Carbapenem Resistance and Carbapenemase Detection in the Clinical Microbiology Lab

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Disclosures

- None

Objectives

1. Describe mechanisms of carbapenem resistance

2. Explain various methods for detecting carbapenemases in the clinical lab

3. Discuss the importance of carbapenemase detection for treatment of infections

Definitions

- **Carbapenem**:
  - A class of antibiotics

- **Carbapenem Resistance**:
  - A type of antimicrobial resistance occurring primarily among Gram-negative organisms. Considered a major, ongoing, global public health issue
  - Organisms with this type of resistance are termed “carbapenem-resistant” (CR)

- **Carbapenemase**:
  - An enzyme that confers carbapenem resistance to organisms

Carbapenemases

- **<u>Carbapenemases</u>**: β-lactamase enzymes capable of hydrolyzing carbapenem antibiotics
- Also able to hydrolyze other antibiotic classes:
  - Penicillins
  - Cephalosporins
  - Monobactams

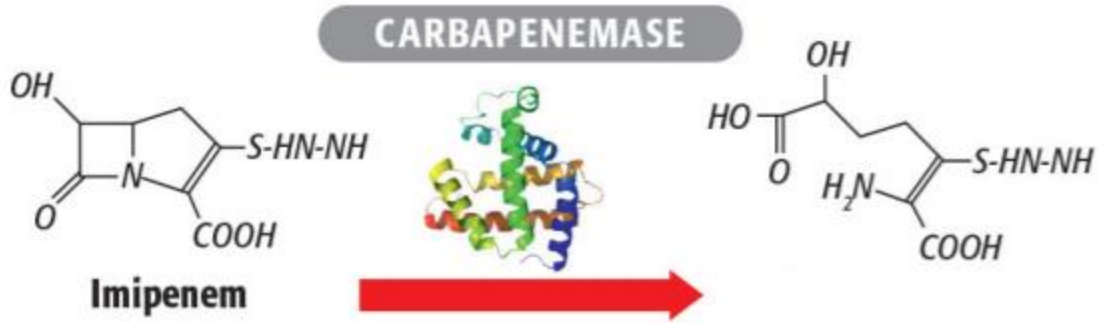
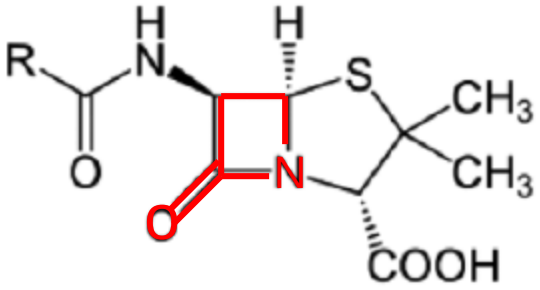


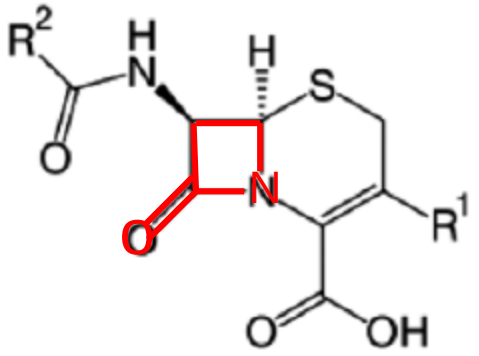
Image modified from https://www.biomerieux.co.uk/sites/clinic/files/carbapenem_resistance_booklet.pdf

Antibiotic Chemical Structures

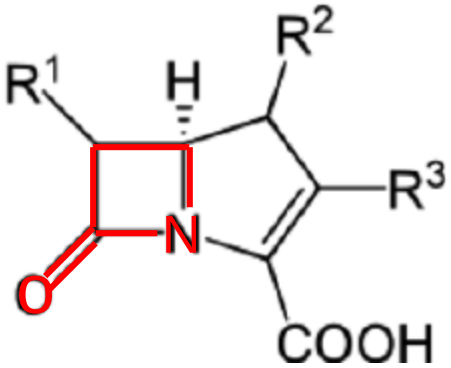
a. Core structure of penicillins



b. Core structure of cephalosporins



c. Core structure of carbapenems



d. aztreonam chemical structure

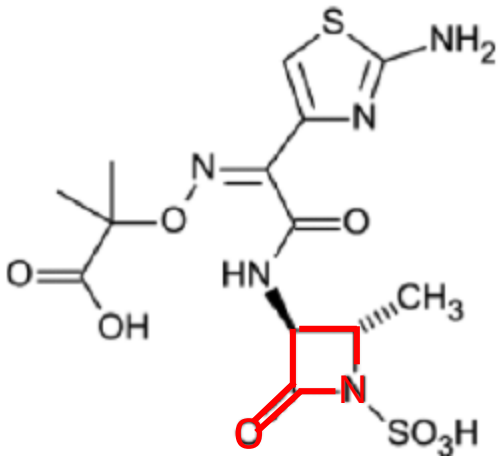


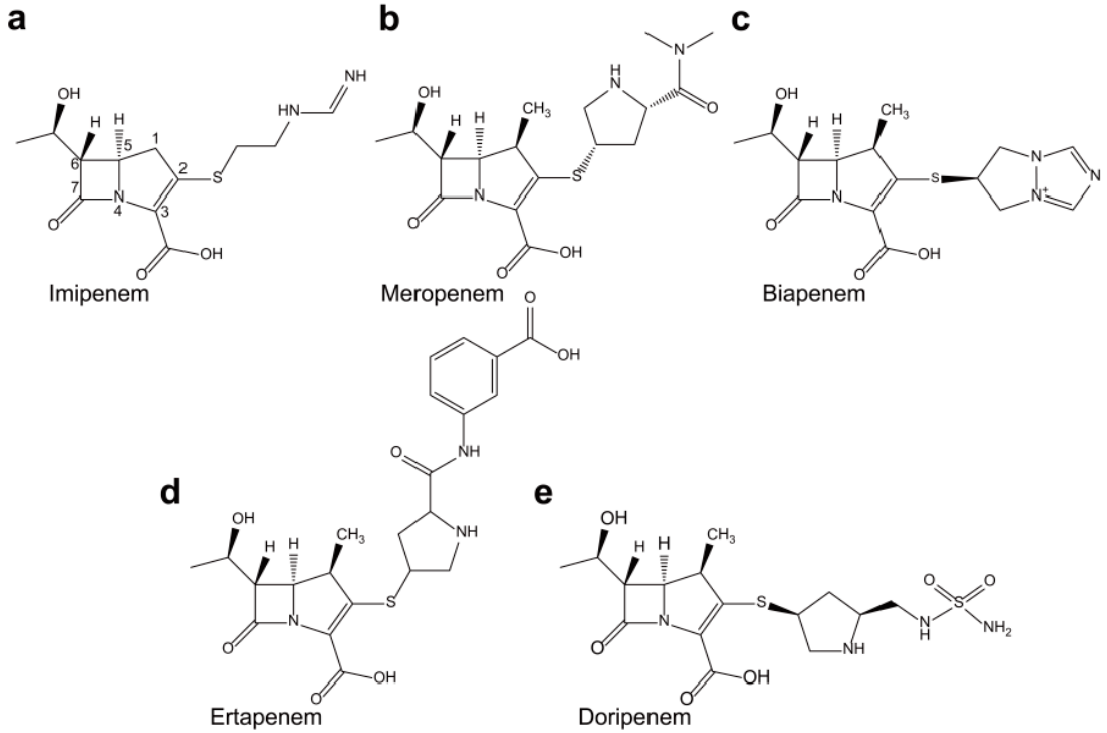
Image modified from Terico & Gallagher (2014) J Pharm Pract

Carbapenemase-Producing Organisms

- Carbapenemase-producing isolates of *Enterobacteriaceae:*
  - Intermediate (I) or resistant (R) to one or more carbapenems
  - Usually R to one or more 3rd generation cephalosporins

