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laboratory medicine.*

## PEARLS OF LABORATORY MEDICINE

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Title: Laboratory detection of carbapenem resistance in  
Gram-negative bacteria

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DOI: 10.15428/CCTC.2017.274571



# Objectives

- Review the mode of action of  $\beta$ -lactam antibiotics
- Describe different classes and subclasses of  $\beta$ -lactam antibiotics
- Review  $\beta$ -lactam resistance mechanisms and classes of  $\beta$ -lactamases
- Discuss methods for detecting carbapenem resistance and indications for carbapenemase testing in Gram-negative bacteria
- Explain methods for detecting carbapenemases



# Common Gram-negative rods

- *Enterobacteriaceae*
  - *Escherichia*
  - *Shigella*
  - *Citrobacter*
  - *Salmonella*
  - *Edwardsiella*
  - *Klebsiella*
  - *Enterobacter*
  - *Serratia*
  - *Proteus*
  - *Providencia*
  - *Morganella*
  - *Yersinia*
- Glucose non-fermenters:
  - *Pseudomonas aeruginosa*
  - *Acinetobacter* spp.
  - *Stenotrophomonas maltophilia*
- Diverse infections:
  - Pneumonia
  - Wound or surgical site
  - Meningitis
  - Gastrointestinal
  - Bloodstream



# Commonly used $\beta$ -lactam antibiotics

## Penicillins

Amoxicillin  
Ampicillin

## $\beta$ -lactam inhibitor combinations

Amoxicillin-clavulanate  
Ampicillin-sulbactam  
Piperacillin-tazobactam  
Ceftolozane-tazobactam  
Ceftazidime-avibactam

## Cephalosporins

Cefazolin (1<sup>st</sup>)  
Cefuroxime (2<sup>nd</sup>)  
Cefotaxime (3<sup>rd</sup>)  
Ceftazidime (3<sup>rd</sup>)  
Ceftriaxone (3<sup>rd</sup>)  
Cefpodoxime (3<sup>rd</sup>)  
Cefepime (4<sup>th</sup>)

