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

Webinar

November 8th, 2017

Microbial Diseases Laboratory
Healthcare-Associated Infections Program
California Department of Public Health

# 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)

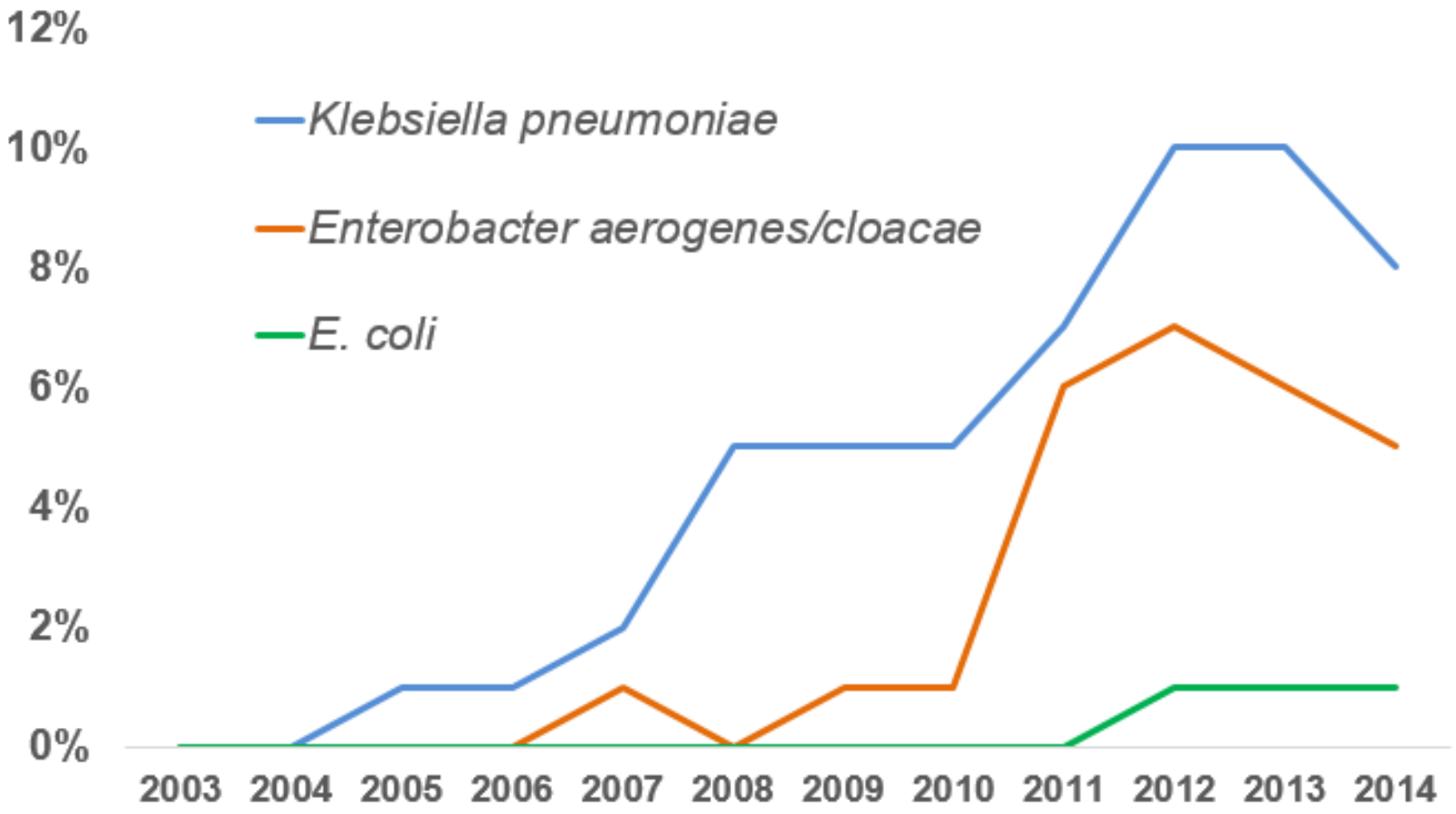
**CDC 2015 Surveillance Definition of CRE**

- 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

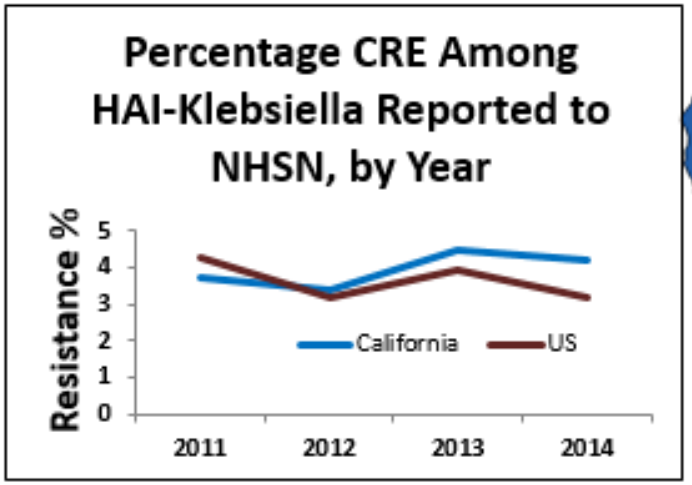



Source: Center for Disease Dynamics, Economics, and Policy (CDDEP)
Isolate level data was obtained from The Surveillance Network (TSN)



California Department of PublicHealth

# CRE Among Healthcare-Associated Infections







Source: Antibiotic Resistance Patient Safety Atlas
Data from National Healthcare Safety Network (NHSN), 2011-2014