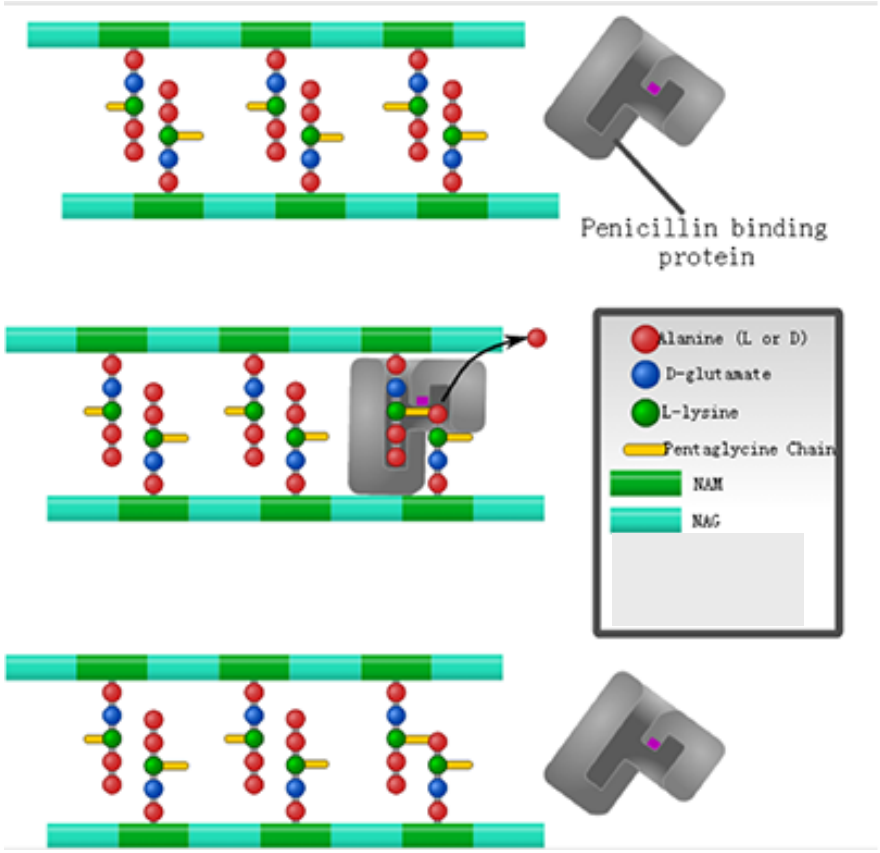
Carbapenems

- Broad-spectrum β-lactam antibiotics, bactericidal (targets the cell wall)
- Indications for treatment:
  - Used in treatment of severe GN infections (also cover GP and anaerobes)
  - Considered “drugs of last resort”



Jeon et al (2015) Int. J. Mol. Sci.;
CDC Antibiotic Threat Report (2013)

Carbapenem Mechanism of Action



- PBPs play a role in synthesis of the bacterial cell wall peptidoglycan layer
- Carbapenems enter the cell through outer membrane porins
- Inhibit the cross-linking of peptidoglycan
  - Transpeptidase inhibition
- Prevents inhibition of autolytic enzymes, leading to cell death
- Carbapenems bind to many different PBPs

Image modified from Wikipedia

Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. Antimicrob Agents Chemother. 2011;55(11):4943–4960. doi:10.1128/AAC.00296-11

Carbapenems – A History

- Carbapenem discovery was driven by the search for β-lactamase inhibitors in the 1970s
  - 1976: Discovery of thienamycin, the first carbapenem
    - A compound produced by environmental organism *Streptomyces cattleya*
    - Differs from penicillins and cephalosporins: secondary ring does not contain sulfur
    - Broad antimicrobial activity:
      - β-lactamase inhibitor, active against most Gram negatives tested, anti-*Staphylococcal* activity, excellent anaerobic coverage

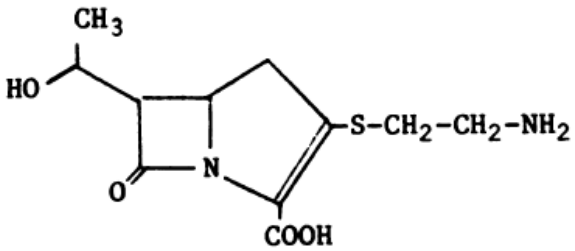
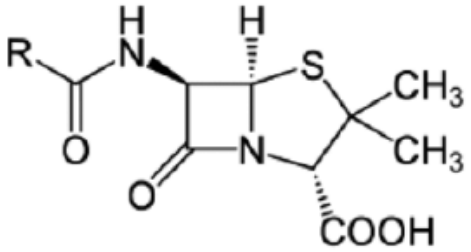
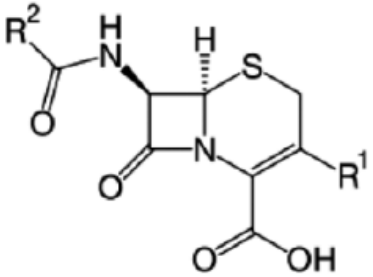


FIG. 1. *Structure of thienamycin.*



Structure of penicillins



Structure of cephalosporins

Images modified from Terico & Gallagher (2014) J Pharm Pract

Antibacterial properties: Tally, F. P., Jacobus, N. V., Gorbach, S. L., Antimicrob. Agents Chemother. 1978, 14, 436– 438

Carbapenems – A History

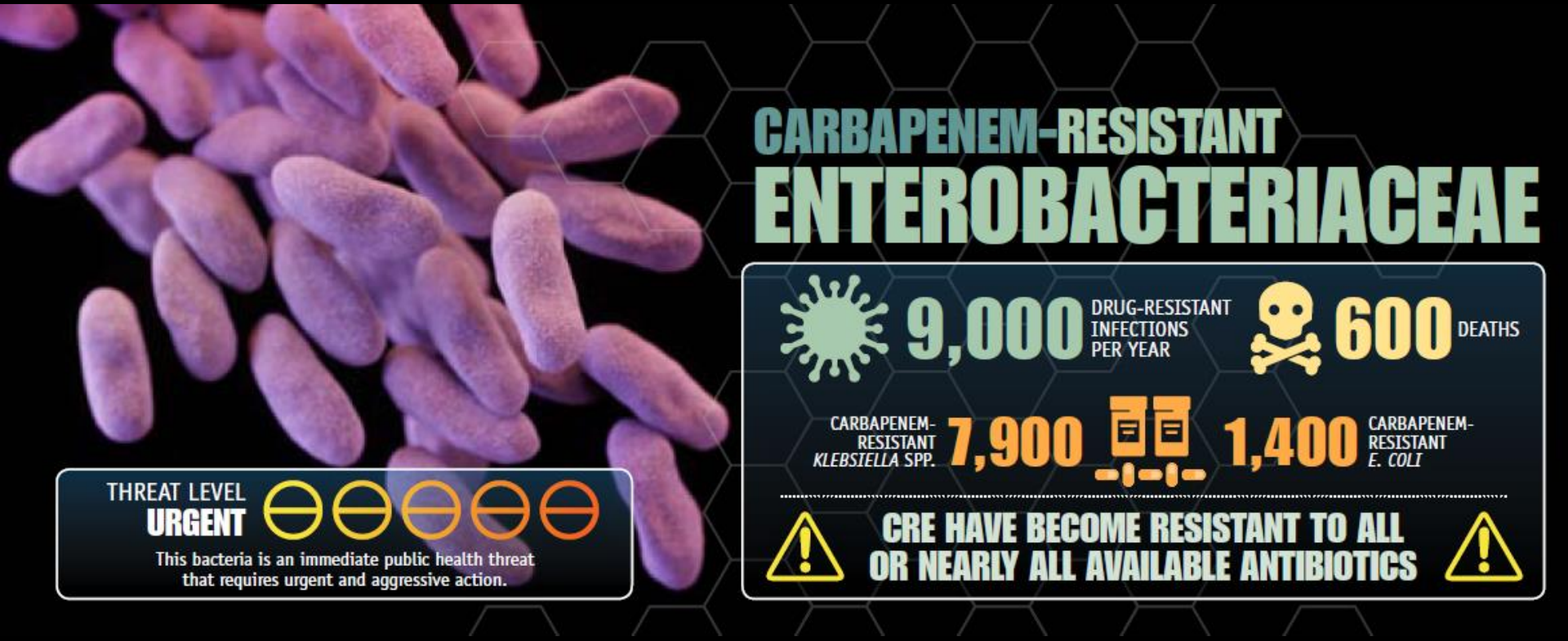
- 1980 – US patent for imipenem was granted to Burton Christensen and colleagues
  - Approved for use in the United States in 1985

