California Antimicrobial Resistance Laboratory Network: Carbapenemase Testing at the CDPH Microbial Diseases Laboratory: New Tests and Submission Options

Webinar
November 8th, 2017
Presenters

- **Sam Horwich-Scholefield, MPH CIC**
  Antimicrobial Use and Resistance Coordinator
  Healthcare-Associated Infections Program
- **Stephanie Abromaitis, Ph.D.**
  Foodborne & Waterborne Diseases Section
  Microbial Diseases Laboratory
- **Peng Zhang, Ph.D.**
  Bacterial Diseases Section
  Microbial Diseases Laboratory
- **Hillary Berman Watson, Ph.D. MPH**
  Core Laboratory
  Microbial Diseases Laboratory
- **Robin Hogue, CLS PHM**
  Bacterial Diseases Section
  Microbial Diseases Laboratory
- **Erin Epson, MD**
  Assistant Chief/ Public Health Medical Officer
  Healthcare-Associated Infections Program
Objectives

• Review the role of laboratory testing in preventing the spread of carbapenemase-producing organisms
• Describe phenotypic and molecular tests for carbapenemase detection available at MDL
• Present different carbapenemase submission and testing scenarios
• Provide detailed instructions on specimen submission
Enterobacteriaceae

- Gram negative bacteria - normal human gut flora
  - *Citrobacter* spp.
  - *E. coli*
  - *Enterobacter* spp.
  - *Klebsiella* spp
  - *Morganella* spp.
  - *Proteus* spp.
  - *Salmonella* spp.
  - *Serratia* spp.

- Causative agents of various types of infections
  - UTI, wound infections, pneumonia, bacteremia

- Transmission, outbreaks in healthcare settings
Carbapenem-Resistant Enterobacteriaceae

• Carbapenem antibiotics generally reserved for Enterobacteriaceae that are resistant to other antibiotics

• Infections caused by carbapenem-resistant Enterobacteriaceae (CRE) are more difficult to treat and associated with high mortality

• Risk factors for CRE include healthcare exposures, medical devices and antibiotic use
Carbapenem-Resistant Enterobacteriaceae (CRE)

CDC 2015 Surveillance Definition of CRE

• Any Enterobacteriaceae that is either:
  – Resistant to at least one carbapenem antibiotic
  - OR -
  – Demonstrated to produce carbapenemase (e.g., KPC, NDM, OXA, VIM, IMP)
Carbapenem Resistance in the US

Source: Center for Disease Dynamics, Economics, and Policy (CDDEP)
Isolate level data was obtained from The Surveillance Network (TSN)
CRE Among Healthcare-Associated Infections

Source: Antibiotic Resistance Patient Safety Atlas
Data from National Healthcare Safety Network (NHSN), 2011-2014
Percentage CRE Among HAI Reported to NHSN, 2014-2015, California Acute Care Hospitals (N=342)
Different Types of CRE

• **Carbapenemase-producing CRE (CP-CRE)**
  – produce enzymes that make carbapenems ineffective (e.g., KPC, NDM, OXA, VIM, IMP)

• **Non-carbapenemase producing CRE (non CP-CRE)**
  – resistant by other mechanisms (e.g., ESBL or AmpC combined with porin loss mutation)
CRE Iceberg

Patients test 'CRE'-positive by antimicrobial susceptibility testing

6 Permutations of Nosocomial CRE

- CP | Non-CP
- CP | Non-CP
- CP | Non-CP

Transmission-mediated exogenous acquisition
Dual: antibiotic pressure and exogenous exposure
Antibiotic selective pressure-induced endogenous acquisition or enrichment

Patients enter hospital CRE-negative

CP-CRE are a Public Health Threat

- Carbapenemases can be transmitted between bacteria; increased incidence of CRE in the US is due to CP-CRE
- Higher mortality with invasive CP-CRE infections
  - Adjusted odds of dying more than 4 times greater for CP-CRE compared with non-CP-CRE
- CDC identifies CRE as urgent public health threat

Source: Guh et al Epidemiology of CRE, 2012-2013 JAMA 2015
Source: Tamma et Mortality with CP-CRE bacteremia CID 2017
Carbapenemase Testing