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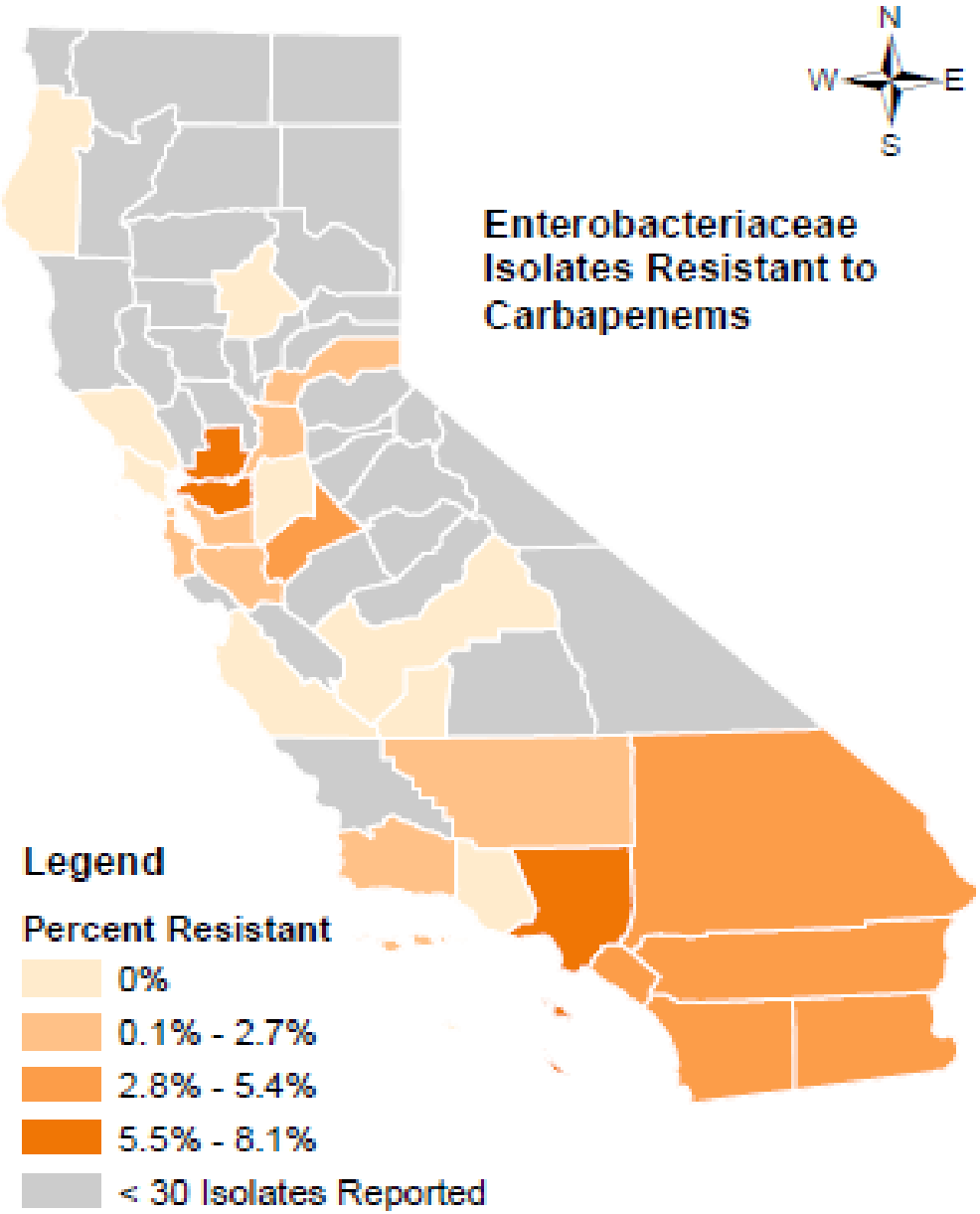
# Percentage CRE Among HAI Reported to NHSN, 2014-2015, California Acute Care Hospitals (N=342)



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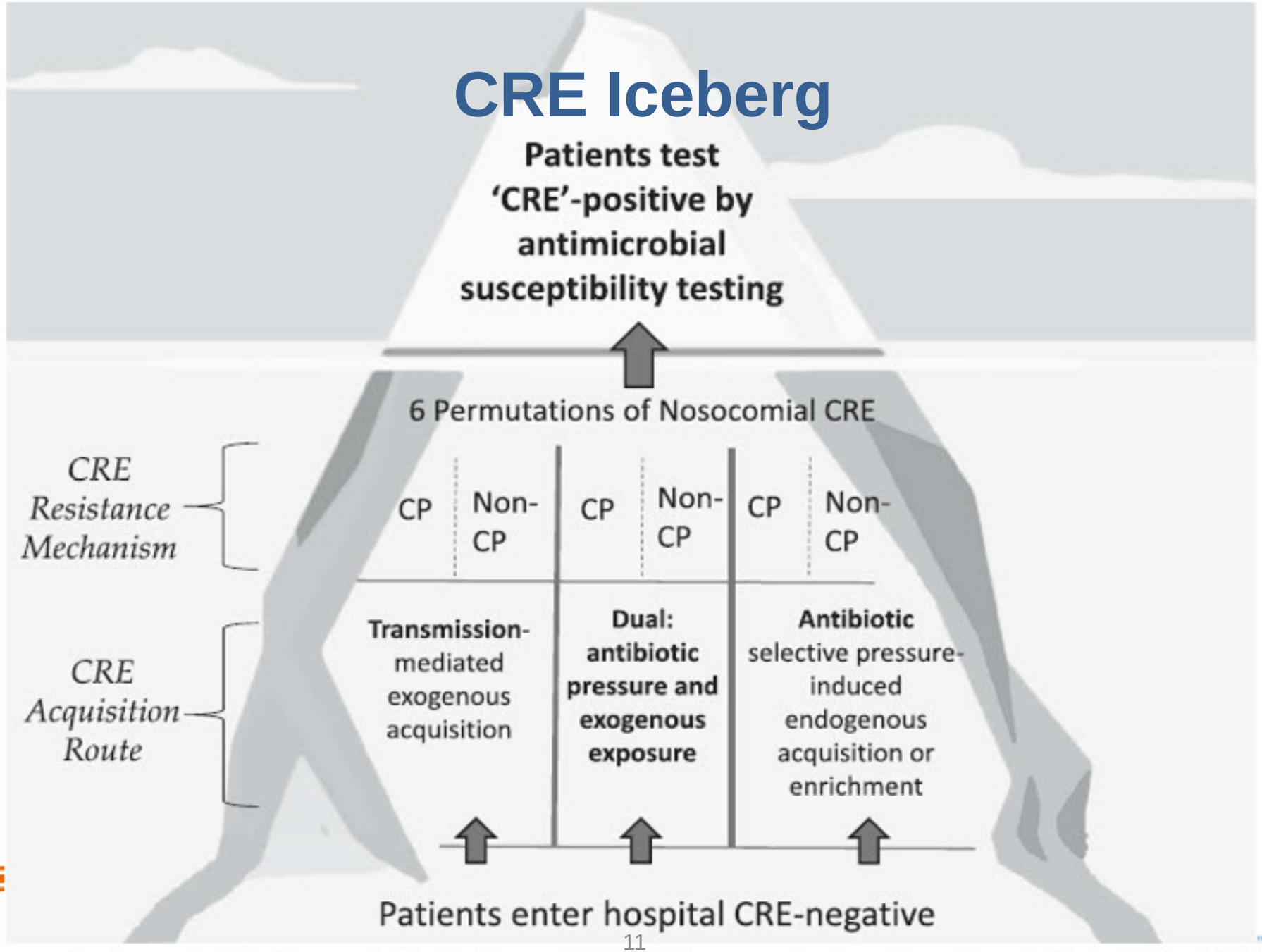
# Different Types of CRE

- **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






Source: Goodman et al Infection control implications of CRE resistance mechanisms Expert Rev. Anti Infect. Ther. (2016)