- 1984 – Makoto Sunagawa and colleagues filed a patent with the European Patent Office for meropenem synthesis
  - Approved for use in the United States in 1996

- 1998 - Resistance to imipenem identified

US FDA www.accessdata.fda.gov
Christensen et al. U.S. Patent 4,194,047
Meropenem synthesis: Sunagawa, M., Matsumura, H., Inoue, T., Fukasawa, M., Masatomo, K., Kato, M., EU-A EP126587, ***1984***

CDC Antibiotic Threat Report - 2013

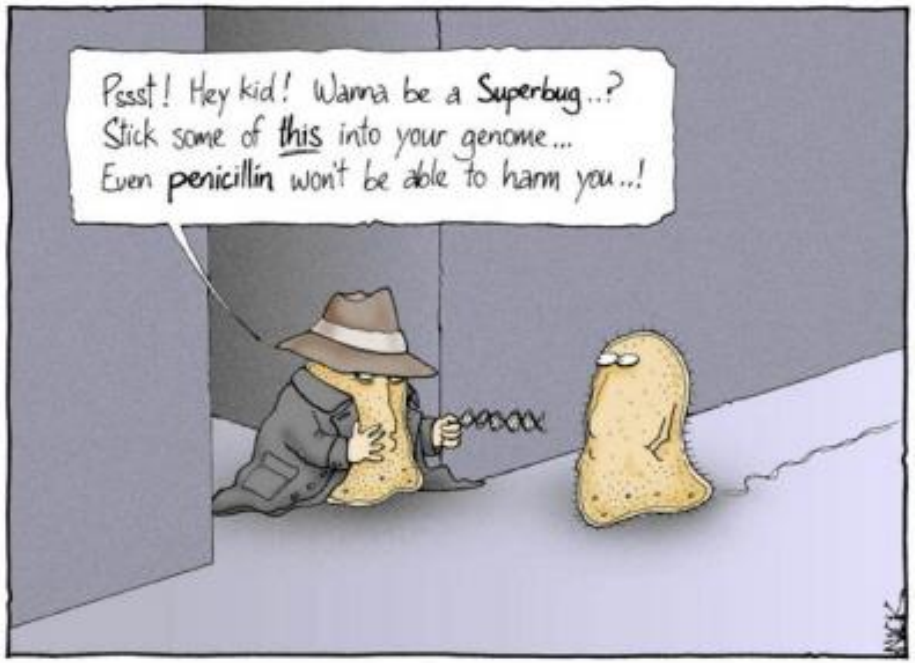


Common Carbapenem-Resistant Organisms

- Carbapenem-resistant *Enterobacteriaceae*
  - *Klebsiella* spp.
  - *Escherichia coli*
  - *Serratia* spp.
- *Pseudomonas* spp.
- *Acinetobacter* spp.
- *Enterobacter cloacae* complex
- *Stenotrophomonas maltophila*

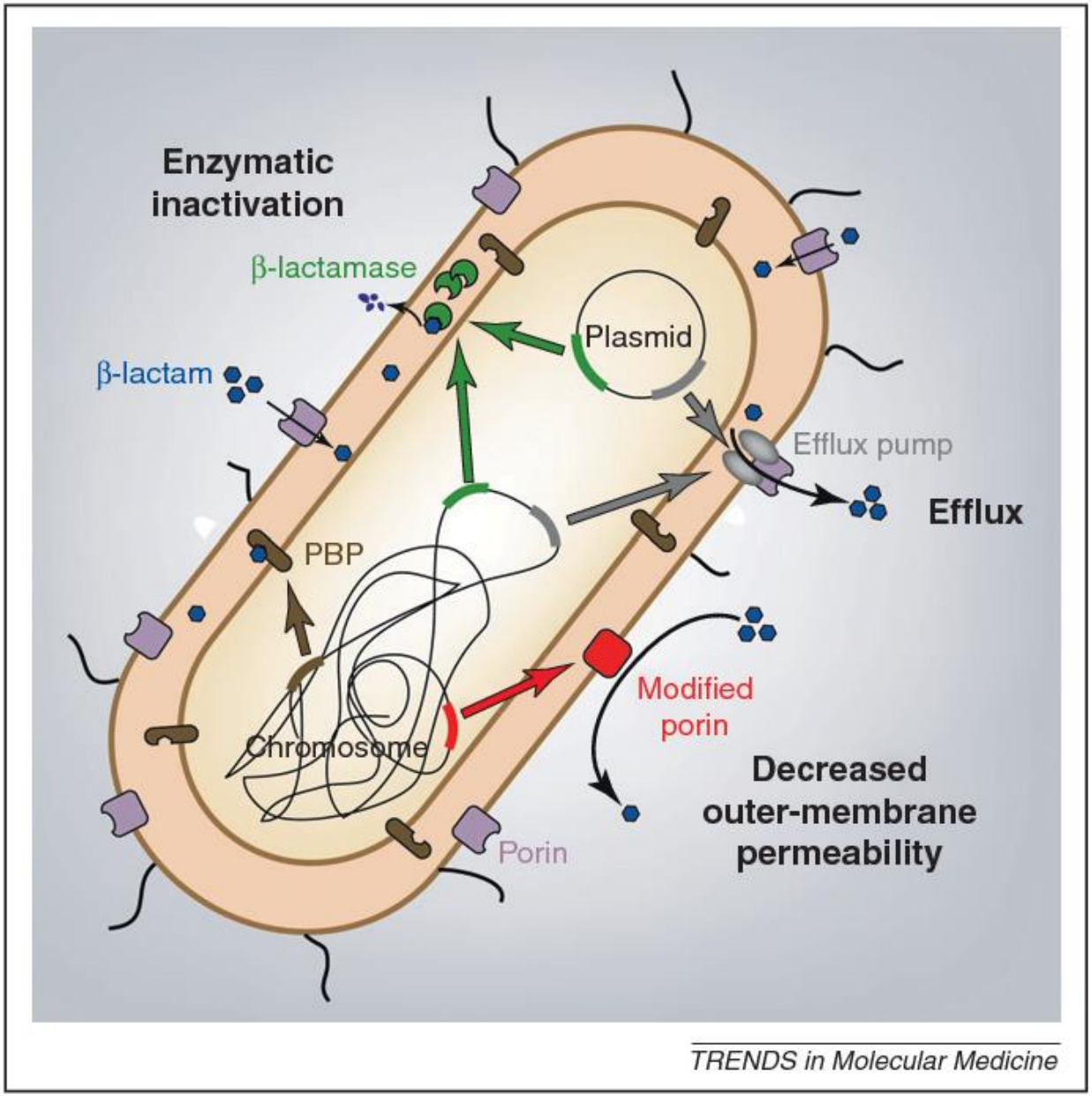
Mechanisms of Carbapenem Resistance

- Intrinsic
  - Chromosomally encoded carbapenemases
- Acquired
  - Decreased outer membrane permeability
  - Overexpression of efflux pumps
  - Horizontally transferred carbapenemases



It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

Meletis G. Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Infect Dis. 2016;3(1):15–21. doi:10.1177/2049936115621709;
Codjoe FS, Donkor ES. Carbapenem Resistance: A Review. Med Sci (Basel). 2017;6(1):1. Published 2017 Dec 21. doi:10.3390/medsci6010001


Enzymatic inactivation
β-lactamase
β-lactam
Plasmid
Efflux pump
Efflux
PBP
Chromosome
Modified porin
Decreased outer-membrane permeability
Porin
TRENDS in Molecular Medicine

Carbapenemases

- **<u>Carbapenemases</u>**: β-lactamase enzymes capable of hydrolyzing carbapenem antibiotics
- Also able to hydrolyze other antibiotic classes:
  - Penicillins
  - Cephalosporins
  - Monobactams