## Cephameycins

Cefoxitin  
Cefotetan

## Monobactams

Aztreonam

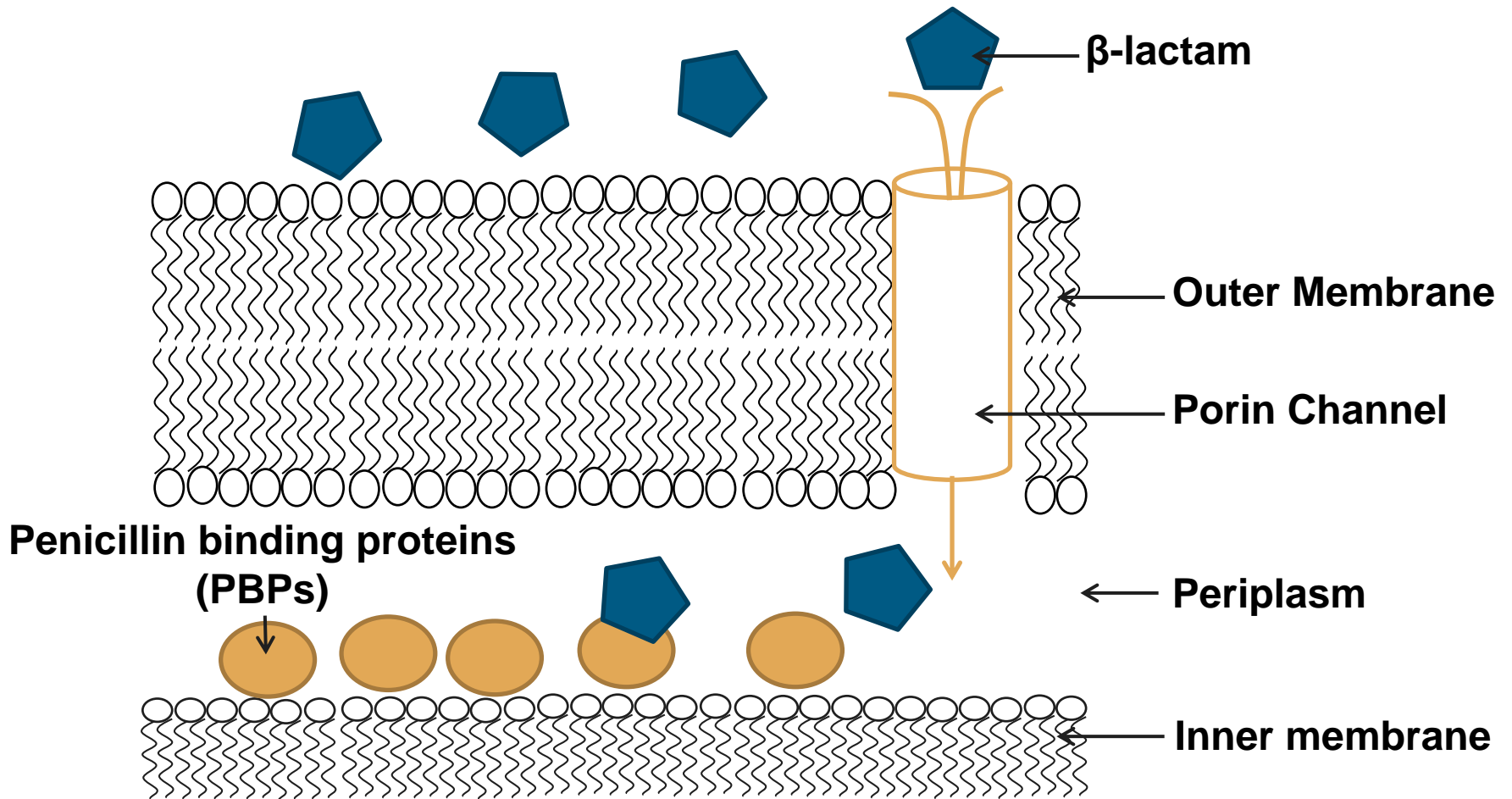
## Carbapenems

Doripenem  
Ertapenem  
Imipenem  
Meropenem

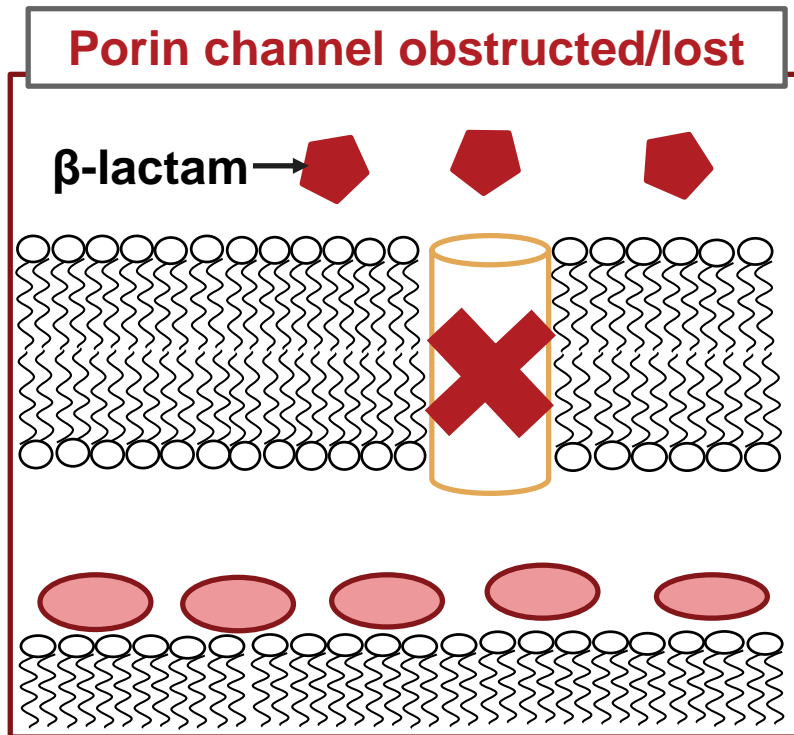
- Broadest spectrum of activity
- Carbapenem-resistant Enterobacteriaceae (CRE) are usually resistant to all  $\beta$ -lactam agents