# 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

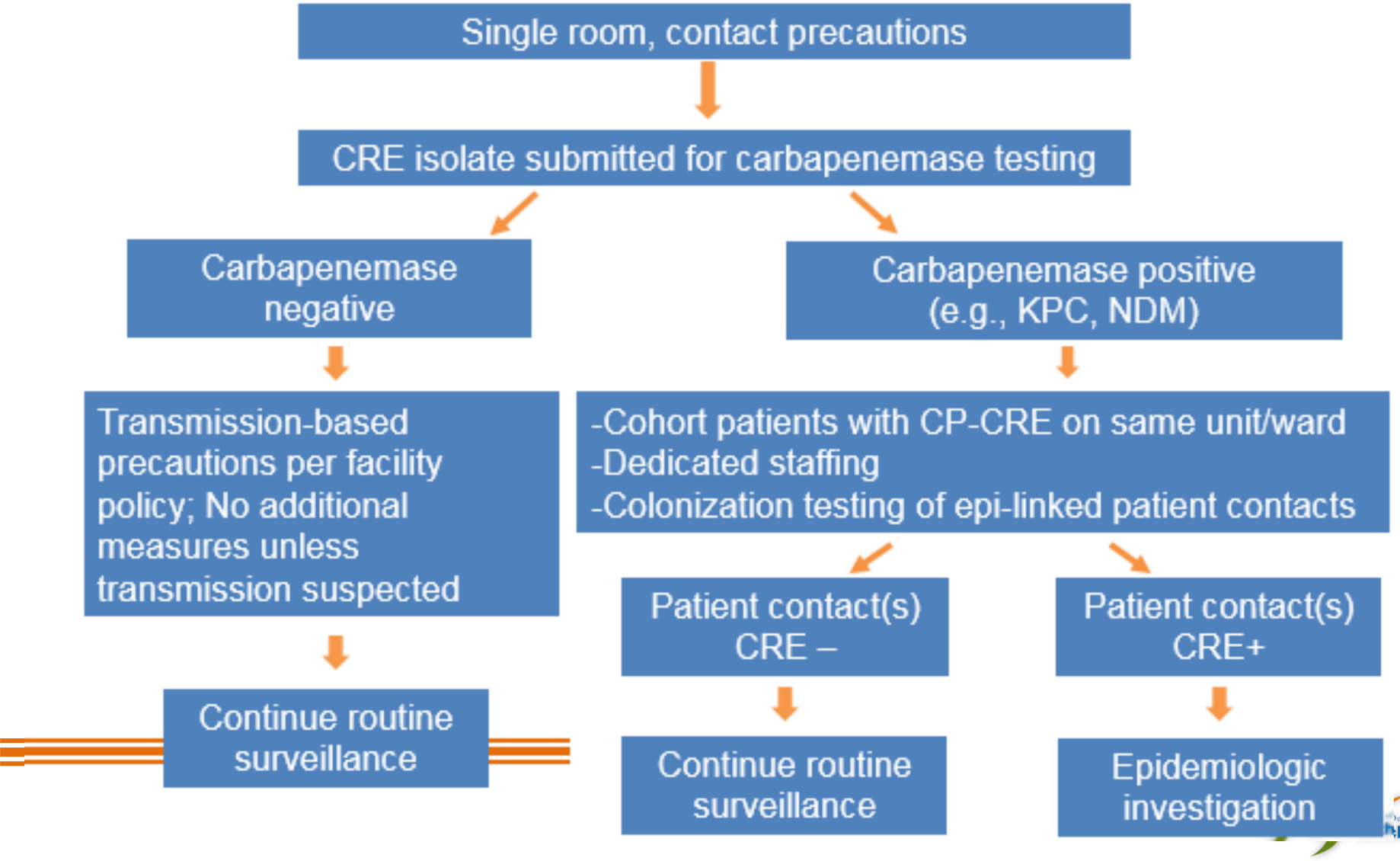
Source: Guh et al Epidemiology of CRE, 2012-2013 JAMA 2015
Source: Tamma et Mortality with CP-CRE bacteremia CID 2017

# 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



# 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

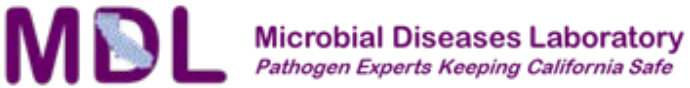
- Discrepant results, e.g., positive phenotypic test and negative genotypic test, might represent a novel carbapenemase

# Carbapenemase Gene Detection by Cepheid Xpert® Carba-R Assay

**Stephanie Abromaitis, Ph.D.**

Section Chief - Foodborne & Waterborne Diseases Section

Microbial Diseases Laboratory Program



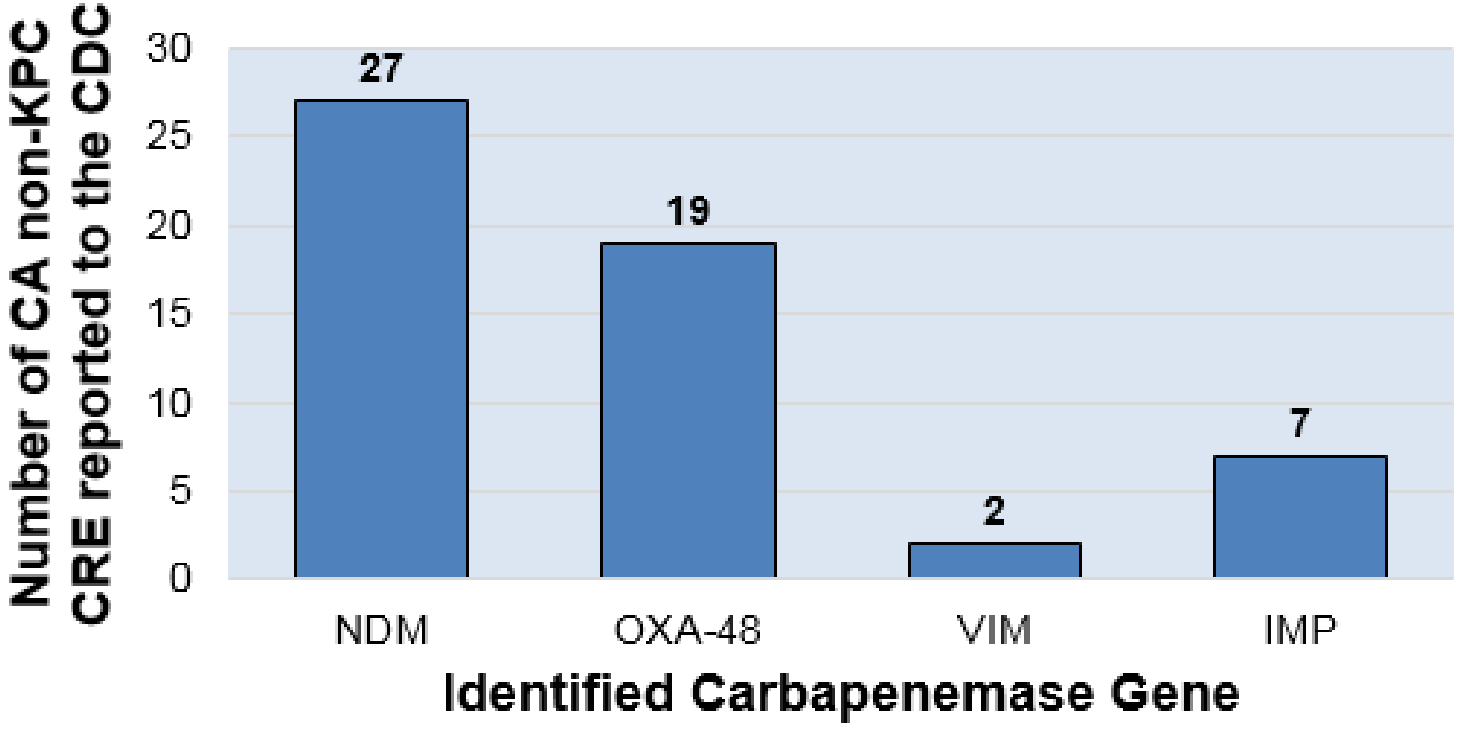
# Overview:

