



**Weill Cornell  
Medicine**

# **What You Should Be Doing And What's To Come**

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Michigan Department of Health and Human Services

CRE Surveillance and Prevention Conference

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# Disclosures

- **Accelerate Diagnostics, Inc.**, research funding
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- **Hardy Diagnostics**, research funding
- **Roche Molecular Systems, Inc.**, advisory board

# Importance of Antibiotics



Antibiotics (such as carbapenems) are unique among all therapeutic agents in that the use of the agent in one patient can compromise its efficacy in another → carbapenem-resistant *Enterobacteriaceae*

# Carbapenem-Resistant *Enterobacteriaceae*

Carbapenem-Resistant  
*Enterobacteriaceae* (**CRE**):  
resistant to any carbapenem

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graph TD; A["Carbapenem-Resistant Enterobacteriaceae (CRE): resistant to any carbapenem"] --> B["Carbapenemase-Producing CRE (CP-CRE): Resistant to carbapenems due to production of a carbapenemase (e.g., IMP, KPC, NDM, OXA-48, VIM)"]; A --> C["Non-CP-CRE: Resistant to carbapenems, but do not produce a carbapenemase. Resistant to carbapenems by alternative mechanisms (e.g., cephalosporinase [ESBL, AmpC] production in conjunction with altered porin permeability or efflux pumps)"];
```

Carbapenemase-Producing  
CRE (**CP-CRE**):  
Resistant to carbapenems  
due to production of a  
carbapenemase (e.g., IMP,  
KPC, NDM, OXA-48, VIM)