• CP-CRE warrant measures to assess and prevent further transmission in healthcare settings

• Carbapenemase testing to distinguish CP-CRE from non-CP CRE informs
  – Better understanding of your hospital’s CRE epidemiology
  – Immediate infection control interventions
  – Epidemiologic investigation
  – Public health response actions
Scenario: Hospitalized Patient Identified with CRE

Single room, contact precautions

CRE isolate submitted for carbapenemase testing

Carbapenemase negative

Transmission-based precautions per facility policy; No additional measures unless transmission suspected

Continue routine surveillance

Carbapenemase positive (e.g., KPC, NDM)

-Cohort patients with CP-CRE on same unit/ward
-Dedicated staffing
-Colonization testing of epi-linked patient contacts

Patient contact(s) CRE –
Continue routine surveillance

Patient contact(s) CRE+
Epidemiologic investigation
Different Types of Carbapenemase Testing

- **Phenotypic testing:** identifies whether or not an isolate is a carbapenemase producer
  - Modified carbapenem inactivation method (mCIM)

- **Molecular testing:** identifies the specific type of carbapenemase present
  - Real-time PCR testing using Cepheid Xpert® Carba-R, Whole Genome Sequencing

- Discrepant results, e.g., positive phenotypic test and negative genotypic test, might represent a novel carbapenemase
Carbapenemase Gene Detection by Cepheid Xpert® Carba-R Assay

Stephanie Abromaitis, Ph.D.
Section Chief - Foodborne & Waterborne Diseases Section
Microbial Diseases Laboratory Program
Overview:

• What the Xpert® Carba-R detects
• How the Xpert® Carba-R works
• MDL verification summary
What the Xpert® Carba-R Detects

- The Xpert® Carba-R detects and differentiates gene sequences for the carbapenemase resistance genes
  - blaKPC (KPC)
  - blaNDM (NDM)
  - blaVIM (VIM)
  - blaIMP (IMP)
  - blaOXA-48 like (OXA-48)
California non-KPC CRE Reported to the Centers for Disease Control and Prevention (CDC)

Numbers reflect totals as of June 2017
What the Xpert® Carba-R Detects

- There are multiple variants of each carbapenemase gene
- Not all variants of each of the “Big Five” carbapenemases are detected by the Xpert® Carba-R

<table>
<thead>
<tr>
<th>Carbapenemase</th>
<th>Variants Detected by Xpert® Carba-R</th>
<th>Variants Not Detected by Xpert® Carba-R</th>
<th>Untested Variants (partial list)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP</td>
<td>IMP-1, 2, 6, 10, 11</td>
<td>IMP-7, 13, 14</td>
<td>IMP-3, 8, 9, 19, 20, 21, 22, 24, 25, 27, 30, 31, 33, 37, 40, 42</td>
</tr>
</tbody>
</table>

Adapted from Cepheid Xpert® Carba-R 510(k) Substantially Equivalent documents
How the Xpert® Carba-R Works

- Automated system for
  - DNA extraction
  - Template amplification
  - Target sequence detection via real-time PCR
MDL Verification Summary

- Tested isolates grown on blood agar
  - 32 carbapenemase-positive
  - 10 carbapenemase-negative
- Included:
  - 13 species
  - 19 carbapenemase gene variants
  - Isolates encoding non-carbapenemase beta-lactamases

<table>
<thead>
<tr>
<th>Verification Results</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy = 97.6%</td>
<td>Sensitivity = 96.9%</td>
</tr>
<tr>
<td>Reproducibility = 100%</td>
<td>Specificity = 100%</td>
</tr>
</tbody>
</table>
MDL Xpert® Carba-R Testing Thus Far

36 isolates received 08/30/2017 to 10/31/2017

<table>
<thead>
<tr>
<th>Carbapenemase Gene Detected</th>
<th>Number of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC+</td>
<td>9 (25%)</td>
</tr>
<tr>
<td>NDM+</td>
<td>2 (5.5%)</td>
</tr>
<tr>
<td>VIM+</td>
<td>1 (2.8%)</td>
</tr>
<tr>
<td>No Detect</td>
<td>24 (66.7%)</td>
</tr>
</tbody>
</table>
Carbapenemase Production Detection by Modified Carbapenem Inactivation Method (mCIM)