- 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
  - *bla*KPC (**KPC**)
  - *bla*NDM (**NDM**)
  - *bla*VIM (**VIM**)
  - *bla*IMP (**IMP**)
  - *bla*OXA-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

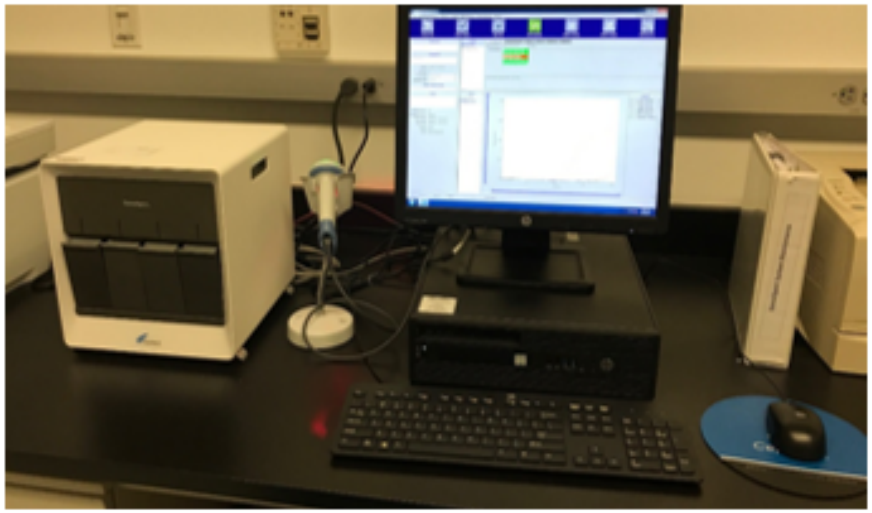
- There are multiple variants of each carbapenemase gene
- Not all variants of each of the “Big Five” carbapenemases are detected by the Xpert® Carba-R

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

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



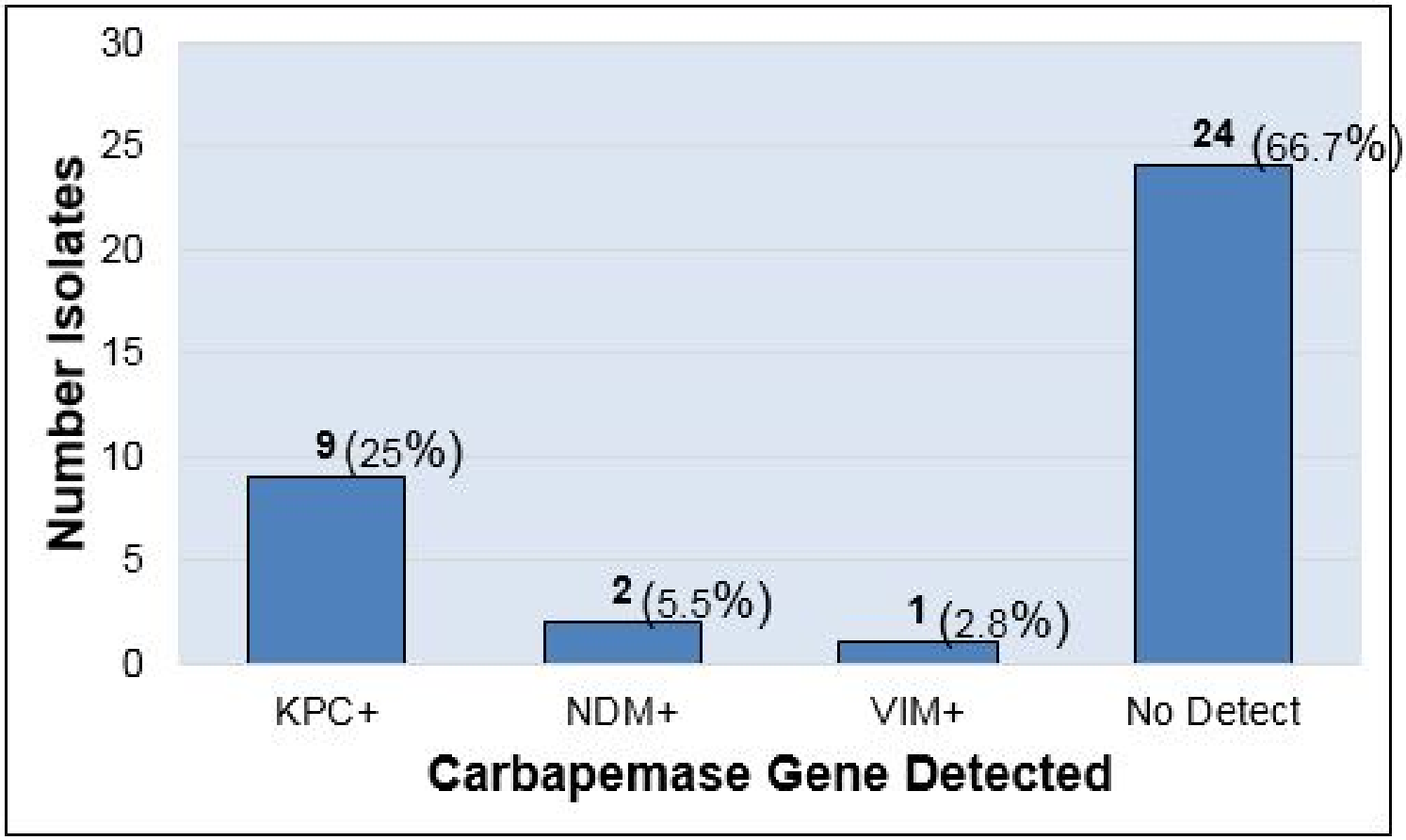
# MDL Verification Summary

- 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

| Verification Results | |
|---|---|
| Accuracy = 97.6% | Sensitivity = 96.9% |
| Reproducibility = 100% | Specificity = 100% |

# MDL Xpert® Carba-R Testing Thus Far

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



# Carbapenemase Production Detection by Modified Carbapenem Inactivation Method (mCIM)

**Peng Zhang, Ph.D.**

Section Chief – Bacterial Diseases Section

Microbial Diseases Laboratory Program

MDL Microbial Diseases Laboratory

*Pathogen Experts Keeping California Safe*

California Department of PublicHealth

# Phenotypic Testing for Carbapenemase Production

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

New in 2017!