*\*Collectively, CP-CRE and non-Enterobacteriaceae (e.g., Pseudomonas aeruginosa and Acinetobacter baumannii) that produce carbapenemases are called Carbapenemase-Producing Organisms (CPOs)*

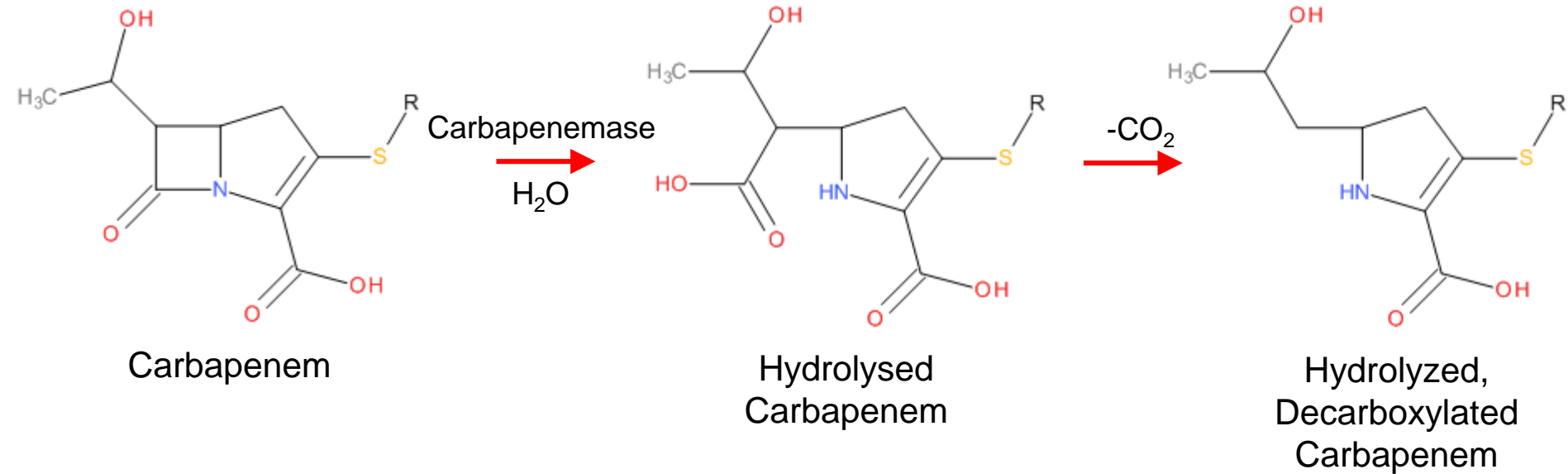
## **Non-CP-CRE:**

Resistant to carbapenems,  
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(e.g., cephalosporinase  
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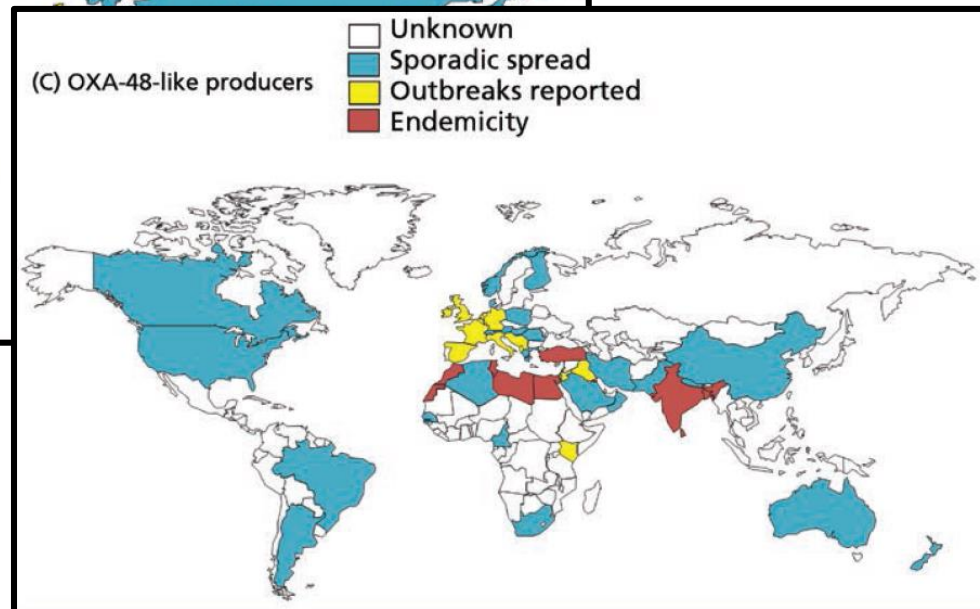
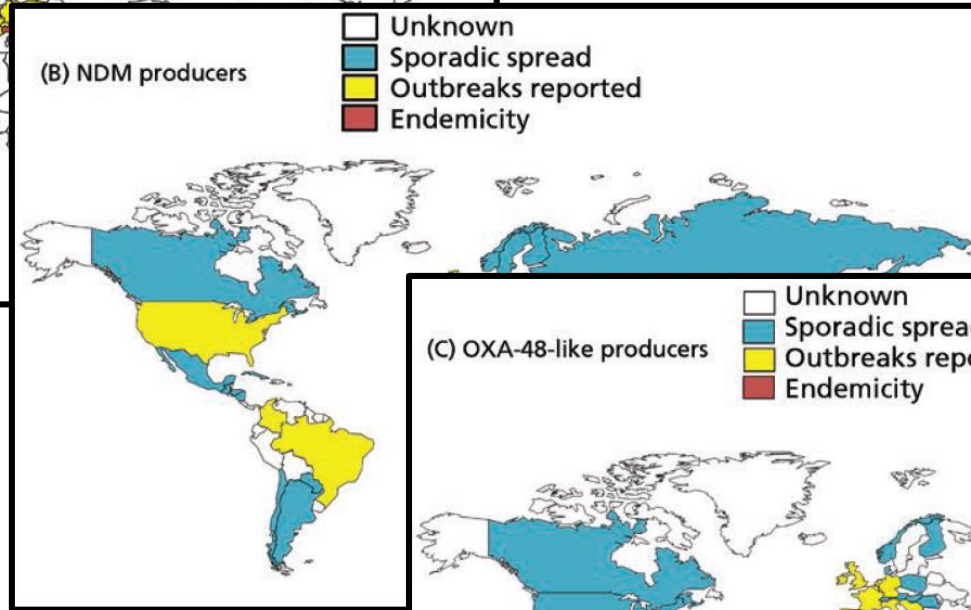
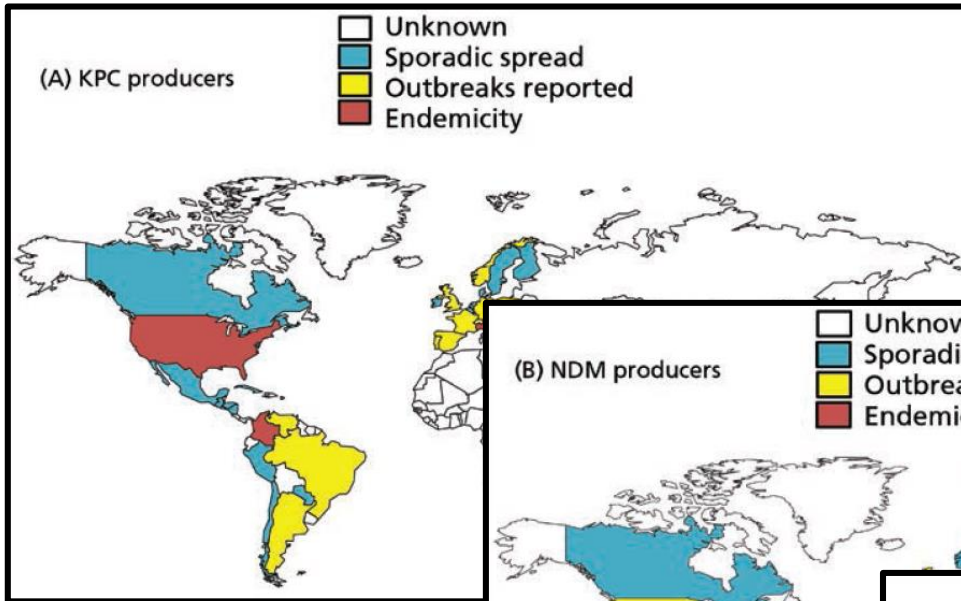
# Carbapenemases

- Carbapenemases hydrolyse carbapenems: potent, broad-spectrum antibiotics
- Gram-negatives in whom carbapenemase production is of serious concern:
  - *Enterobacteriaceae*
  - *Pseudomonas aeruginosa*
  - *Acinetobacter baumannii*
- Carbapenemases (Ambler classification):
  - Class A (e.g., KPC): serine-based hydrolytic mechanism
  - Class B (e.g., NDM): metallo- $\beta$ -lactamases (MBLs), zinc ion-based hydrolytic mechanism
  - Class D (e.g., OXA-48-like): serine-based hydrolytic mechanism
- Inhibitors (not-therapeutic):
  - KPC enzymes (class A), phenylboronic acid
  - MBLs (class B), EDTA

# Carbapenemase Mechanism of Action



# Distribution of Carbapenemases



# People Travel: Epidemiology Changes

## Scientists find new superbug spreading from India

Recommend 126 people recommend this.



By Kate Kelland and Ben Hirschler  
LONDON | Wed Aug 11, 2010 5:45pm EDT

(Reuters) - A new superbug from India could spread around the world -- in part because of medical tourism -- and scientists say there are almost no drugs to treat it.