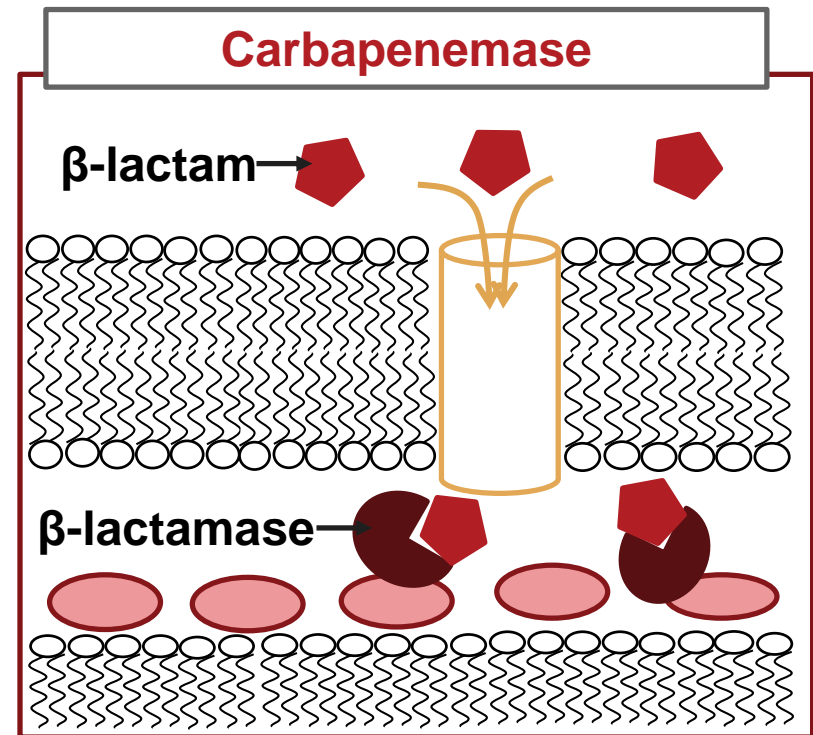
# Mode of action



# Mechanisms of carbapenem resistance



Usually with ESBL or AmpC  
 Non-carbapenemase producer  
**non-CP**



Carbapenemase producer  
**CPO or CP-CRE**  
 Hydrolyze penicillins, cephalosporins,  
 monobactams, and carbapenems



# β-lactamases: Ambler classification

Class	Examples	Produced by:	Notes
<b>A</b>	ESBLs <b>KPC</b>	<i>Enterobacteriaceae</i>	Endemic in US
<b>B</b>	Metallo-β-lactamases <b>NDM, VIM, IMP</b>	<i>Enterobacteriaceae</i> <i>P. aeruginosa</i> <i>A. baumannii</i> <i>S. maltophilia</i>	Endemic in India/Asia, increasing in US
<b>C</b>	AmpC	<i>Enterobacteriaceae</i> Some non-fermenters	Inducible in some genera
<b>D</b>	<b>OXA</b>	<i>A. baumannii</i> <i>Enterobacteriaceae</i>	Hydrolyze carbapenems Endemic in Asia, Europe