CLSI M100 27th ed.
CLSI AST News Update, Vol 2(1), June 2017

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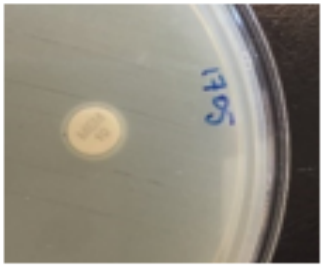
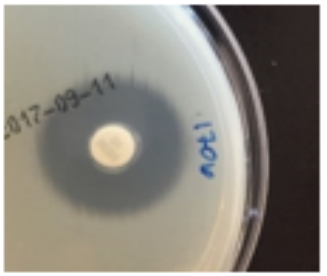
# Phenotypic Testing for Carbapenemase Production

- **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.

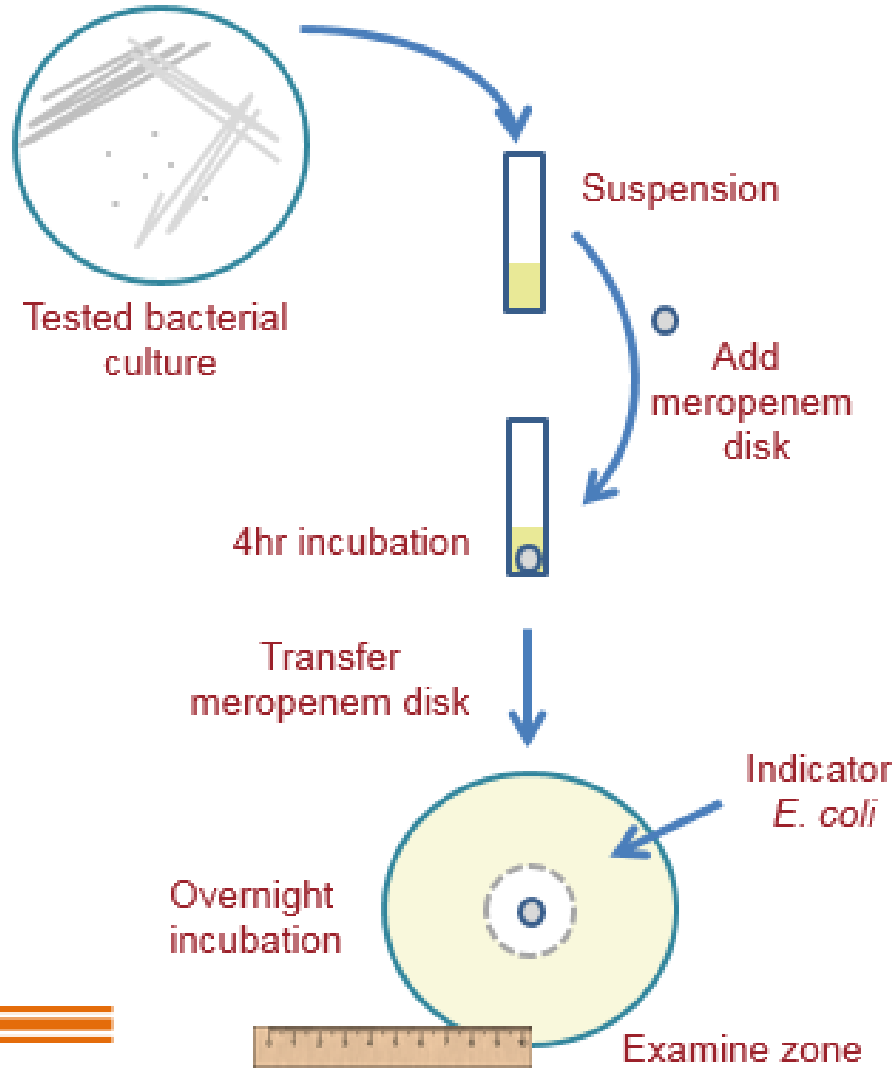


➢**Non-carbapenemase producer**
Meropenem in the disk retains its activity and inhibits the growth of indicator *E. coli*. A zone of inhibition around the disk.



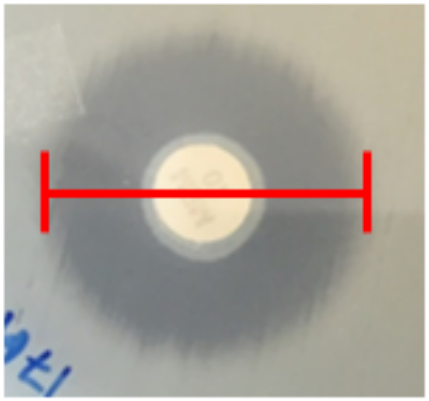
# Phenotypic Testing for Carbapenemase Production

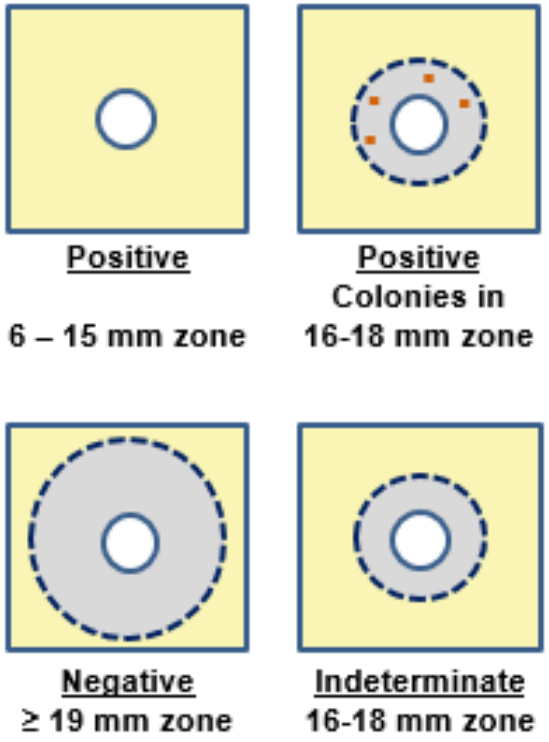
- **How is mCIM Performed?**



# 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 ≥19 mm.
  - **Indeterminate**: zone 16-18 mm.






CLSI M100 27th ed.

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# 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**

| | Accuracy | Sensitivity | Specificity | Reproducibility |
|---|---|---|---|---|
| **CRE** | 100.0% | 100% | 100.0% | 96% |
| **CRPA** | 96.7% | 100% | 93.3% | 100% |

California Department of PublicHealth

# 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*



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# Bacterial Whole Genome Sequencing and CRE