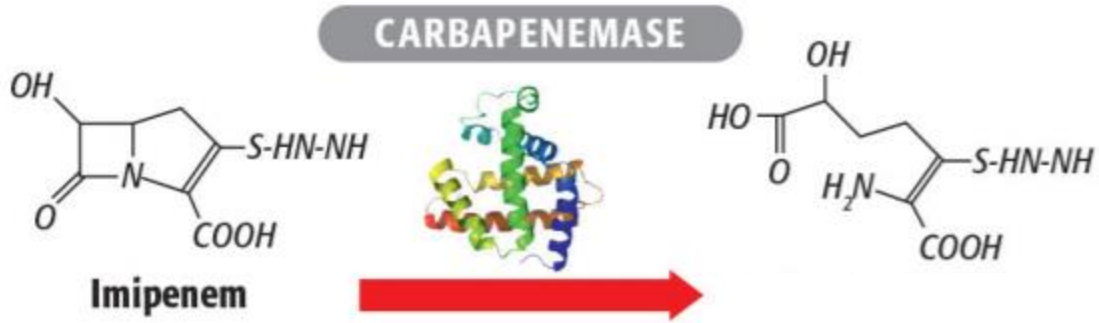
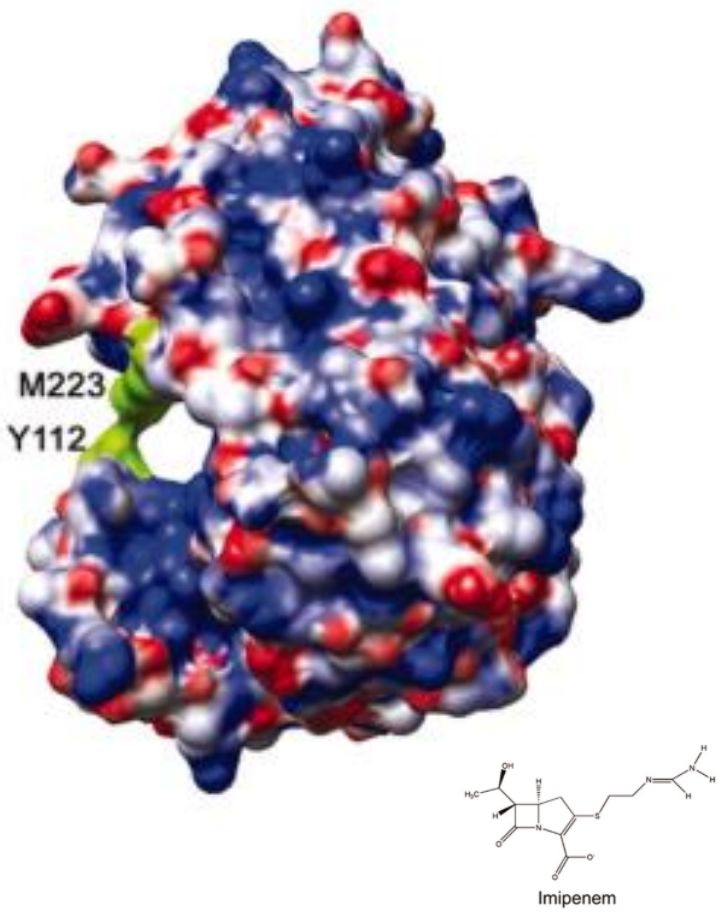
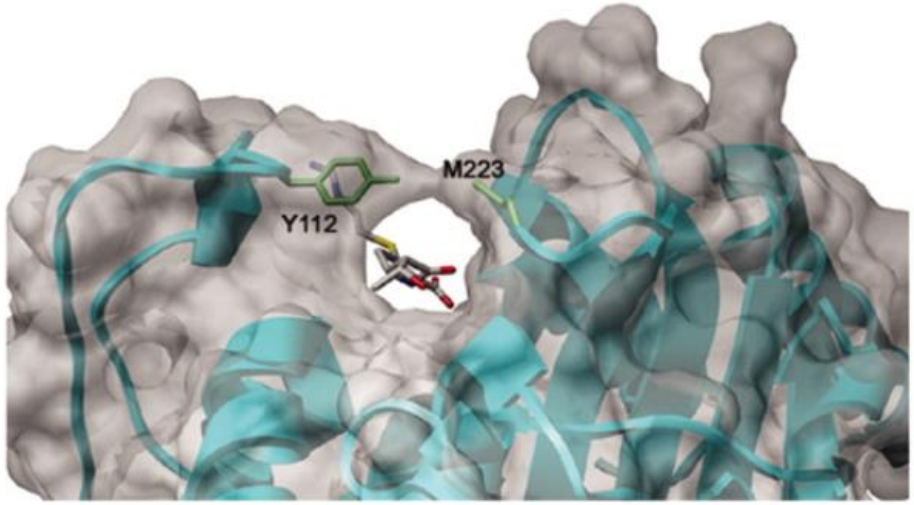


Image modified from https://www.biomerieux.co.uk/sites/clinic/files/carbapenem_resistance_booklet.pdf

Carbapenem-Carbapenemase Complex





Images modified from Santillana et al (2007) PNAS

Ambler Classification of β-Lactamases

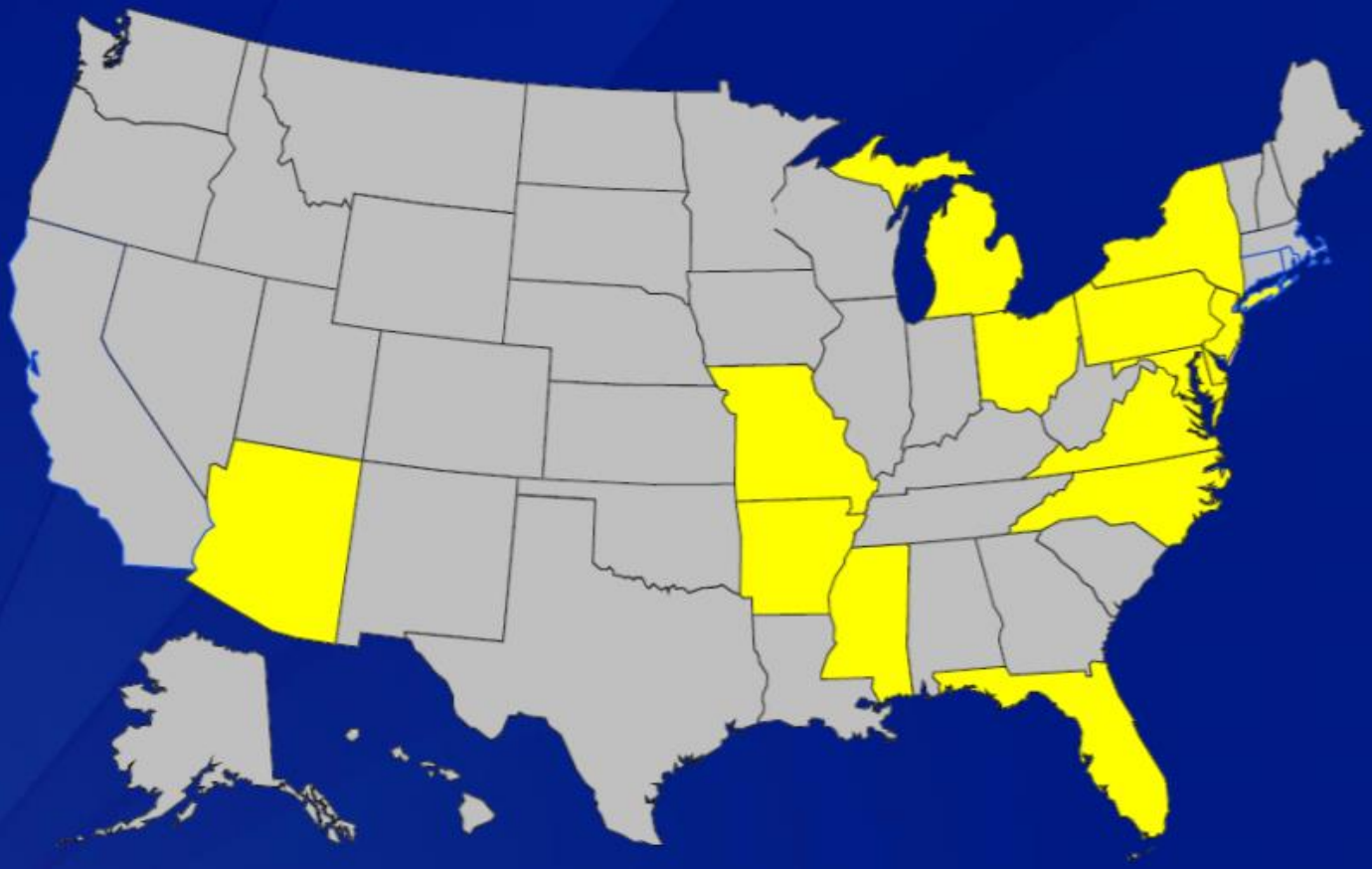
Ambler Class	Antibiotic Inactivation	Inhibition	Representative Enzymes
A (Serine carbapenemases)	Penicillins, cephalosporins, carbapenems, and aztreonam	β-lactamase inhibitors (Clavulanate, Avibactam) + ceftazidime or aztreonam	KPCs
B (Metallo-β-lactamases (MBLs))	All β-lactams except aztreonam	EDTA	IMP, VIM, NDM
D (Oxacillinases; serine carbapenemases)	Penicillins, 1st gen cephalosporins, weak against carbapenems	Avibactam	OXA-48 and derivatives