# Laboratory detection of carbapenem resistance: *Enterobacteriaceae*

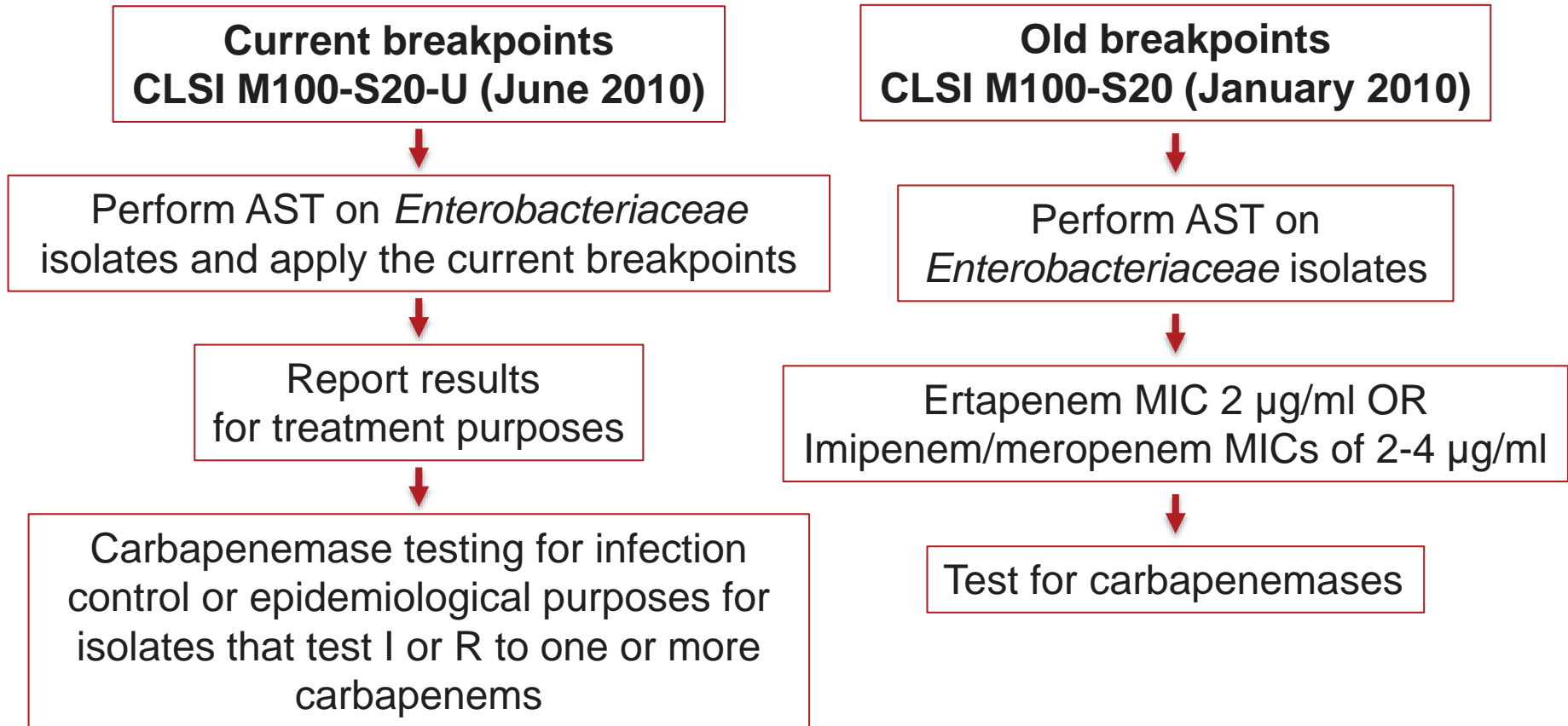
- Outlined by the Clinical Laboratory Standards Institute (CLSI)
- Disk diffusion and broth microdilution
- June 2010: *Enterobacteriaceae* breakpoints for carbapenems were lowered
- Breakpoints were revised based on evaluation of:
  - PK-PD properties, clinical data, and MIC data
- Routine carbapenemase testing does not need to be performed to guide treatment

Current Interpretive Categories and MIC Breakpoints (µg/mL) <i>Enterobacteriaceae</i>			
Agent	S	I	R
Doripenem Imipenem Meropenem	≤ 1	2	≥ 4
Ertapenem	≤ 0.5	1	≥ 2





