosa*) 2017
      - ~97% sensitivity and ~99% specificity for Enterobacteriaceae
  - Carba NP CLSI Approved in 2015
  - Modified Hodge
    - test to be retired in 2018
CIM- Carbapenem Inactivation Method

- First described: Van der Zwaluw et al PLoS ONE 2015 10 (3) 1-13

- mCIM Enterobacteriaceae 1 µl loop of inoculum or P. aeruginosa 10 µl loop, into 2 ml of TSB with 10 µg meropenem disk; incubate 4 hr.
mCIM Result Possibilities

Positive mCIM (colonies in 16-18 mm zone)

Indeterminant mCIM (colonies in ≥19 mm zone)

Negative mCIM (≥ 19 mm zone)

Positive mCIM (6-15 mm zone)

- ≤15 mm zone of inhibition, positive for carbapenemase
- 16-18 mm zone of inhibition, indeterminant
- ≥19 mm zone of inhibition, negative for carbapenemase
Carba NP

- pH change when imipenem cleaved by carbapenemase
  - Change in color of pH indicator
- Phenol red indicator: Positive = Red $\rightarrow$ Yellow
- Same-day results (~2 hrs.)
- Strong carbapenemase results within 10 min
- Decreased sensitivity with weak carbapenemase i.e. OXA-48-like

Commercial methods—not FDA approved
- RapidEC (biomérieux) – pending approval
- Neo Rapid Carba and Blue Carba (Roscoe)
# Carba NP vs mCIM

<table>
<thead>
<tr>
<th></th>
<th>CarbaNP</th>
<th>mCIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSI approval</td>
<td>Enterobacteriaceae, <em>P. aeruginosa</em>, <em>Acinetobacter</em> spp.</td>
<td>Enterobacteriaceae, <em>P. aeruginosa</em></td>
</tr>
<tr>
<td>Incubation time</td>
<td>2h</td>
<td>4h +18-24h overnight</td>
</tr>
<tr>
<td>Pros</td>
<td>quick TAT; approved for <em>Acinetobacter</em> spp.</td>
<td>Easier to perform and interpret; inexpensive; increased sensitivity for weak carbapenemases</td>
</tr>
<tr>
<td>Cons</td>
<td>Difficult to interpret; test solution with imipenem needs to be made up fresh; decreased sensitivity for weak carbapenemases</td>
<td>CLSI not approved for <em>Acinetobacter</em>;</td>
</tr>
</tbody>
</table>

M100 Tables 3C and D
## PCR assays

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Type</th>
<th>Source</th>
<th>Targets</th>
<th>Instrumentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real-time PCR KPC/NDM</td>
<td>PCR</td>
<td>CDC</td>
<td>bla_\text{KPC}/bla_\text{NDM}</td>
<td>ABI 7500</td>
</tr>
<tr>
<td>Real-time PCR OXA-48</td>
<td>PCR</td>
<td>CDC</td>
<td>bla_\text{OXA-48-like}</td>
<td>ABI 7500</td>
</tr>
<tr>
<td>Real-time PCR VIM</td>
<td>PCR</td>
<td>CDC</td>
<td>bla_\text{VIM}</td>
<td>ABI 7500</td>
</tr>
<tr>
<td>Real-time PCR IMP</td>
<td>PCR</td>
<td>CDC</td>
<td>bla_\text{IMP}</td>
<td>ABI 7500</td>
</tr>
<tr>
<td>Real-time PCR mcr-1</td>
<td>PCR</td>
<td>CDC</td>
<td>mcr-1</td>
<td>ABI 7500</td>
</tr>
<tr>
<td>X-pert Carba-R</td>
<td>PCR</td>
<td>Cepheid</td>
<td>bla_KPC, bla_NDM, bla_OXA-48-like, bla_VIM, bla_IMP-1 group</td>
<td>GeneXpert®</td>
</tr>
</tbody>
</table>
Cepheid GeneXpert Carba-R Assay