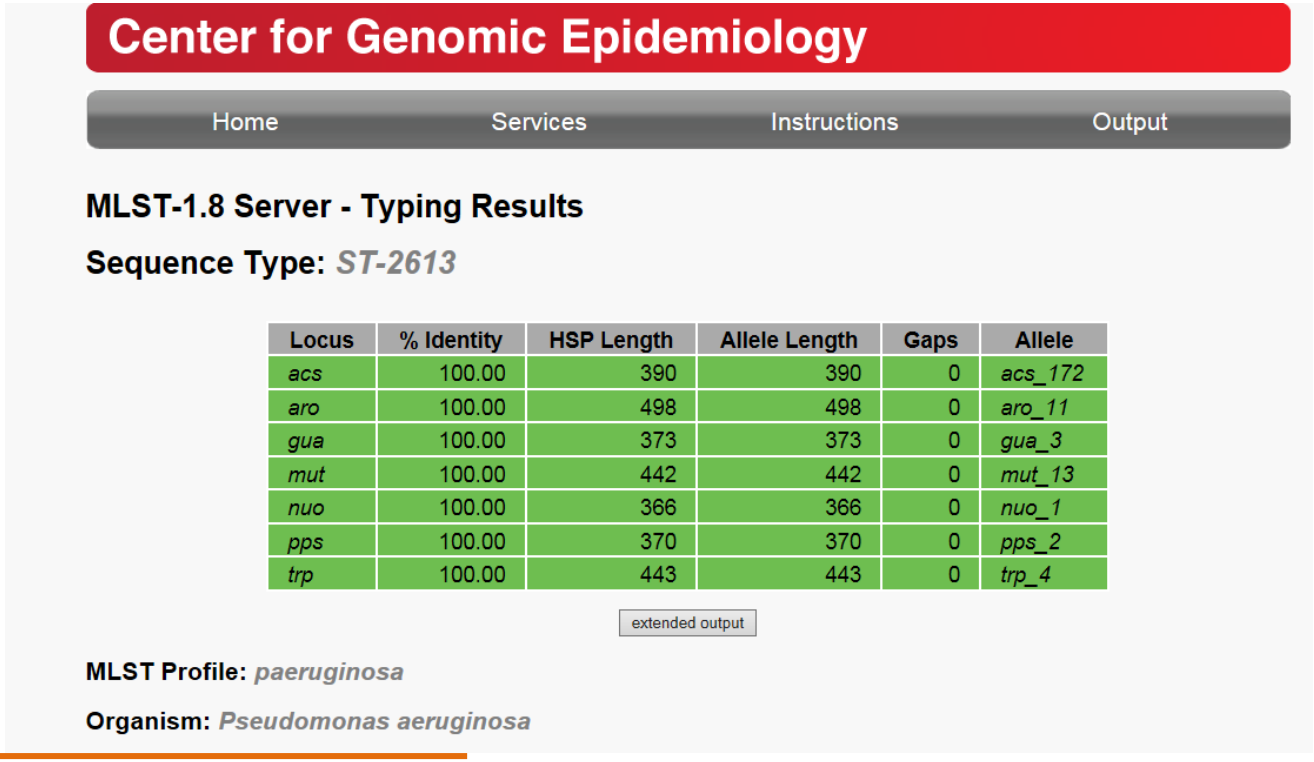
- MDL offers a CLIA validated WGS assay on the Illumina MiSeq sequencing platform

- 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



Center for Genomic Epidemiology

Home | Services | Instructions | Output

**MLST-1.8 Server - Typing Results**

**Sequence Type:** *ST-2613*

| Locus | % Identity | HSP Length | Allele Length | Gaps | Allele |
|---|---|---|---|---|---|
| *acs* | 100.00 | 390 | 390 | 0 | *acs_172* |
| *aro* | 100.00 | 498 | 498 | 0 | *aro_11* |
| *gua* | 100.00 | 373 | 373 | 0 | *gua_3* |
| *mut* | 100.00 | 442 | 442 | 0 | *mut_13* |
| *nuo* | 100.00 | 366 | 366 | 0 | *nuo_1* |
| *pps* | 100.00 | 370 | 370 | 0 | *pps_2* |
| *trp* | 100.00 | 443 | 443 | 0 | *trp_4* |

extended output

**MLST Profile:** *paeruginosa*

**Organism:** *Pseudomonas aeruginosa*

# Bacterial Whole Genome Sequencing and CRE

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

**Center for Genomic Epidemiology**

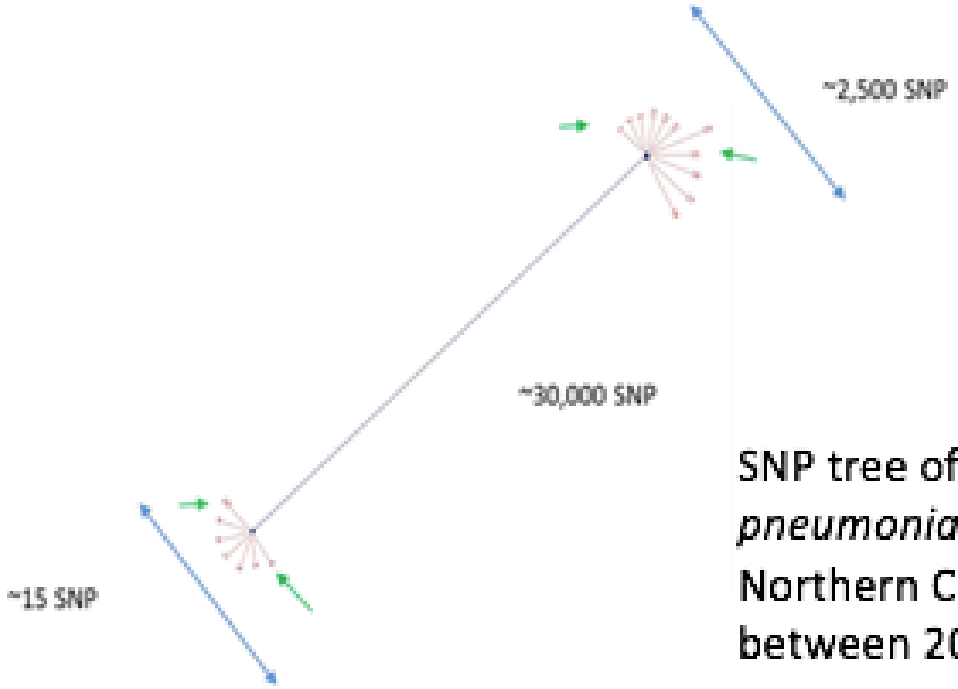
**ResFinder-2.1 Server - Results**

| Beta-lactam | | | | | | |
|---|---|---|---|---|---|---|
| Resistance gene | % Identity | HSP length/Query | Contig | Position in contig | Predicted phenotype | Accession number |
| *blaNDM-1* | 100.00 | 813 / 813 | 17C00071_S14_L001_R1_001_14 _(paired)_trimmed_(paired) _contig_57 | 5152..5964 | Beta-lactam resistance | FN396876 |