# Workflow for carbapenem resistance testing



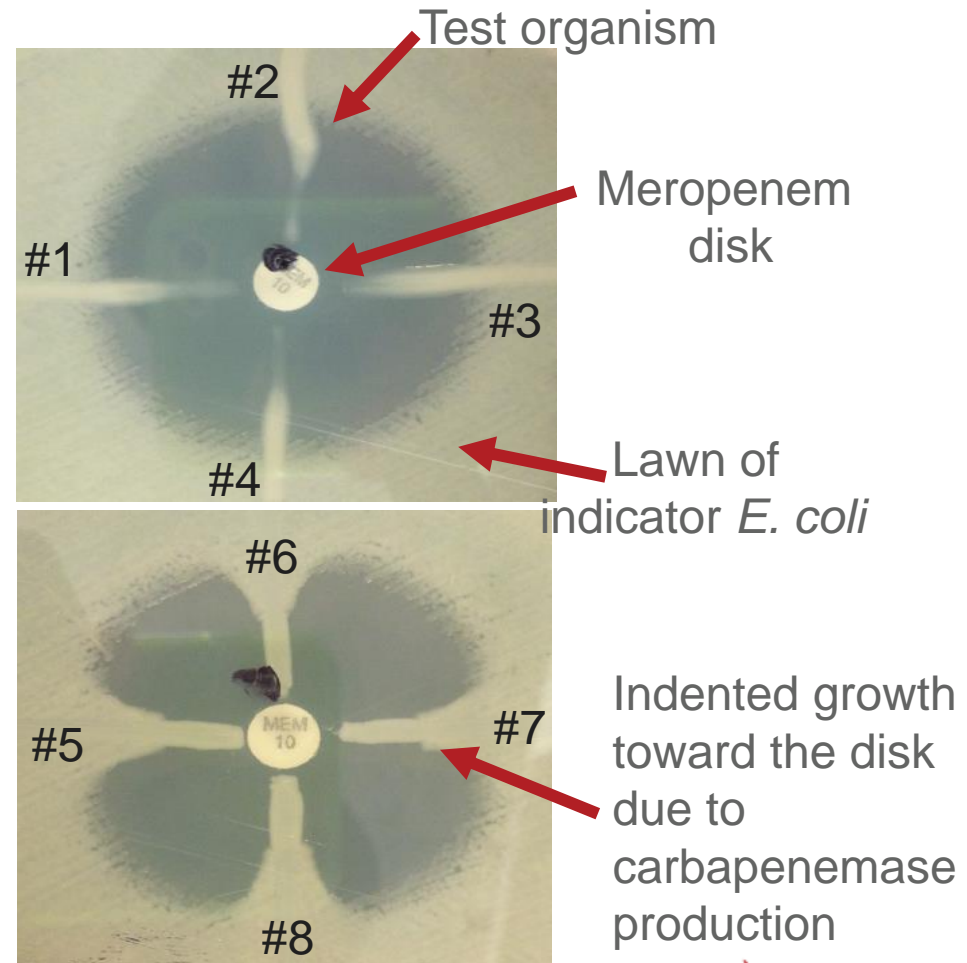
## CLSI recommended tests for carbapenemases

- Phenotypic testing:
  - Modified Hodge Test (MHT)\*
    - \*Future CLSI guidelines will not recommend the MHT
  - Carba NP
  - Modified carbapenem inactivation method (mCIM)
- Molecular testing
- Reporting for phenotypic tests:
  - Positive:
    - Carbapenemase positive or carbapenemase producer
  - Negative:
    - Carbapenemase not detected

Organism	Carbapenemase testing
<i>Enterobacteriaceae</i>	MHT Carba NP mCIM Molecular testing
<i>P. aeruginosa</i>	Carba NP Molecular testing
<i>Acinetobacter</i> spp.	Carba NP Molecular testing

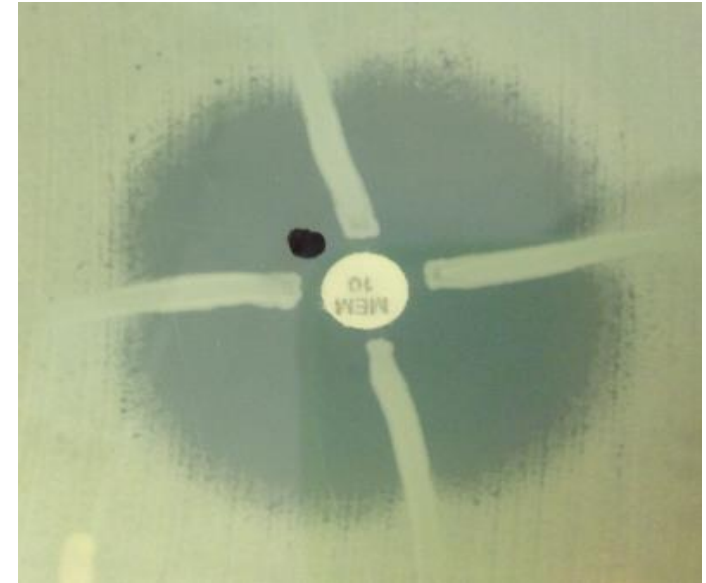
# Modified Hodge Test for carbapenemases

- Lawn of indicator susceptible *E. coli*
- 10 µg ertapenem or meropenem disk
- Test isolate inoculated in a straight line from the disk edge
- Carbapenemase diffuses into agar and hydrolyzes the carbapenem
- Negative: Susceptible *E. coli* cannot grow around the test organism (isolates #1-4)
- Positive: Susceptible *E. coli* can grow toward the carbapenem disk, forming an indentation (isolates #5-8)