1) Biomerieux Carbapenem Resistance Booklet
2) Cadjoe & Donkor (2018) Med Sci (Basel)
3) Queenan & Bush (2007) Clin Micro Rev
4) Ambler (1980) Philos Trans R Soc Lond B Biol Soc
5) Bush & Jacoby (2010) Antimicrob Agents Chemo

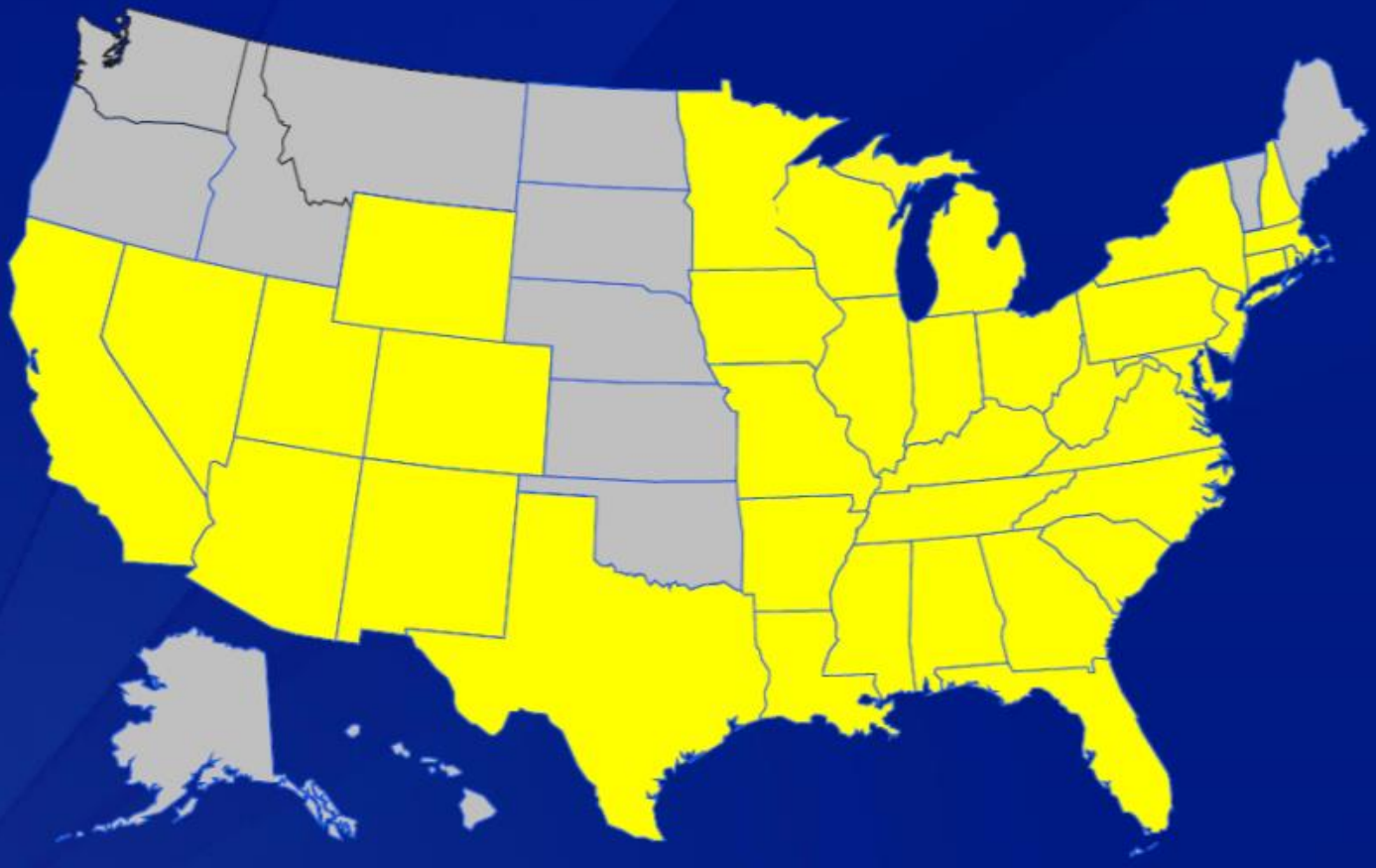
Geographical Distribution of KPC-Producers 2001

Geographical Distribution of KPC-Producers 2005

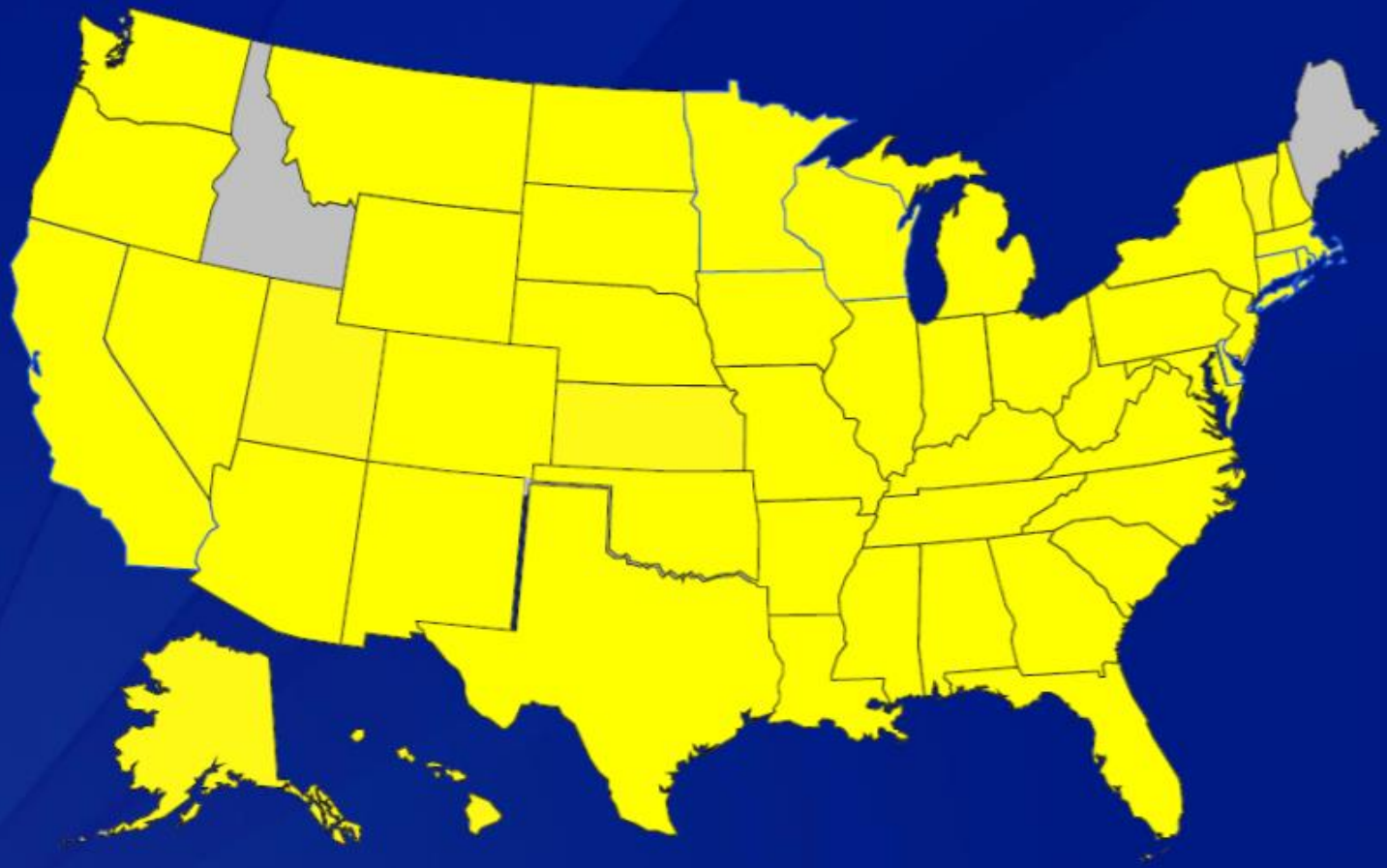
Geographical Distribution of KPC-Producers 2006