Researchers said on Wednesday they had found a new gene called New Delhi metallo-beta-lactamase, or NDM-1, in patients in South Asia and in

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Hospital superbug infections on the decline  
Wed, Aug 11 2010

Analysis & Opinion

On WikiLeaks, Pakistan and Afghanistan; the old iceberg

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# Importance of Detecting Carbapenemases (CP-CRE and CPO)

- Infection control (surveillance and/or outbreak):
  - Within a facility (intra)
  - Between facilities (inter)
- Public health surveillance:
  - Local
  - Regional
  - National
  - International
- Assist therapeutic decision making:
  - No, if using “new” (M100-S21 to -S29) CLSI *Enterobacteriaceae* carbapenem breakpoints (but even with “new” breakpoints some laboratories still perform carbapenemase detection tests [CDTs] for therapeutic and epidemiologic purposes)
  - Yes, if using “old” (M100-S20) CLSI *Enterobacteriaceae* carbapenem breakpoints
  - Yes, if using “novel” antibiotics with carbapenemase-specific activity (e.g., agents active against class A enzymes, but no activity against class B enzymes). Upon recognition of a possible CPO can test for a carbapenemase using a CDT that differentiates between classes a day before “novel” agent AST results available

# Susceptibilities of Carbapenemase-Producing Organisms to $\beta$ -Lactams

$\beta$ -Lactam Class	Class A (e.g., KPC)	Class B (e.g., VIM)	Class D (e.g., OXA-48)
Penicillin	R	R	R
Penicillin- $\beta$ -Lactamase Inhibitors (clavulanate, sulbactam, tazobactam)	R	R	R
1 <sup>st</sup> generation cephalosporins	R	R	R
2 <sup>nd</sup> generation cephalosporins	R	R	R
Cephameycins (Cefoxitin/Cefotetan)	R	R	R
3 <sup>rd</sup> generation cephalosporins	R	R	**V (can test S)
4 <sup>th</sup> generation cephalosporin (Cefepime)	R	R	**V (can test S)
Carbapenems	R	R	R
Aztreonam	R	*S	R
Ceftolozane-Tazobactam	R	R	R
Ceftazidime-Avibactam	S	R	S
Meropenem-Vaborbactam	S	R	R
Imipenem-Relebactam	S	R	R
Cefiderocol	S	S	S

S, susceptible; R, resistant; V, variable

\*Isolates often encode other  $\beta$ -lactamases that confer resistance to aztreonam

\*\*Isolates can test susceptible to broad-spectrum cephalosporins then develop resistance (OXA-48 + porin mutation), or encode other  $\beta$ -lactamases that hydrolyze broad-spectrum cephalosporins

# Optimal Characteristics of CDTs Related to their Intended Role

Role	Diagnostic Characteristics	Turnaround Time	Information Needed
Therapeutic guidance	Highly sensitive; highly specific	Fast as possible (hours/1-2 days)	<ul style="list-style-type: none"> <li>• Presence/absence of carbapenemase</li> <li>• Differentiation between carbapenemase class (<i>e.g.</i>, serine vs MBL)</li> </ul>
Intra-facility infection control	Highly sensitive; specific	Fast as possible (1-2 days)	<ul style="list-style-type: none"> <li>• Presence/absence of carbapenemase</li> </ul>
Inter-facility infection control	Highly sensitive; specific	Results available at discharge	<ul style="list-style-type: none"> <li>• Differentiation between carbapenemase class?</li> </ul>
Public health surveillance	Sensitive; highly specific	Not critical for clinical care (batched)	<ul style="list-style-type: none"> <li>• Confirmation of carbapenemase activity</li> <li>• Identification of specific resistance mechanism</li> </ul>

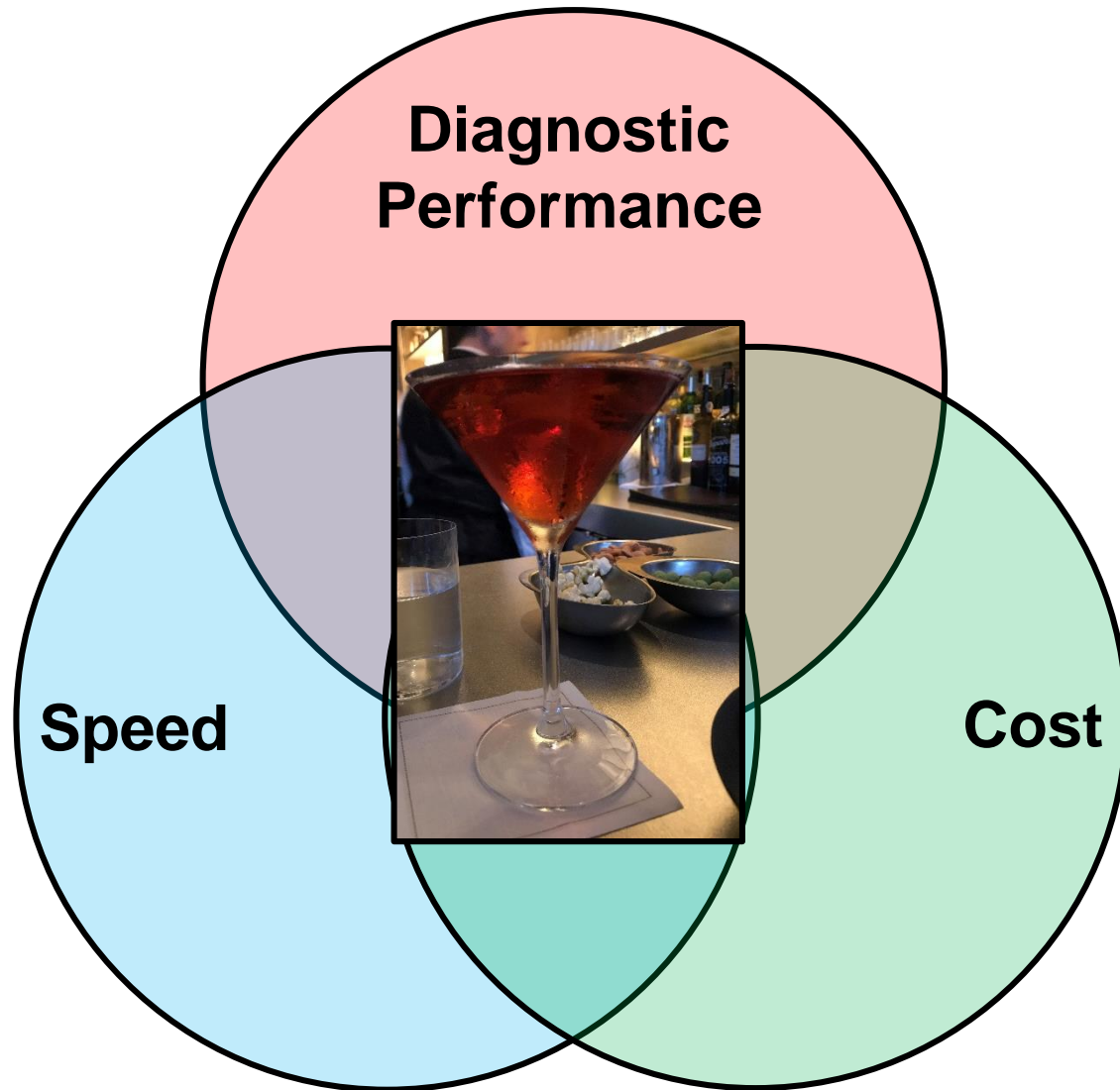
# Detecting Carbapenem Resistance in Clinical Laboratories

Test Type	Turnaround Time	Cost
<b>Direct from clinical specimen/positive (blood) culture broth:</b>		
Carbapenemase gene detection (PCR/microarray)	Day 1 (2 h)	\$\$\$
Direct antibiotic susceptibility testing (AST)	Day 1 (8 h)	\$-\$\$\$
Chromogenic/selective media	Day 2 (overnight)	\$

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Direct antibiotic susceptibility testing (AST)	Day 1 (8 h)	\$-\$\$\$
Chromogenic/selective media	Day 2 (overnight)	\$
<b>Bacterial isolate:</b>		
Rapid AST	Day 2 (5 h)	?