| 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

MDL Microbial Diseases Laboratory

*Pathogen Experts Keeping California Safe*

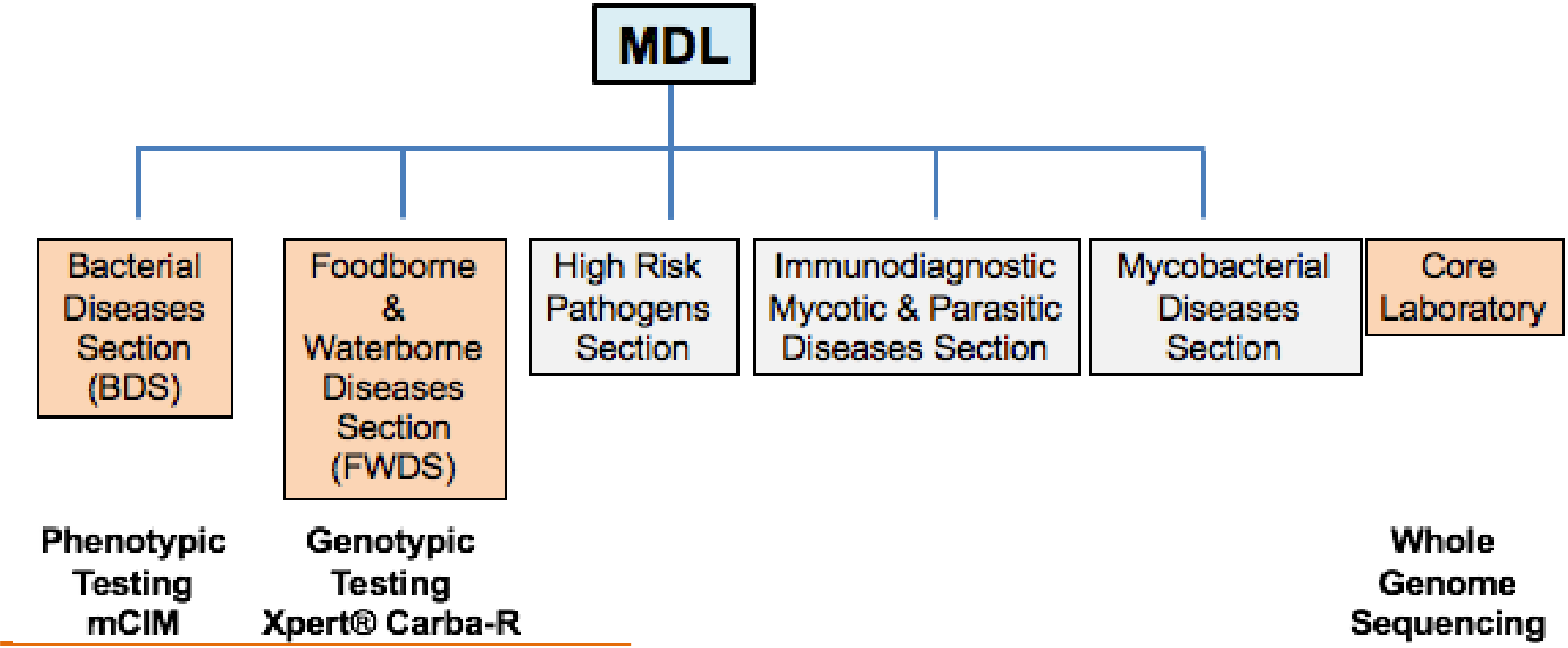
California Department of PublicHealth

# 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)



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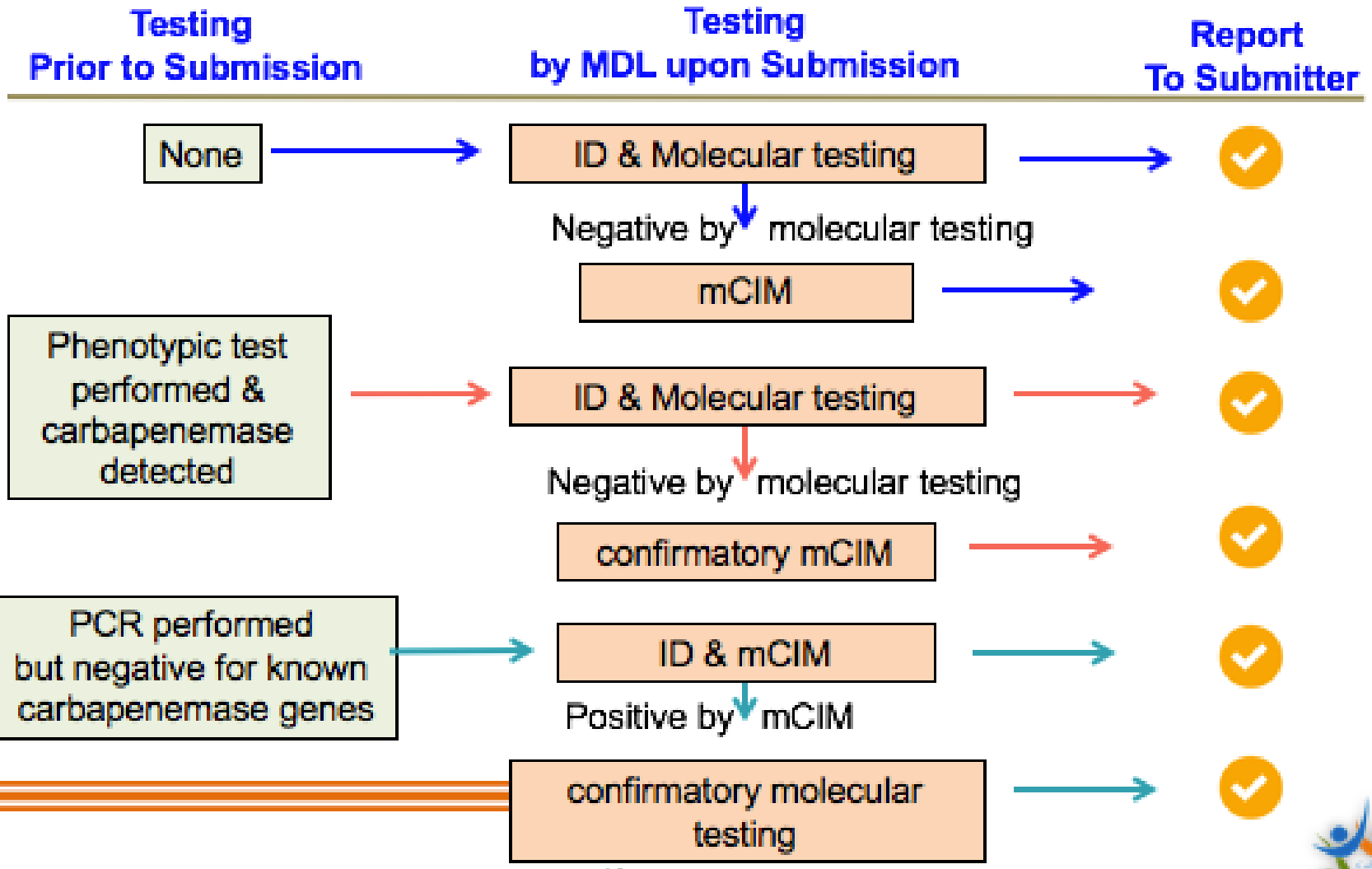
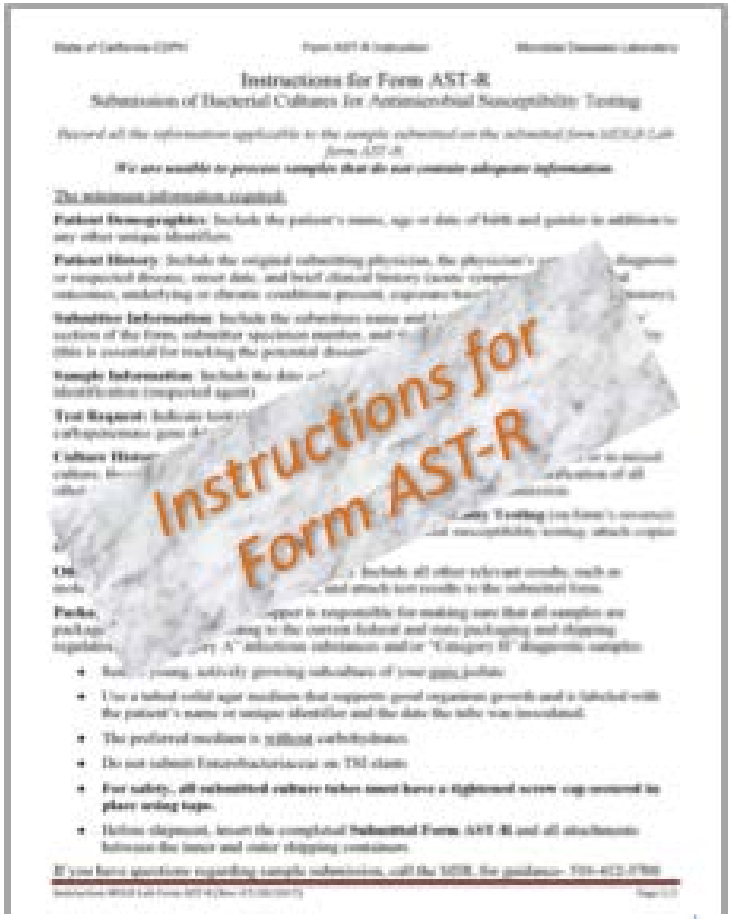
# Submission Instructions