Geographical Distribution of KPC-Producers 2010



Geographical Distribution of KPC-Producers 2015



Patients with KPC-producing *Carbapenem-resistant Enterobacteriaceae* (CRE) reported to the Centers for Disease Control and Prevention (CDC) as of December 2017, by state

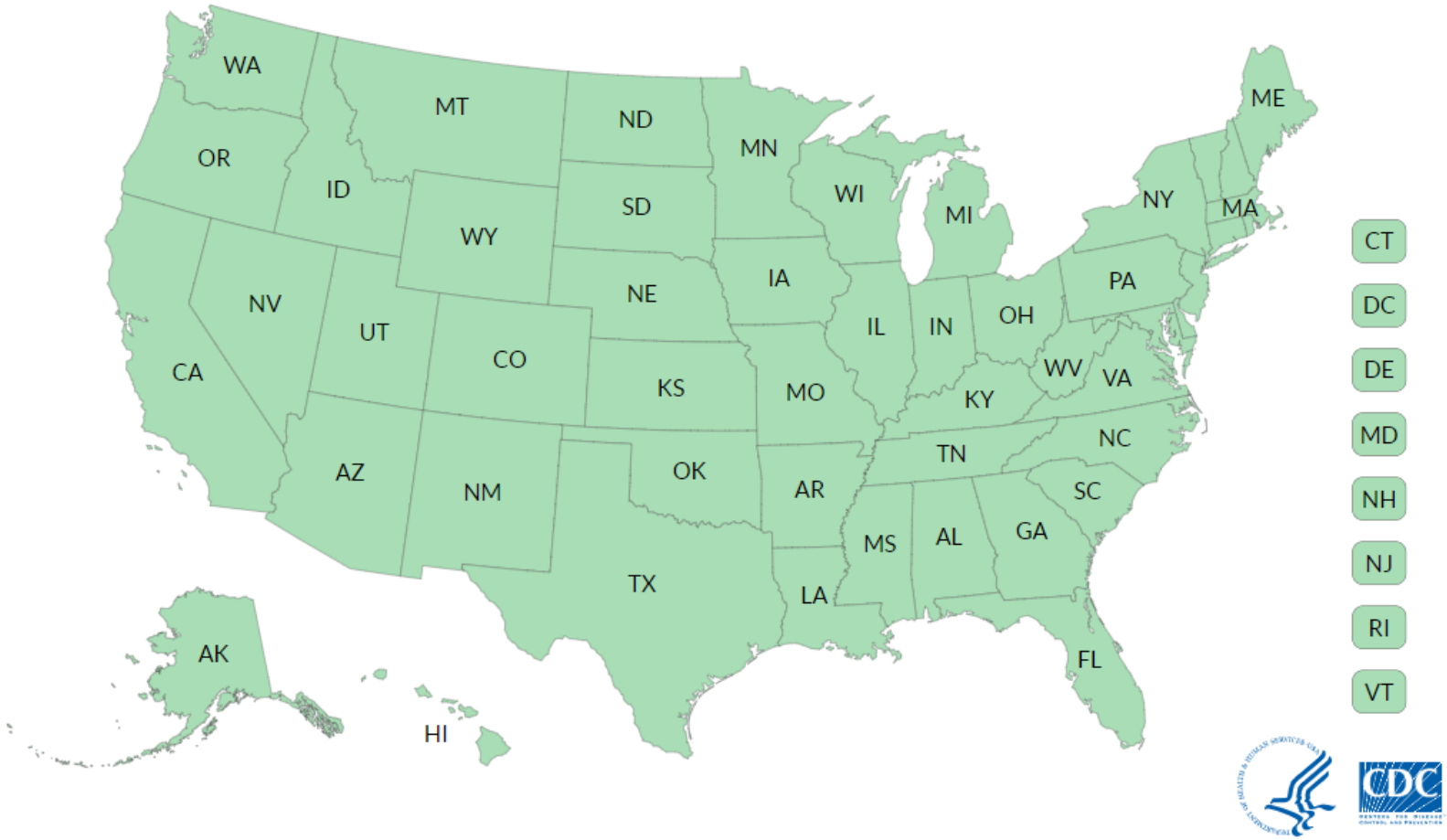


Table. A selection of carbapenemase detection tests that are currently available

Test (manufacturer)	Method	Specimen type	Turnaround time*	Carbapenemase gene(s) detected	Regulatory status
Phenotypic tests					
Carba NP	Color indicator of carbapenem (imipenem) hydrolysis	Isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa*	Same day	Not applicable	Commercial version U.S. FDA-cleared Rapidec Carba NP (bioMérieux); there is also a CLSI-recommended method
mCIM	Growth of carbapenem-susceptible indicator strain around meropenem disk incubated with a suspected carbapenemase-producing test strain	Isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa*	Next day	Not applicable	Laboratory-developed test
eCIM	Growth of carbapenem-susceptible indicator strain around meropenem disk incubated with a suspected carbapenemase-producing test strain in the presence and absence of EDTA	Isolates of *Enterobacteriaceae* (modification of mCIM that affords differentiation between serine- and metal-dependent carbapenemases)	Next day	Not applicable	Laboratory-developed test
Matrix-assisted laser desorption ionization time of flight mass spectrometry	Detection of carbapenem degradation products	Bacterial isolates	Same day	Not applicable	Laboratory-developed test
Genotypic tests					
FilmArray blood culture identification panel (BioFire Diagnostics)	Polymerase chain reaction	Positive blood culture broth with Gram-negative rods	Same day	bla_{KPC}	FDA cleared
Verigene Gram-negative blood culture test (Luminex)	Microarray	Positive blood culture broth with Gram-negative rods	Same day	bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{VIM}	FDA cleared
Xpert Carba-R (Cepheid)	Polymerase chain reaction	Rectal swabs, also isolates of *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*	Same day	bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{VIM}	FDA cleared