Rapid carbapenemase test (e.g., Carba NP and associated variants)	Day 2 (2 h)	\$-\$\$
Lateral flow immunochromatographic test for carbapenemases (e.g., Carba 5)	Day 2 (0.3 h)	?
Carbapenemase gene detection (PCR/microarray)	Day 2 (2 h)	\$\$\$
Conventional AST	Day 3 (18 h)	\$
Carbapenemase test (e.g., modified Hodge Test, modified carbapenem inactivation method and associated variants)	Day 3 (18 h)	\$

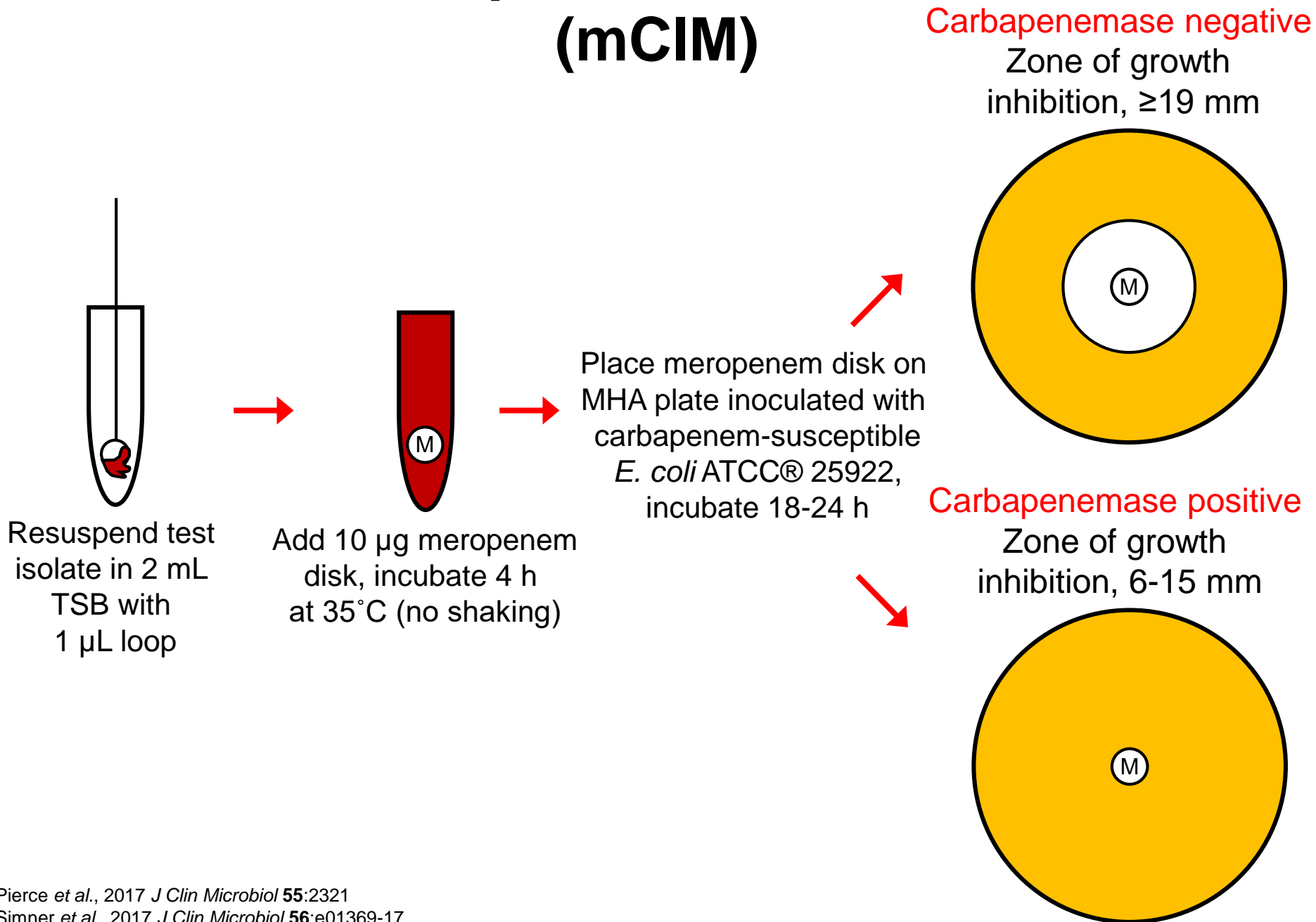
# Implementing a Diagnostic Test



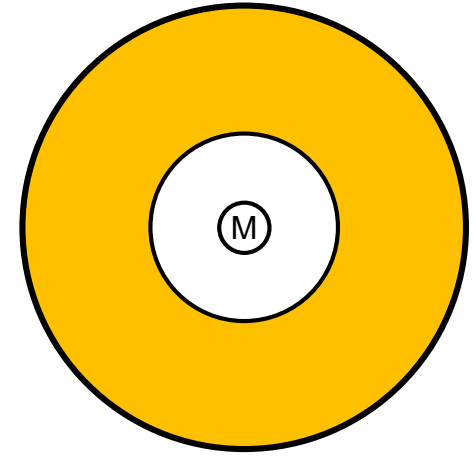
# CDT Flavors

- Phenotypic CDTs:
  - Growth-based (disk/gradient diffusion inhibitor combinations, mCIM and variants)
  - Biochemical-based (Carba NP and variants)
  - Lateral flow immunoassays (Carba 5)
  - Mass spectrometry-based assays
- Genotypic CDTs:
  - Targeted multiplex assays (PCR, microarray)
  - Whole-genome sequencing/metagenomics

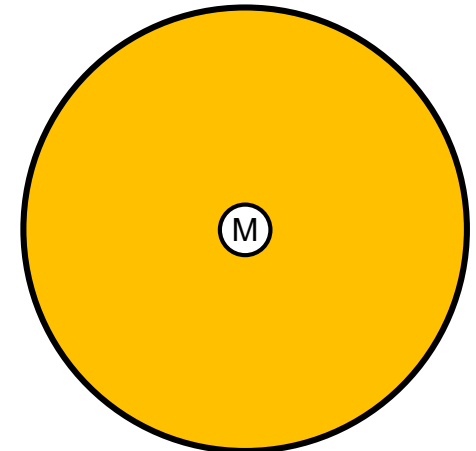
# Modified Carbapenem Inactivation Method (mCIM)



Carbapenemase negative  
Zone of growth inhibition,  $\geq 19$  mm



Carbapenemase positive  
Zone of growth inhibition, 6-15 mm



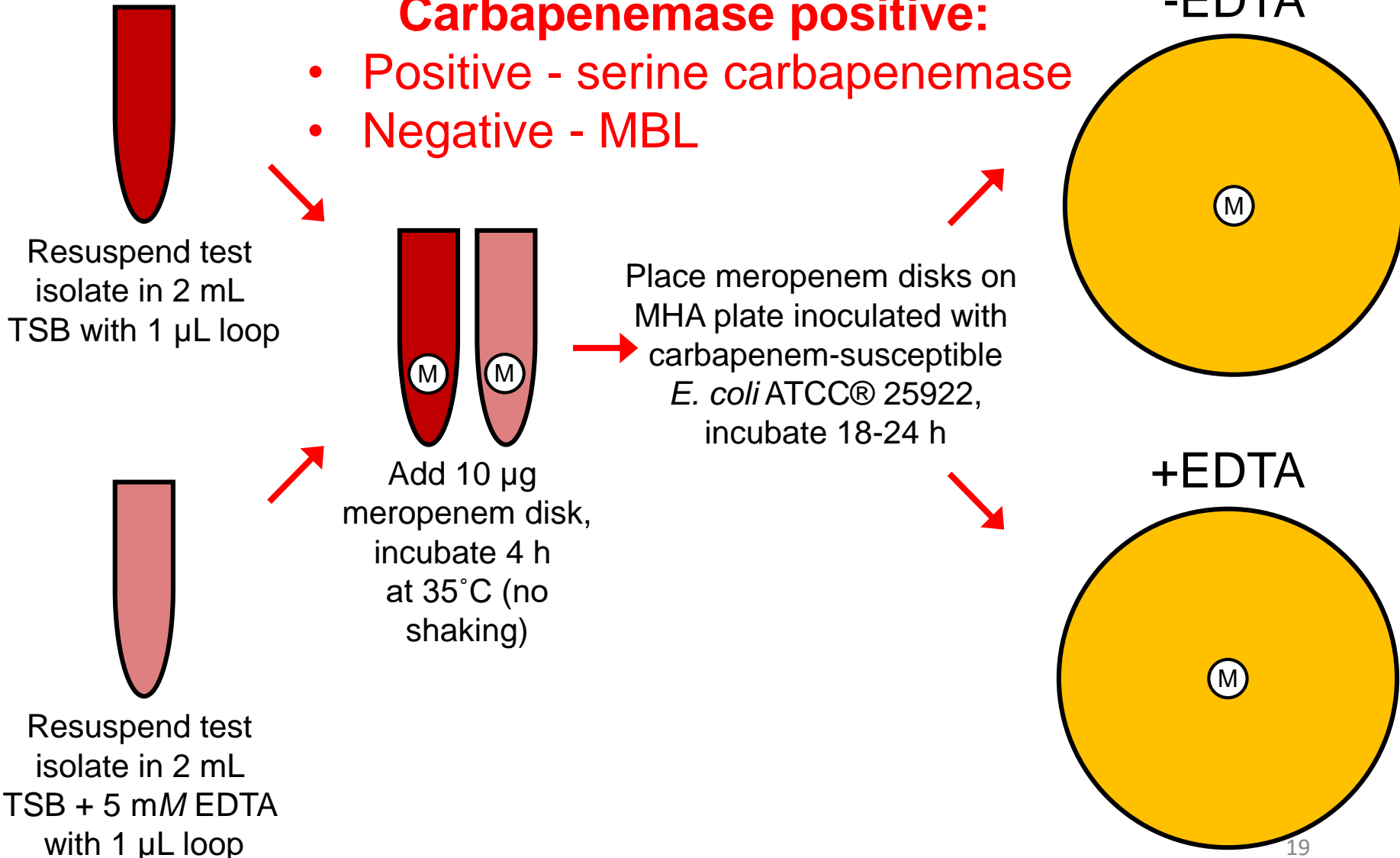
# Modified Carbapenem Inactivation Method (mCIM)

- Multicenter evaluation with *Enterobacteriaceae* (9 sites)
- Overall diagnostic performance:
  - Sensitivity (range), 93-100%
  - Specificity (range), 97-100%
- Good performance with class A, B, and D enzymes
- Easily implemented in any laboratory anywhere, but does require overnight incubation