# MHT: advantages and limitations

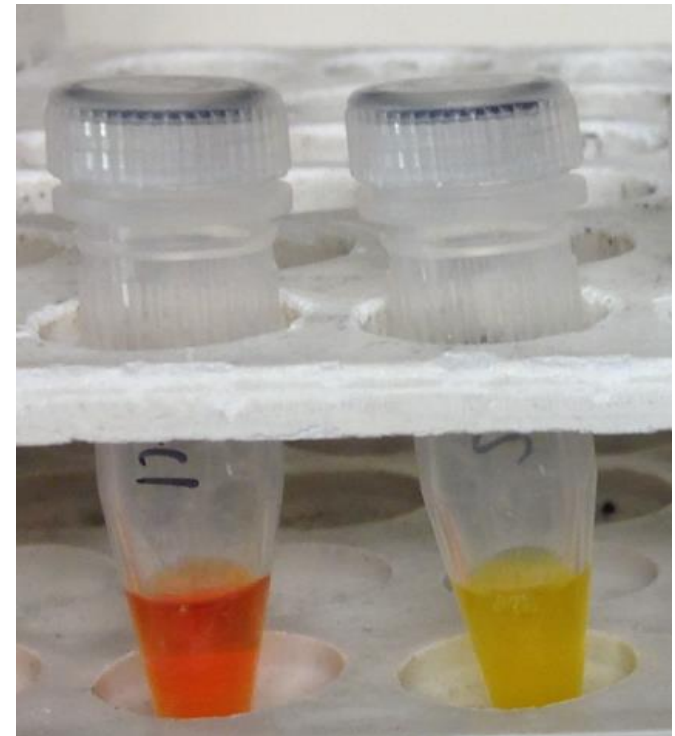
- Advantages:
  - Simple to perform
  - No special reagents or media necessary
- Limitations:
  - False-positive results can occur in isolates that produce ESBL or AmpC enzymes
  - Poor sensitivity for NDM



NDM  
False -

# Carba-NP

- Emulsify test isolate in microcentrifuge tube containing extraction reagent
- Add imipenem and pH indicator solutions
- Incubate for  $\leq 2$  hours
- Positive: color change from red or red-orange to light orange, dark yellow, or yellow
- Negative: tube remains red or red-orange
- Invalid: color change from red/red-orange to orange

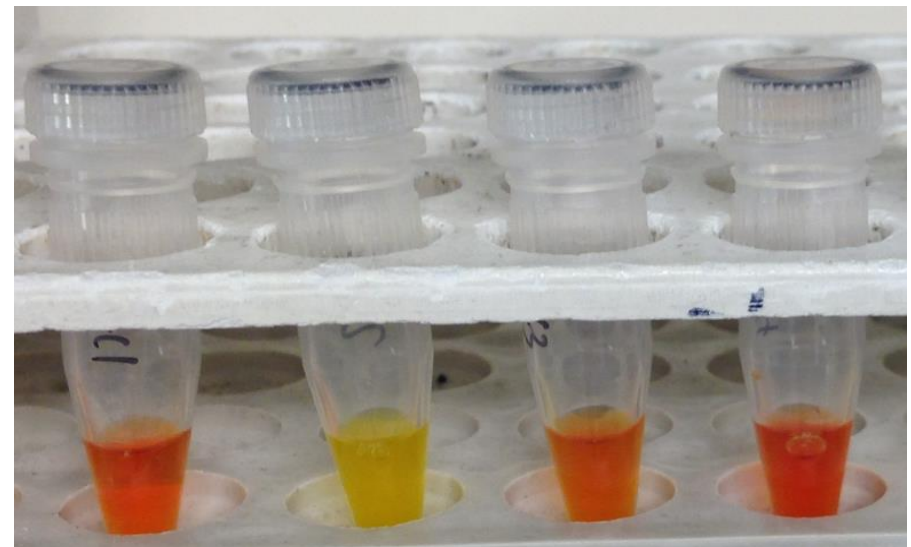


Negative

KPC+

# Carba-NP: advantages and limitations

- Advantages:
  - Rapid: results in  $\leq 2$  hours
  - Can be performed on:
    - *Enterobacteriaceae*
    - *P. aeruginosa*
    - *Acinetobacter* spp.
- Limitations:
  - Reagents
  - Invalid results
  - OXA-type carbapenemases not often detected