Peng Zhang, Ph.D.
Section Chief – Bacterial Diseases Section
Microbial Diseases Laboratory Program
### Phenotypic Testing for Carbapenemase Production

<table>
<thead>
<tr>
<th>Phenotypic Test Used for Epidemiological or Infection Control-Related Testing</th>
<th>Modified Hodge Test (MHT)</th>
<th>Carba NP</th>
<th>Modified Carbapenem Inactivation Method (mCIM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organisms</strong></td>
<td>* Only applies to <em>Enterobacteriaceae</em></td>
<td><em>Enterobacteriaceae</em>&lt;br&gt;<em>P. aeruginosa</em>&lt;br&gt;<em>Acinetobacter</em> spp.</td>
<td>* Currently applies to <em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td><strong>Strengths</strong></td>
<td>* Simple to perform&lt;br&gt;  * No special reagents or media necessary</td>
<td>* Rapid</td>
<td>* Simple to perform&lt;br&gt;* No special reagents or media necessary</td>
</tr>
<tr>
<td><strong>Limitations</strong></td>
<td>* False positives with some <em>Enterobacter</em> spp. possessing AmpC enzymes and porin alterations&lt;br&gt;* False negatives with NDM-1 carbapenemases</td>
<td>* Special reagents are needed&lt;br&gt;* Poor sensitivity for detection of OXA-48 carbapenemases</td>
<td>* Poor sensitivity and specificity for carbapenemases in <em>Acinetobacter</em></td>
</tr>
</tbody>
</table>

*New in 2017!*

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CLSI M100 27th ed.<br>CLSI AST News Update, Vol 2(1), June 2017
Phenotypic Testing for Carbapenemase Production

• **How does mCIM work?**
  
  – Meropenem in a disk is inactivated (hydrolyzed) by the carbapenemase produced by bacteria in a bacterial suspension.
  
  – The inactivation of meropenem is determined by transferring and incubating the disk on a plate with meropenem-susceptible indicator *E. coli*.

  ➢ **Carbapenemase producer**
  Meropenem in the disk is inactivated and allows indicator *E. coli* to grow. No zone or very small zone of inhibition around the disk.

  ➢ **Non-carbapenemase producer**
  Meropenem in the disk retains its activity and inhibits the growth of indicator *E. coli*. A zone of inhibition around the disk.
Phenotypic Testing for Carbapenemase Production

- **How is mCIM Performed?**

  1. Tested bacterial culture
  2. Add meropenem disk
  3. 4 hr incubation
  4. Transfer meropenem disk
  5. Overnight incubation
  6. Indicate \textit{E. coli}
  7. Examine zone
Phenotypic Testing for Carbapenemase Production

- **How is mCIM Result Interpreted?**
  - **Carbapenemase positive:** zone 6-15 mm or presence of colonies within a 16-18 mm zone.
  - **Carbapenemase negative:** zone $\geq 19$ mm.
  - **Indeterminate:** zone 16-18 mm.

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CLSI M100 27th ed.
Phenotypic Testing for Carbapenemase Production

- **Validation of mCIM for CRE and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA)**
  - **CRE**
    - FDA-CDC AR bank isolates: 80
    - Carbapenemase type: KPC, NDM, VIM, IMP, OXA, SME, IMI
  - **CRPA**
    - FDA-CDC AR bank isolates: 30
    - Carbapenemase type: KPC, NDM, VIM, IMP, SPM

- **Validation Results**

<table>
<thead>
<tr>
<th></th>
<th>Accuracy</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRE</td>
<td>100.0%</td>
<td>100%</td>
<td>100.0%</td>
<td>96%</td>
</tr>
<tr>
<td>CRPA</td>
<td>96.7%</td>
<td>100%</td>
<td>93.3%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Carbapenemase Gene Detection and Genetic Relatedness by WGS

Hillary Berman Watson, Ph.D. MPH
Research Scientist - Core Laboratory
Microbial Diseases Laboratory

MDL Microbial Diseases Laboratory
Pathogen Experts Keeping California Safe
Bacterial Whole Genome Sequencing and CRE

• MDL offers a CLIA validated WGS assay on the Illumina MiSeq sequencing platform

• In some cases this additional genetic testing may be useful.
  – In potential outbreak or cluster investigations, WGS genotyping can help clarify routes of transmission.