- Recommended by CLSI (M100-S29) for detection of carbapenemase-producing *Enterobacteriaceae* (also recommended for *P. aeruginosa*, but use 10  $\mu$ L-loopful of *P. aeruginosa* test isolate)

# EDTA-mCIM (eCIM): Differentiation between Serine Carbapenemases and MBLs

## Carbapenemase positive:

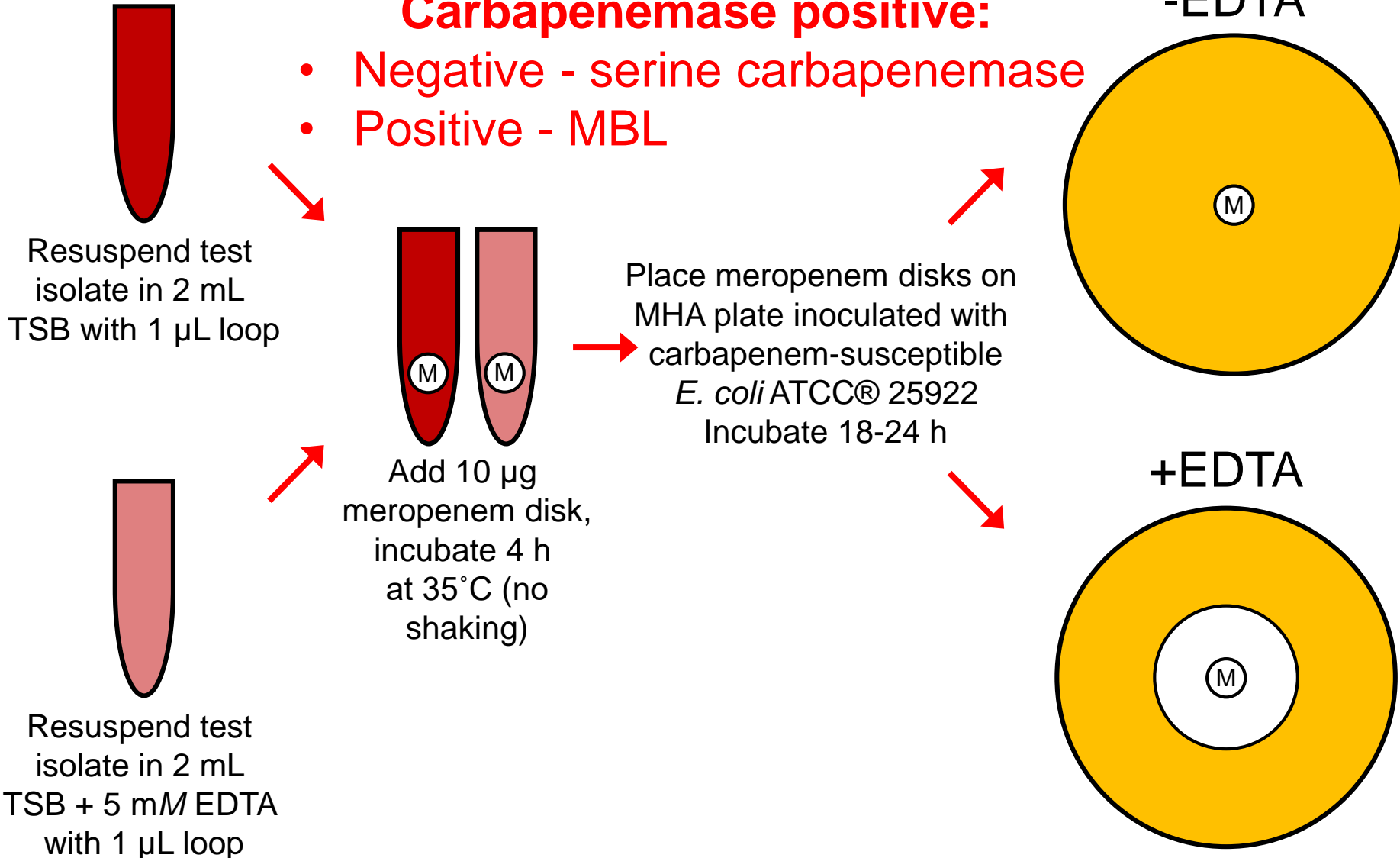
- Positive - serine carbapenemase
- Negative - MBL



# EDTA-mCIM (eCIM): Differentiation between Serine Carbapenemases and MBLs

## Carbapenemase positive:

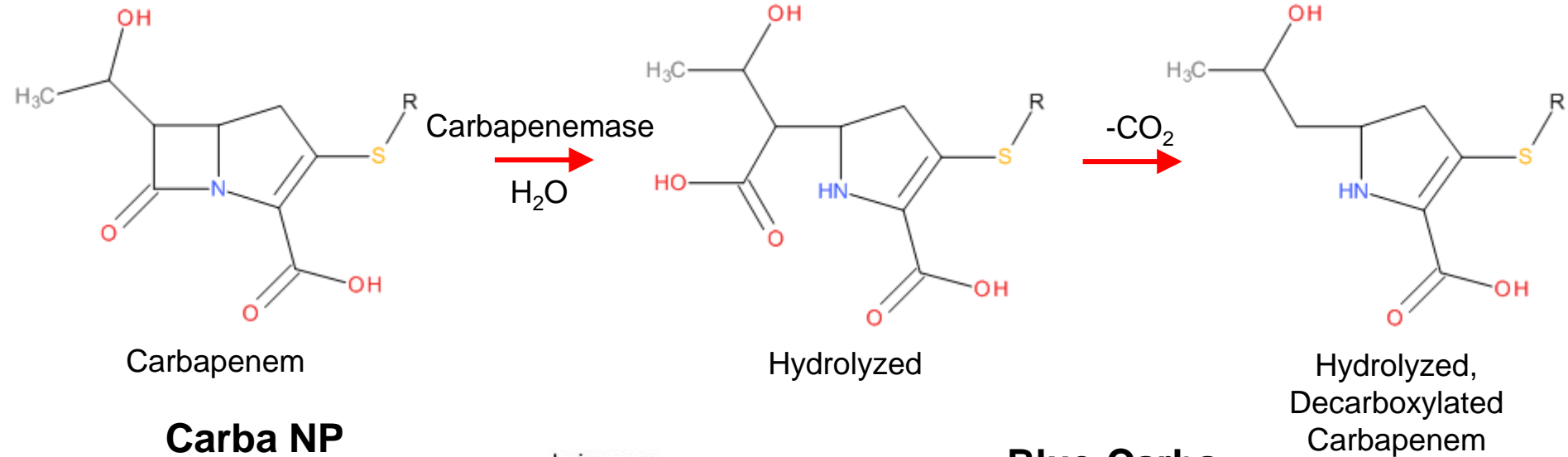
- Negative - serine carbapenemase
- Positive - MBL



# EDTA-mCIM (eCIM): Differentiation between Serine and Metal-Dependent Carbapenemases

- Diagnostic performance for MBLs:
  - Sensitivity, 100%
  - Specificity, 100%
- Easily implemented in any laboratory anywhere, but requires overnight incubation
- Recommended by CLSI (M100-S29) for detection of carbapenemase-producing *Enterobacteriaceae*: **only interpreted if mCIM is positive**

# Carba NP (and Variants): pH-Based Tests

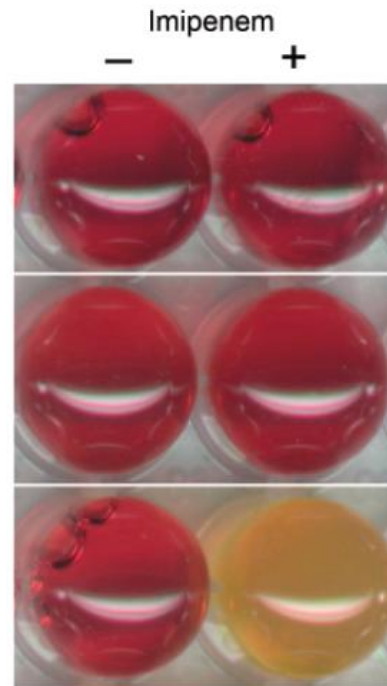


## Carba NP (uses extracts of bacterial isolates)

No inoculation

Non-carbapenemase producer

Carbapenemase producer



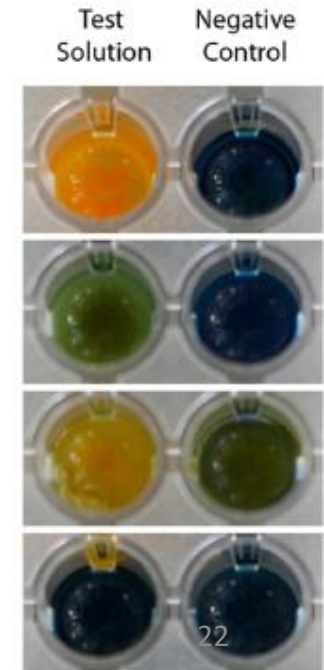
## Blue-Carba (uses bacterial isolates)

*E. coli* NDM-1

*A. baumannii* OXA-23

*K. pneumoniae* OXA-48

*E. coli* non-carbapenemase producer



# Carba NP

- Multicenter evaluation with *Enterobacteriaceae* and non-fermenters (7 sites)
- Overall diagnostic performance for all isolates:
  - Sensitivity (range), 70.6-89.1%