*Time to results from setting up the assay

Phenotypic Tests

CarbaNP

mCIM

eCIM

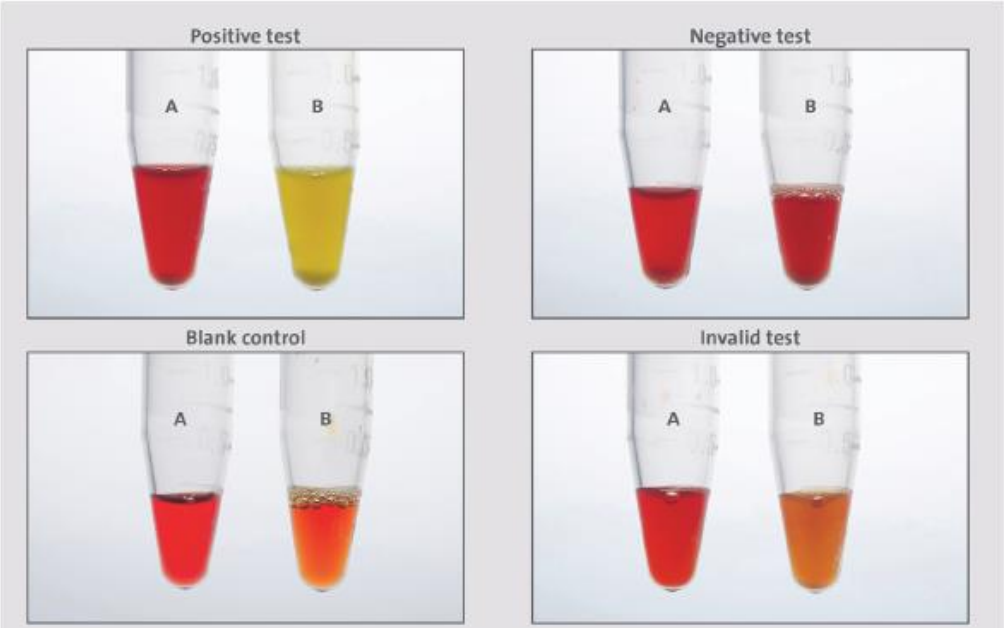
MALDI-TOF

CarbaNP

- **Purpose**:
  - To test for suspected carbapenemase production in *Enterobacteriaceae* and *Pseudomonas aeruginosa*
    - In laboratories using carbapenem breakpoints from M100 S20 (2010)
    - *Enterobacteriaceae* ex: imipenem or meropenem MICs 2-4 µg/mL, or ertapenem MIC 2 µg/mL
  - Infection control or epidemiological purposes
    - *Enterobacteriaceae* and *P. aeruginosa* that are not S to one or more carbapenems
- **Principle and Method**:
  - Detection of carbapenemase production by imipenem hydrolization
  - Colorimetric microtube assay

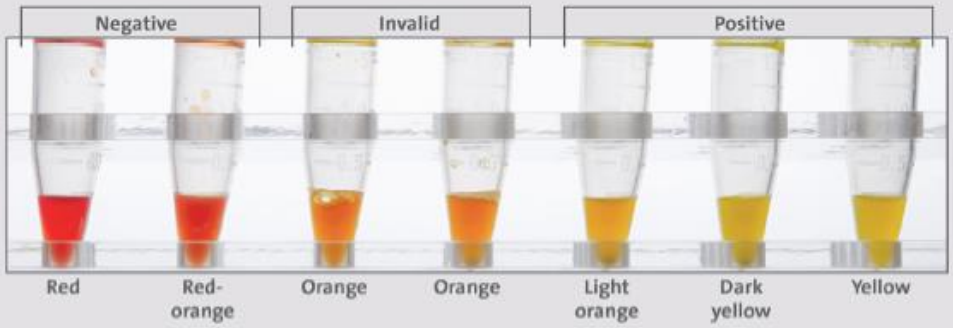
CarbaNP

- Two tubes per patient
- Emulsify one loop of bacteria in bacterial protein extract in each tube
- Add solution A to tube A, and solution B (A + imipenem) to tube B
- Incubate at 35°C for up to 2 hours
- Any tube positive before 2 hours can be reported as positive



"No carbapenemase detected"

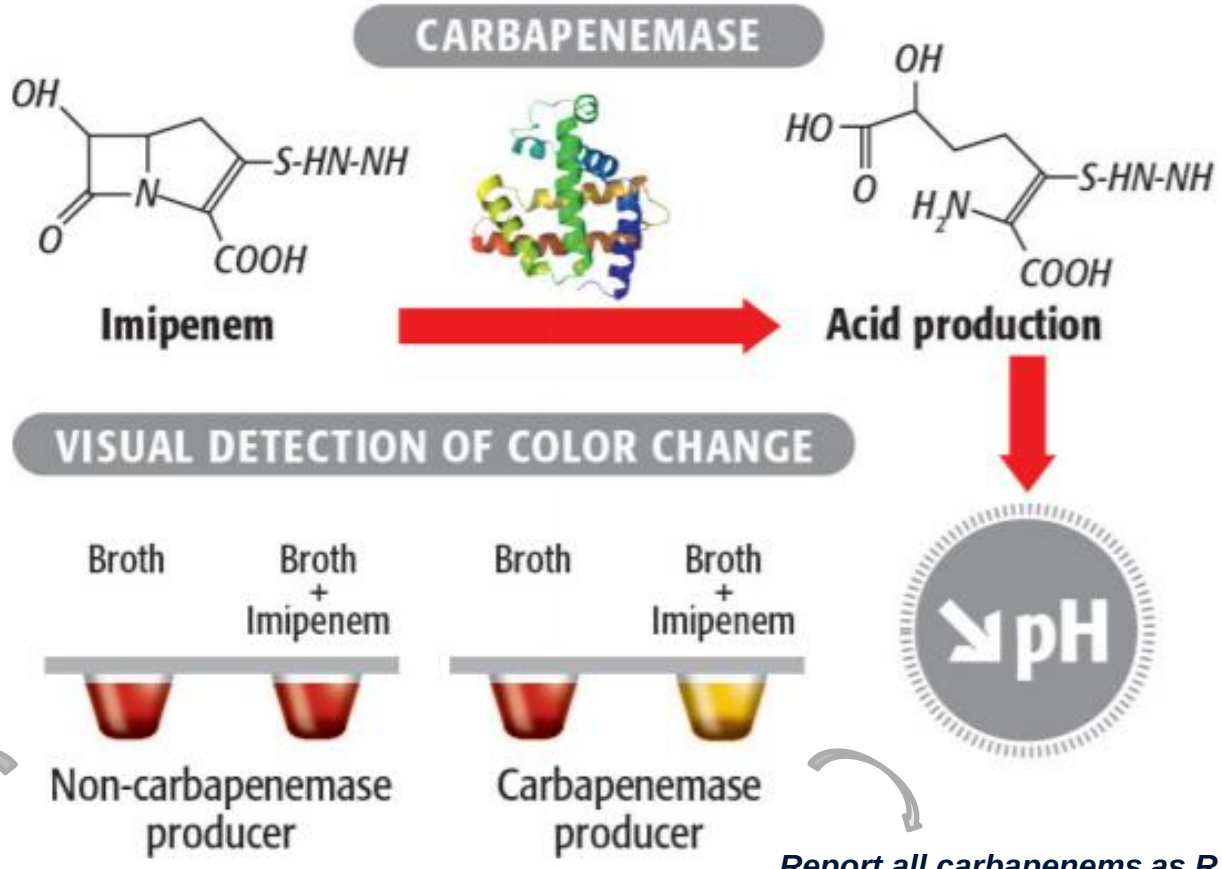
"Carbapenemase detected"



Results for Patient and QC Tubes		
Tube "a": Solution A (serves as internal control)	**Tube "b": Solution B**	**Interpretation**
Red or red-orange	Red or red-orange	Negative, no carbapenemase detected
Red or red-orange	Light orange, dark yellow, or yellow	Positive, carbapenemase producer
Red or red-orange	Orange	Invalid
Orange, light orange, dark yellow, or yellow	Any color	Invalid

CLSI M100 29th Ed. 2019

Principle of Colorimetric Detection of Carbapenemases



Interpret carbapenems using M100-S20 (if not using updated breakpoints)

Report all carbapenems as R (if not using updated breakpoints)

Image modified from https://www.biomerieux.co.uk/sites/clinic/files/carbapenem_resistance_booklet.pdf

CarbaNP

ADVANTAGES

- Rapid: ~2hrs
- High sensitivity and specificity for detecting KPC, NDM, VIM, IMP, SPM, and SME-type carbapenemases
- Detection in *Enterobacteriaceae* and *Pseudomonas aeruginosa*