## Different Isolate Submission and Testing Scenarios



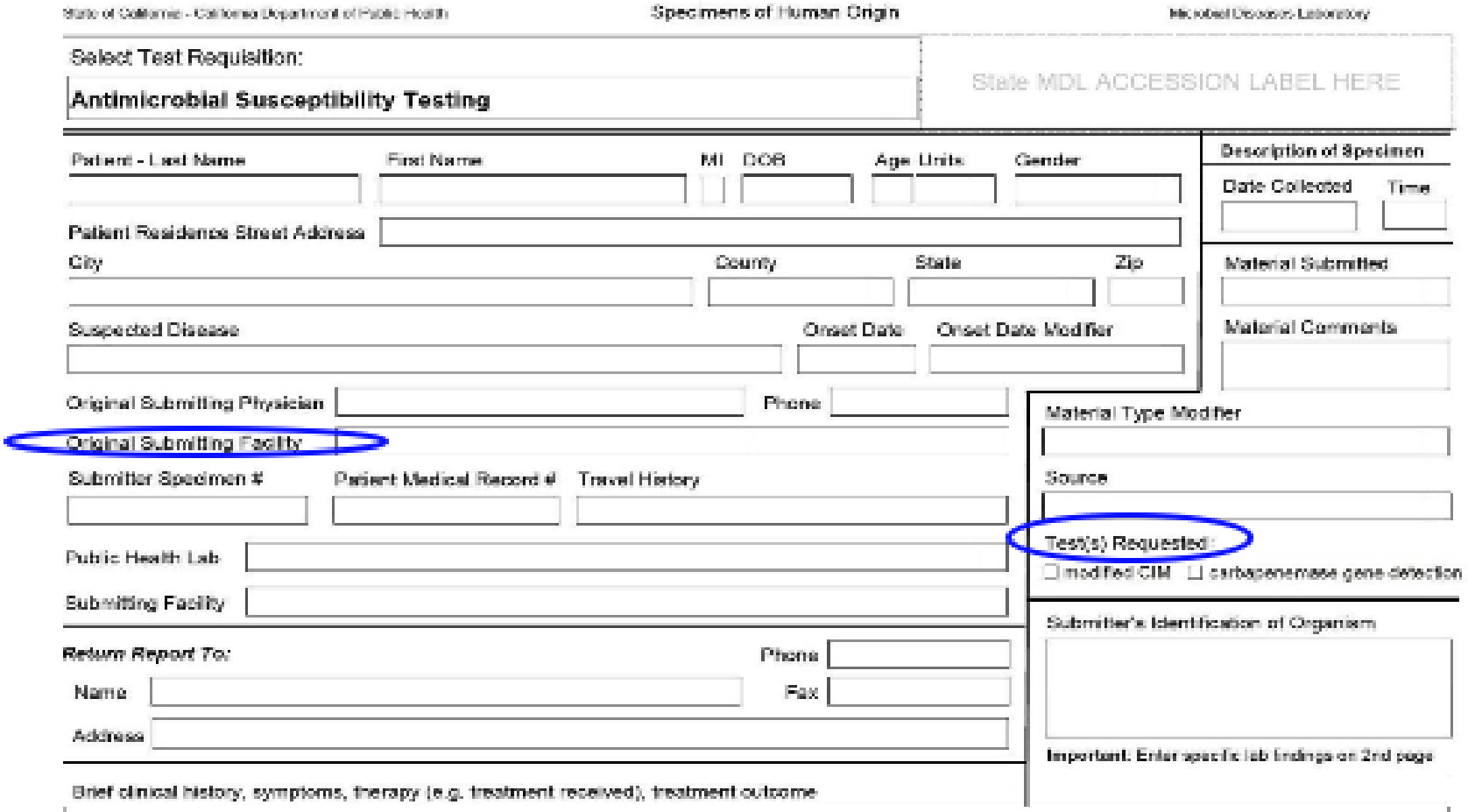
# Submission Instructions

## Submittal Form: MDLB Lab Form AST-R

# Submission Instructions

Submittal Form: MDLB Lab Form AST-R



# Submission Instructions

Submittal Form: MDLB Lab Form AST-R

**Antimicrobial Susceptibility Testing** - *Submitter's Laboratory Findings*

Culture made from original clinical sample were: ☐ Pure ☐ Mixed

If mixed list other organisms:

Laboratory colony counts where applicable (e.g., urine):

Number of times this organism was isolated from patient:

Medium on which primary growth was obtained:

Medium on which organisms is being submitted: Date Inoculated:

Condition of incubation prior to mailing: Temperature Atmosphere Length

**Method used for Identification:**

*Please attach a copy of biochemical 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
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Peng Zhang, BDS Section Chief Peng.Zhang@cdph.ca.gov

**Xpert® Carba-R**

MDL Foodborne and Waterborne Diseases Section (FWDS) Laboratory: (510) 412-3796
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Stephanie Abromaitis, FWDS Section Chief Stephanie.Abromaitis@cdph.ca.gov

**Whole genome sequencing:**

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Vishnu Chaturvedi, MDL Lab Director Vishnu.Chaturvedi@cdph.ca.gov

**CDPH Healthcare-Associated Infections (HAI) Program:**

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Erin Epson, Assistant Chief, Public Health Medical Officer Erin.Epson@cdph.ca.gov