  - Specificity (range), 93-100%
- Poor performance with OXA-48-like enzymes and OXA enzymes (e.g., OXA-23) associated with *A. baumannii*
- Readily implemented in any laboratory anywhere (same day results)
- Some reagents must be made fresh (with “home brew” method)
- Recommended by CLSI (M100-S29) for detection of carbapenemase-producing *Enterobacteriaceae* and *P. aeruginosa* (*Acinetobacter* species removed from M100-S28 [2018])

# Lateral Flow Immunoassays (LFIsAs)

- Carba 5 (NG Biotech) LFIA that detects the following enzymes:
  - IMP
  - KPC
  - NDM
  - OXA-48-like
  - VIM
- Specimen: bacterial isolates
- Preparation, 5 min; turnaround time, 15 min
- Sensitivity, 97.3%; specificity, 99.8%
- Not FDA-cleared (yet)



# Flavors of CDTs

- Phenotypic CDTs:
  - Growth-based (disk/gradient diffusion inhibitor combinations, mCIM and variants)
  - Biochemical-based (Carba NP and variants)
  - Lateral flow immunoassays (Carba 5)
  - Mass spectrometry-based assays
- Genotypic CDTs:
  - Targeted multiplex assays (PCR, microarray)
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# FDA-Cleared Genotypic CDTs

Test (Preparation/ Turnaround time)	Specimen	Type	IMP (B)	KPC (A)	NDM (B)	OXA (D)	VIM (B)
Unyvero LRT Panel, Curetis USA, Inc. (2 min/4-5 h)	<ul style="list-style-type: none"> <li>Tracheal aspirates</li> </ul>	PCR		✓	✓	✓ (OXA-23) (OXA-24) (OXA-48) (OXA-58)	✓
ePlex BCID-GN Panel, GenMark Diagnostics, Inc. (2 min/1.5 h)	<ul style="list-style-type: none"> <li>Positive blood culture broths</li> </ul>	PCR	✓	✓	✓	✓ (OXA-48) (OXA-23)	✓