• The only FDA-approved, commercially available CRE detection assay
• Requires the use of the Cepheid Gene Xpert system, Carba-R analysis software, and proprietary kit reagents
• Short turn-around time of ≤1 day
• Regional Lab Colonization Testing:
  – Use of Cepheid dual swab collection kit (Cepheid catalog #900-0370) is encouraged and will be purchased in bulk by CDC and distributed to ARLN regional labs
  – Swabs in transport medium can be stored at 15-28°C for up to 5 days
Case: ID and AST

Isolate sent as *Klebsiella pneumoniae* but MALDI-TOF ID reports *Raoultella ornitholytica*

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ertapenem</td>
<td>MIC= &gt;4 µg/ml</td>
<td>Resistant</td>
</tr>
<tr>
<td>Meropenem</td>
<td>MIC= &gt;8 µg/ml</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>MIC= 16 µg/ml</td>
<td>Resistant</td>
</tr>
<tr>
<td>Colistin</td>
<td>MIC= 4 µg/ml</td>
<td>NWT</td>
</tr>
<tr>
<td>Carba NP</td>
<td>Yellow</td>
<td>Carbapenemase producer</td>
</tr>
<tr>
<td>Real-time PCR</td>
<td>KPC Positive</td>
<td>KPC-producer</td>
</tr>
</tbody>
</table>

Solution/tips

- Report as carbapenemase producer, notify Infection Control
Case: Disk diffusion

- You perform disk diffusion on an *E. cloacae*. After 18h of incubation you notice the following:

- Possible explanations
  - Contamination
  - Resistance in select colonies
Case: Disk Diffusion

- Solution
  - Check purity
  - Repeat testing

- Note if repeat testing gives same result, may be a carbapenemase producer (KPC)
**Case: AST and intrinsic resistance**

*Proteus mirabilis* from urology outpatient clinic is suspected CRE

<table>
<thead>
<tr>
<th>Test: BMD</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ertapenem</td>
<td>MIC = 0.25 µg/ml</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Imipenem</td>
<td>MIC = 8 µg/ml</td>
<td>Resistant</td>
</tr>
<tr>
<td>Meropenem</td>
<td>MIC = 0.5 µg/ml</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>MIC = 16 µg/ml</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>MIC = 1 µg/ml</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Colistin</td>
<td>MIC = &gt;4 µg/ml</td>
<td>Intrinsic Resistance</td>
</tr>
</tbody>
</table>

CRE negative

- Proteus has elevated MICs to imipenem by other mechanisms

Other resistance mechanisms

- Suspect ESBL
  - (possibly CTX_M)
- Proteus intrinsically resistant to colistin
  - M100 Appendix B
Case: Molecular testing

Possible Explanations

- Resistance gene not tested
- (VIM)
- Possible novel resistance mechanism
- Variant of resistant gene not targeted in assays

Solution/Tips

- Send isolate to state lab for further testing/investigation
- Emphasis of the importance of Carbapenemase testing

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCIM</td>
<td>7 mm</td>
<td>Carbapenemase - producing</td>
</tr>
<tr>
<td>PCR</td>
<td>Negative (NDM, KPC)</td>
<td></td>
</tr>
<tr>
<td>Cepheid</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>
Case: Carbapenemase Testing

Long-term care patient has *Escherichia coli* isolated from a urine specimen

Q: What is the likely mechanism?
A: OXA-48

Solution/Tips
- Check purity
- Repeat Carba NP

Solution: Report MIC, do PCR for most common genes, send to State lab
Specimen Handling

- Purity check
  - Colony morphologies
  - Mixed cultures variation same isolate

- Preserve specimens
  - Refrigerate or freeze -70° C preferred
  - Limit repeated subculture, sitting on the bench at room temperature
Questions?

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Brian Yoo – State Support Team
Annie Kim – State Support Team
Allison Brown – State Support Team
James Kamile Rasheed – ARCL Team
David Lonsway – ARCL Team

Clinical and Environmental Microbiology Branch
Thank you!