• In consultation with your Local Public Health department, MDL and the HAI program epidemiologists, isolates received by MDL may be tested for genetic relatedness.
Bacterial Whole Genome Sequencing and CRE

- WGS assay can be used for
  - Species identification and Multilocus Sequence Typing

**MLST-1.8 Server - Typing Results**

**Sequence Type: ST-2613**

<table>
<thead>
<tr>
<th>Locus</th>
<th>% Identity</th>
<th>HSP Length</th>
<th>Allele Length</th>
<th>Gaps</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>aca</td>
<td>100.00</td>
<td>390</td>
<td>390</td>
<td>0</td>
<td>aca_172</td>
</tr>
<tr>
<td>aro</td>
<td>100.00</td>
<td>498</td>
<td>498</td>
<td>0</td>
<td>aro_11</td>
</tr>
<tr>
<td>gua</td>
<td>100.00</td>
<td>373</td>
<td>373</td>
<td>0</td>
<td>gua_3</td>
</tr>
<tr>
<td>mut</td>
<td>100.00</td>
<td>442</td>
<td>442</td>
<td>0</td>
<td>mut_13</td>
</tr>
<tr>
<td>nuc</td>
<td>100.00</td>
<td>366</td>
<td>366</td>
<td>0</td>
<td>nuc_1</td>
</tr>
<tr>
<td>pps</td>
<td>100.00</td>
<td>370</td>
<td>370</td>
<td>0</td>
<td>pps_2</td>
</tr>
<tr>
<td>trp</td>
<td>100.00</td>
<td>443</td>
<td>443</td>
<td>0</td>
<td>trp_4</td>
</tr>
</tbody>
</table>

**MLST Profile:** *P. aeruginosa*

**Organism:** *Pseudomonas aeruginosa*
Bacterial Whole Genome Sequencing and CRE

- WGS assay can be used for:
  - Antimicrobial Resistance Gene Detection and Identification

### ResFinder-2.1 Server - Results

<table>
<thead>
<tr>
<th>Beta-lactam</th>
<th>% Identity</th>
<th>HSP length/Query</th>
<th>Contig</th>
<th>Position in contig</th>
<th>Predicted phenotype</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>blaNDM-1</em></td>
<td>100.00</td>
<td>813 / 813</td>
<td>17C00071_S14_L001_R1_001_14_(paired)<em>trimmed</em>(paired)_contig_57</td>
<td>5152..5964</td>
<td>Beta-lactam resistance</td>
<td>FN396876</td>
</tr>
</tbody>
</table>

**Colistin**

No resistance genes found.
Bacterial Whole Genome Sequencing and CRE

- WGS assay can be used for:
  - Phylogenetic Analysis including Phylogenetic Trees

SNP tree of NDM+ *Klebsiella pneumoniae* isolates from Northern California collected between 2015-2017
Submission Instructions

Robin Hogue, CLS, PHM
Section Supervisor – Bacterial Diseases Section
Microbial Diseases Laboratory Program
Submission Instructions

• Specimen/Isolate Requirements

– Identified to at least the genus level
– Confirmed as *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter* species or *Pseudomonas aeruginosa*
– Resistant to at least one carbapenem, i.e., imipenem, ertapenem, doripenem, or meropenem
– Pure culture
– Other *Enterobacteriaceae* organisms are on a case by case basis after consultation with HAI program
Submission Instructions

CRE/CRPA Antimicrobial Susceptibility Testing Performed in Microbial Diseases Laboratory (MDL)

- Bacterial Diseases Section (BDS)
- Foodborne & Waterborne Diseases Section (FWDS)
- High Risk Pathogens Section
- Immunodiagnostic Mycotic & Parasitic Diseases Section
- Mycobacterial Diseases Section
- Core Laboratory