FilmArray BCID, BioFire Diagnostics, LLC (2 min/1 h)	<ul style="list-style-type: none"> <li>Positive blood culture broths</li> </ul>	PCR		✓			
FilmArray Pneumonia Panel, BioFire Diagnostics, LLC (2 min/1 h)	<ul style="list-style-type: none"> <li>Bronchoalveolar lavage</li> <li>Endotracheal aspirates</li> <li>Sputa</li> </ul>	PCR	✓	✓	✓	✓ (OXA-48)	✓
Verigene BC-GN, Luminex Corporation (5 min/2 h)	<ul style="list-style-type: none"> <li>Positive blood culture broths</li> </ul>	Array	✓	✓	✓	✓ (OXA-48)	✓
Xpert Carba R, Cepheid, Inc. (2-5 min/1 h)	<ul style="list-style-type: none"> <li>Rectal swabs</li> <li>Pure bacterial colonies*</li> </ul>	PCR	✓	✓	✓	✓ (OXA-48)	✓

\*Pure colonies of *Enterobacteriaceae*, *P. aeruginosa*, *A. baumannii*

# Multicenter Evaluation of a Genotypic CDT

- Prospective multicenter study evaluation of Xpert Carba R at 4 sites with rectal swabs<sup>1</sup>
  - 633 specimens:
    - Clinical specimens, 383
    - Contrived specimens, 250
  - Diagnostic performance:
    - Sensitivity, 96.6%
    - Specificity, 98.6%
- Excellent performance with bacterial isolates (100% sensitivity and specificity)<sup>2,3</sup>
- Requires GeneXpert system: same day results with clinical material – rectal swabs – and bacterial isolates recovered in culture)

<sup>1</sup>Tato *et al.*, 2016 *J Clin Microbiol* **54**:1814

<sup>2</sup>McMullen *et al.*, 2016 *Clin Chem* **63**:723

<sup>3</sup>Miller *et al.*, 2017 *J Clin Microbiol* **55**:1827