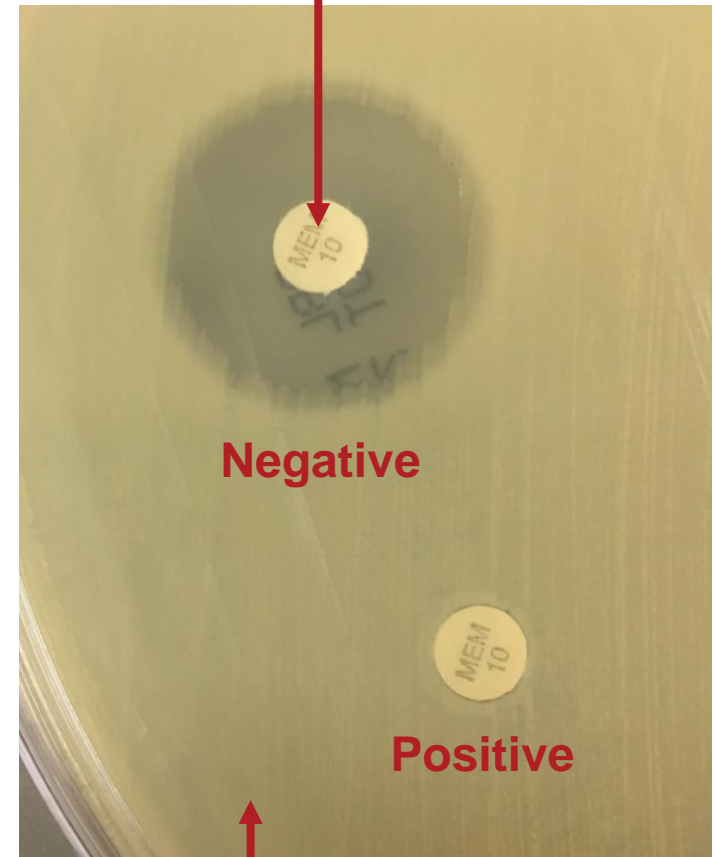


Negative KPC OXA48 OXA181

# mCIM

- Suspend test isolate in tryptic soy broth
- Add a 10 µg meropenem disk to the tube
- Incubate for 4 hours
- Inoculate MHA plate with indicator susceptible *E. coli*
- Remove the meropenem disk from the tube and place on the inoculated plate
- Positive: zone of 6-15 mm or presence of colonies within a 16-18 mm zone
- Negative: Zone  $\geq 19$  mm
- Indeterminate: Zone of 16-18 mm

Meropenem disk  
incubated with test isolate



Lawn of  
indicator *E. coli*



# mCIM: advantages and limitations

- Advantages:
  - No special reagents or media are required
  - Highly sensitive and specific
  - CLSI now endorses this test for *P. aeruginosa*
- Limitations:
  - Requires overnight incubation
  - Not all carbapenemase-producing *Enterobacteriaceae* are mCIM positive
    - OXA-232 producing *K. pneumoniae*





# Molecular testing: advantages and limitations

- Can be performed on *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* spp.
- Determines presence or type of carbapenemase
- FDA-cleared: Cepheid Xpert Carba-R
  - Detects KPC, NDM, VIM, IMP-1, OXA-48
- Advantage:
  - Rapid
  - Distinguishes type of carbapenemase
- Limitations:
  - False-negatives if test isolate has a carbapenemase that is not targeted
  - More expensive



# Summary

- Using the current carbapenem breakpoints, carbapenemase testing is performed if requested by infection control or for epidemiological purposes
- Using the old carbapenem breakpoints, carbapenemase testing is required in isolates that have an ertapenem MIC 2 µg/ml or imipenem/meropenem MICs of 2-4 µg/ml
- Tests for carbapenemases are the MHT, Carba-NP, mCIM, and molecular testing
  - MHT will no longer be recommended by CLSI
- Challenges to carbapenemase testing:
  - Ertapenem resistance is often due to AmpC or ESBL
  - *Proteus*, *Providencia*, and *Morganella* have intrinsically elevated imipenem MICs
  - No phenotypic test detects all carbapenemase resistance mechanisms



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