For more information please contact Centers for Disease Control and Prevention
1600 Clifton Road NE, Atlanta, GA 30333
Telephone: 1-800-CDC INFO (232-4636)/TTY: 1-888-232-6348
E-mail: cdcinfo@cdc.gov Web: http://www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
<table>
<thead>
<tr>
<th>Real-time assay</th>
<th>Format</th>
<th>Chemistry</th>
<th>Targets&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IAC&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mmx kit</th>
<th>Run time</th>
<th>Validation</th>
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</thead>
<tbody>
<tr>
<td>KPC/NDM</td>
<td>Multiplex</td>
<td>Taqman (FAM-BHQ1 and HEX-BHQ1)</td>
<td>KPC 1-27; NDM 1-16</td>
<td>16S (Cy5-BHQ3)</td>
<td>2x Quanti-Fast Probe PCR Kit&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45 min</td>
<td>4 KPC+, 9 NDM+, 1 KPC-/NDM+, 38 KPC-/NDM-</td>
</tr>
<tr>
<td>OXA-48-like</td>
<td>Multiplex</td>
<td>Taqman (FAM-BHQ1)</td>
<td>OXA-48, -162,-163, -181,-204, -232,-244, -245,-247</td>
<td>16S (Cy5-BHQ3)</td>
<td>2x Quanti-Fast Probe PCR Kit</td>
<td>45 min</td>
<td>10 OXA-48-like+, 30 OXA-48-like-</td>
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<tr>
<td>VIM</td>
<td>Multiplex</td>
<td>Taqman (FAM-BHQ1)</td>
<td>VIM 1-6; 8-46</td>
<td>16S (Cy5-BHQ3)</td>
<td>2x Quanti-Fast Probe PCR Kit</td>
<td>45 min</td>
<td>10 VIM+, 30 VIM-</td>
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<tr>
<td>IMP</td>
<td>Multiplex</td>
<td>Taqman (FAM-BHQ1 and HEX-BHQ1)</td>
<td>IMP 1-58</td>
<td>16S (Cy5-BHQ3)</td>
<td>2x Quanti-Fast Probe PCR Kit</td>
<td>45 min</td>
<td>Pending</td>
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</tbody>
</table>

<sup>a</sup> As determined by <em>in silico</em> analysis of primers
<sup>b</sup> IAC, Internal Amplification Control
<sup>c</sup> Qiagen, Valencia, CA, USA
Carbapenemase testing - Enzyme based

- pH change when imipenem cleaved by carbapenemase
  - Change in color of pH indicator, Same-day results (~2 hrs.)

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Carba NP</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>mCarba NP</td>
<td>96%</td>
<td>100%</td>
<td>AbdelGhani et al. 2015. JCM</td>
</tr>
<tr>
<td>Neo-Rapid Carb</td>
<td>99%</td>
<td>100%</td>
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<tr>
<td>RapidEC</td>
<td>98%</td>
<td>98%</td>
<td>Poirel et al. 2015. JCM</td>
</tr>
<tr>
<td>Blue-Carba</td>
<td>100%</td>
<td>100%</td>
<td>Pires et al. 2013.</td>
</tr>
<tr>
<td>Blue-Carba</td>
<td>100%</td>
<td>100%</td>
<td>Novais et al. 2015. JCM</td>
</tr>
<tr>
<td>Rapid Carb Blue</td>
<td>93.3%</td>
<td>100%</td>
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### Reporting MRSA or MSSA with Oxacillin results

- **S. aureus** – report OX

<table>
<thead>
<tr>
<th>FOX BMD</th>
<th>FOX BMD or DD</th>
<th>meca or PBP2a POS</th>
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</thead>
<tbody>
<tr>
<td>OX S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>OX R</td>
<td>R</td>
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</tbody>
</table>

### CoNS-report OX

<table>
<thead>
<tr>
<th>FOX DD S</th>
<th>FOX DD R</th>
<th>meca or PBP2a POS</th>
</tr>
</thead>
<tbody>
<tr>
<td>OX S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>OX 0.5-2</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>OX ≥4</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>