# Next-Generation Sequencing: Ultimate CDT?

- High-throughput DNA sequencing of bacterial chromosomal and extra-chromosomal DNA (bacterial isolates and clinical material [clinical metagenomics])
- Many platforms/chemistries employed:
  - Illumina
  - Ion Torrent
  - PacBio
  - MinION
- Detect all identifiable carbapenemase genes (highly multiplexed)
- Additional information (open-ended test):
  - Strain relatedness (epidemiology)
  - Plasmid-types (epidemiology/transmission)
  - Cellular permeability: porin mutations/efflux pumps
  - Other resistance determinants (virtual AST)
- Data can be analyzed in real-time or archived for future inquiry
- Requires extensive infrastructure and informatics support

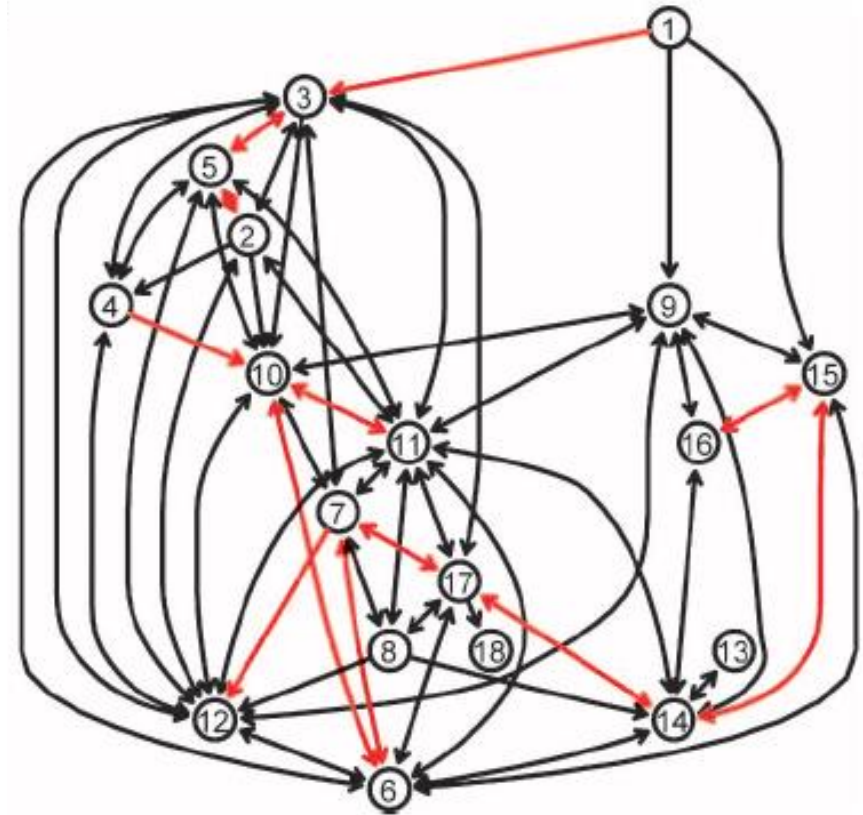
# Next-Generation Sequencing: Ultimate CDT?

- Tracking outbreaks using NGS in conjunction with hospital epidemiology
- KPC-producing *K. pneumoniae* outbreak at NIH Clinical Center
- 1 patient colonized upon admission, linked to colonization in 17 additional patients
- *K. pneumoniae* isolates in US highly related, conventional typing platforms (e.g., PFGE/repPCR) not useful in this setting

# Next-Generation Sequencing: Ultimate CDT?

## Possible Transmission Links Among Patients

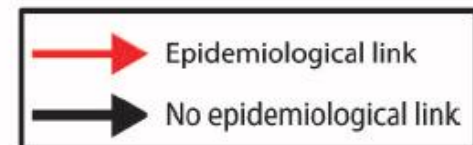
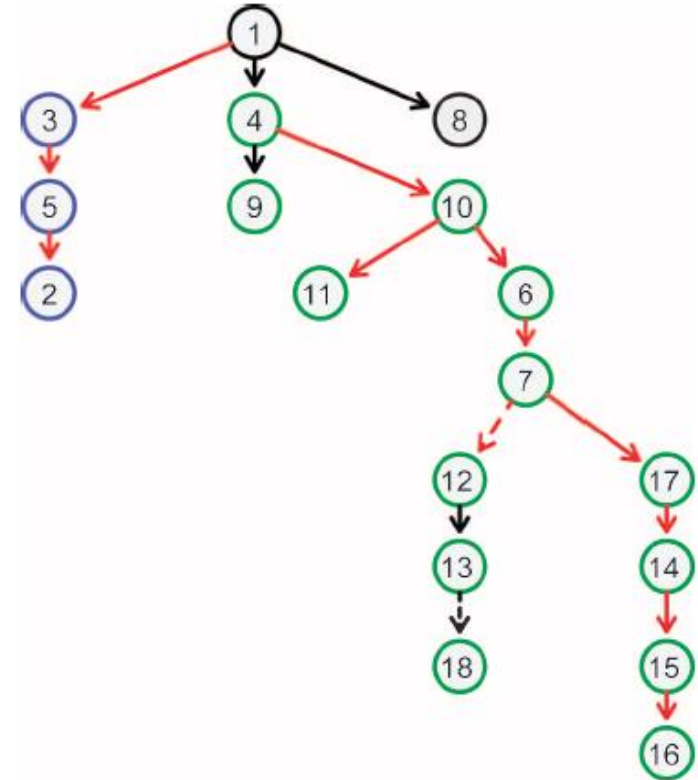
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- KPC-producing *K. pneumoniae* outbreak at NIH Clinical Center
- 1 patient colonized upon admission, linked to colonization in 17 additional patients
- *K. pneumoniae* isolates in US highly related, conventional typing platforms (e.g., PFGE/repPCR) not useful in this setting
- Bacterial whole-genome sequencing identified direct linkage with index patient → **transmission originating from different anatomic sites!**

## Putative Map of *K. pneumoniae* Transmission During Outbreak



# Advantages/Disadvantages of Genotypic Antibiotic Resistance Tests

## Advantages

- Identifies gene(s) encoding exact resistance mechanism
- Rapid results using range of input material:
  - Clinical specimens
  - Bacterial isolates
- Purity not necessary
- Multiplex (multiple antibiotic resistance genes probed simultaneously)

## Disadvantages

- Fail to detect new enzyme variants, novel enzymes, or uncommon enzymes not included in panel
- Presence of gene not always associated with resistance (not expressed)
- Expensive

# What is Really Needed?

## ***Prevalence?***

LFIA/Genotypic assay  
for “Big 5”: IMP, KPC, NDM, OXA-48, VIM

### **Positive:**

- Inform infectious diseases service
- Inform infection control and prevention

### **Negative:**

- Consider functional assay (e.g., eCIM)
- Refer isolate to public health laboratory

# What is Really Needed?

- Routine clinical use:
  - Accurate detection of CRE
  - Awareness of common and unusual resistance mechanisms
  - Method to detect and differentiate between serine carbapenemases and MBLs (LFIA?)
  - Effective communication with infectious diseases/infection control and prevention/antimicrobial stewardship
- Outbreak prevention and control:
  - Rapid, accurate screening method to identify patients colonized with CRE
  - Method to detect and differentiate between serine carbapenemases and MBLs (LFIA?)

# THANK YOU!



# Antibiotic Resistance

- Antibiotics are unique among all therapeutic agents in that the use of the agent in one patient can compromise its efficacy in another
- In the United States each year:
  - 2,000,000 “serious” infections due to multidrug resistant organisms
  - 23,000 deaths due to infection with multidrug resistant organisms
- We are entering (have entered) an era of ineffective antibiotic therapy (post-antibiotic era)