Phenotypic Testing
- mCIM
Genotypic Testing
- Xpert® Carba-R

Whole Genome Sequencing
Submission Instructions

Different Isolate Submission and Testing Scenarios

<table>
<thead>
<tr>
<th>Testing Prior to Submission</th>
<th>Testing by MDL upon Submission</th>
<th>Report To Submitter</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>ID &amp; Molecular testing</td>
<td>☑️</td>
</tr>
<tr>
<td></td>
<td>Negative by molecular testing</td>
<td>☑️</td>
</tr>
<tr>
<td></td>
<td>mCIM</td>
<td>☑️</td>
</tr>
<tr>
<td>Phenotypic test performed &amp; carbapenemase detected</td>
<td>ID &amp; Molecular testing</td>
<td>☑️</td>
</tr>
<tr>
<td></td>
<td>Negative by molecular testing</td>
<td>☑️</td>
</tr>
<tr>
<td></td>
<td>confirmatory mCIM</td>
<td>☑️</td>
</tr>
<tr>
<td>PCR performed but negative for known carbapenemase genes</td>
<td>ID &amp; mCIM</td>
<td>☑️</td>
</tr>
<tr>
<td></td>
<td>Positive by mCIM</td>
<td>☑️</td>
</tr>
<tr>
<td></td>
<td>confirmatory molecular testing</td>
<td>☑️</td>
</tr>
</tbody>
</table>

PublicHealth
Submission Instructions

Submittal Form: MDLB Lab Form AST-R

Instructions for Form AST-R
Submission Instructions
Submission Instructions

Antimicrobial Susceptibility Testing - Submitter’s Laboratory Findings

<table>
<thead>
<tr>
<th>Culture made from original clinical sample were:</th>
<th>True</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>If mixed list other organisms:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory colony counts where applicable (e.g., urine):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of times this organism was isolated from patient:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium on which primary growth was obtained:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium on which organism is being submitted:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date inoculated:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condition of incubation prior to mailing:</td>
<td>Temperature</td>
<td>Atmosphere</td>
</tr>
</tbody>
</table>

Method used for identification:

Please attach a copy of microbial identification test results if it is available

Method used for Antimicrobial Susceptibility Testing:

Please attach a copy of antimicrobial susceptibility test results

Other Test or Comments:

Please attach a copy of all additional test results, including molecular test results
Key Points for Specimen Submission

• Submission forms available by contacting your local public health department

• All isolate submissions should be coordinated and sent through local public health department
  – Comply with local reporting and submission requirements

• Ensure facility information and AST results are communicated with specimen submission
Conclusions

• Carbapenemase testing informs actions to prevent the spread of carbapenemase-producing organisms

• Phenotypic and molecular carbapenemase testing services are available at CDPH MDL and should be coordinated through your local public health department

• CRE isolate submission is encouraged, and may supplement or complement locally available testing
Key Contact Information

**mCIM testing:**
MDL Bacterial Diseases Section (BDS) Laboratory: (510) 412-3903
Robin Hogue, BDS Reference Bacteriology Unit Supervisor
Robin.Hogue@cdph.ca.gov
Peng Zhang, BDS Section Chief Peng.Zhang@cdph.ca.gov

**Xpert® Carba-R**
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Greg Inami, FWDS Detection Unit Supervisor Greg.Inami@cdph.ca.gov
Stephanie Abromaitis, FWDS Section Chief Stephanie.Abromaitis@cdph.ca.gov

**Whole genome sequencing:**
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Hillary Berman-Watson, Research Scientist Hillary.Berman-Watson@cdph.ca.gov
Core Laboratory General Email Address MDL.wgs@cdph.ca.gov
Vishnu Chaturvedi, MDL Lab Director Vishnu.Chaturvedi@cdph.ca.gov

**CDPH Healthcare-Associated Infections (HAI) Program:**
HAI Program: (510) 412-6060
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Erin Epson, Assistant Chief, Public Health Medical Officer Erin.Epson@cdph.ca.gov