DISADVANTAGES

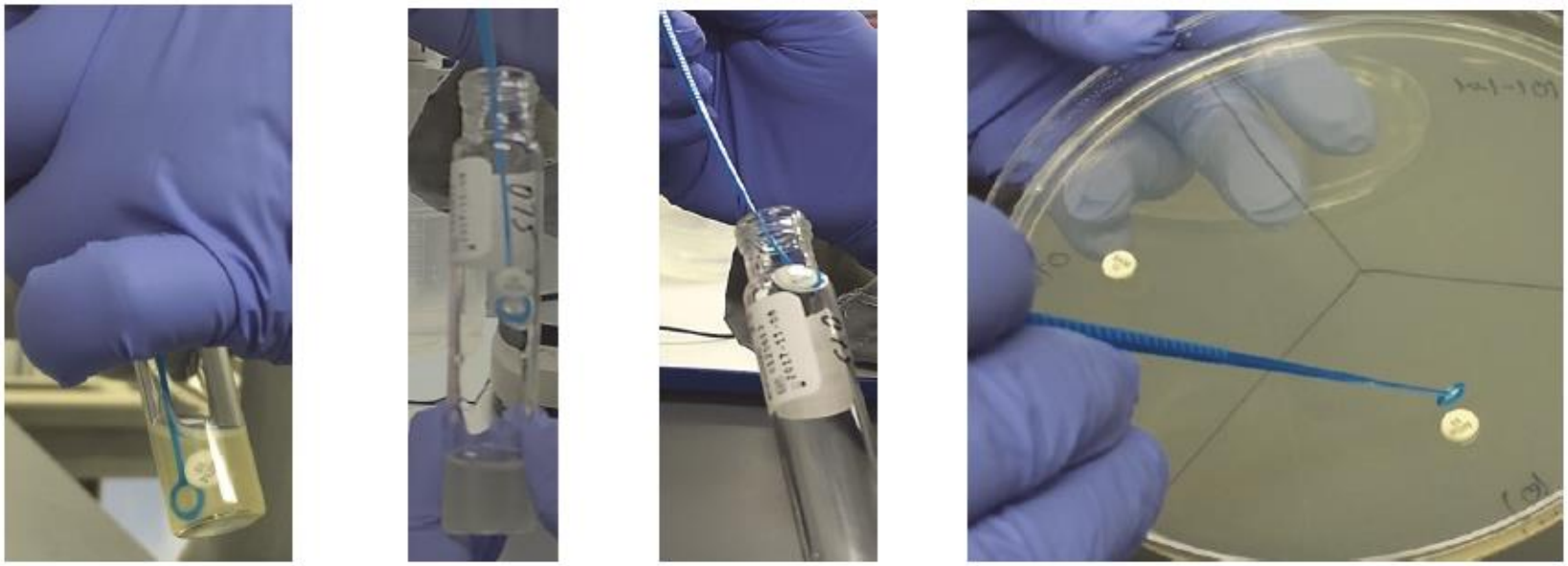
- Reading is subjective
- Need for special reagents
- Potential for invalid results
- Does not distinguish between classes
- Sensitivity for detecting OXA-48 is low (~11%)
- Testing of *Acinetobacter* spp. is not recommended due to poor sensitivity (21%)

Modified Carbapenem Inactivation Method - mCIM

- **Purpose**:
  - To test for suspected carbapenemase production in *Enterobacteriaceae* and *Pseudomonas aeruginosa*
    - In laboratories using carbapenem breakpoints from M100 S20 (2010)
    - *Enterobacteriaceae* ex: imipenem or meropenem MICs 2-4 µg/mL, or ertapenem MIC 2 µg/mL
  - Infection control or epidemiological purposes
    - *Enterobacteriaceae* and *P. aeruginosa* that are not S to one or more carbapenems
- **Principle and Method**:
  - Detection of meropenem disk inactivation
  - Disk diffusion

Modified Carbapenem Inactivation Method - mCIM

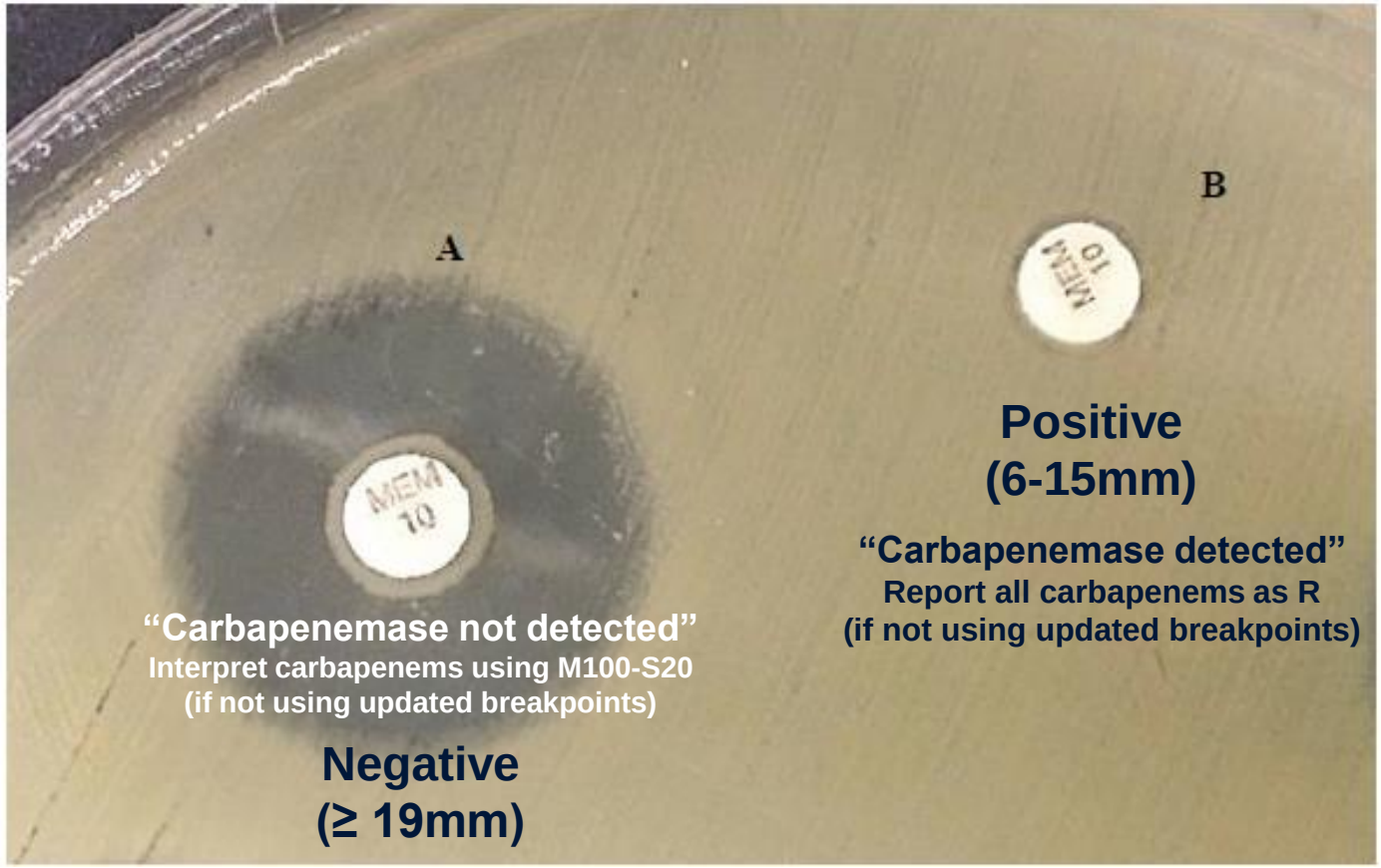
- Make a broth suspension of the isolate to be tested in 2 mL TSB
- Add 10µg meropenem disk to tube & incubate at 35ºC 4hrs
- Remove disk from tube (below) an place on plate inoculated with a 0.5 McFarland lawn of meropenem-susceptible *E. coli*
- Incubate O/N at 35ºC. Measure zones of inhibition after 18-24 hrs



A B C D

mCIM Interpretation

Indeterminate:
- **16-18 mm**
- **≥ 19 mm + pinpoint colonies in the zone**



Neg: No inactivation of meropenem
Pos: inactivates the meropenem

*Can be performed in tandem with eCIM to detect MBLs in *Enterobacteriaceae*

mCIM

ADVANTAGES

- High sensitivity and specificity for detecting KPC, NDM, VIM, IMP, IMI, SPM, SME, and OXA-type carbapenemases among *Enterobacteriaceae*
- High sensitivity and specificity for detecting KPC, NDM, VIM, IMP, IMI, SPM, and OXA-type carbapenemases among *P. aeruginosa*

DISADVANTAGES

- Requires overnight incubation
- Potential for indeterminate results
- Does not distinguish between classes*
- Testing *Acinetobacter* spp. is not recommended (poor specificity and reproducibility)

EDTA-Modified Carbapenem Inactivation Method - eCIM

- **Purpose**:
  - In conjunction with the mCIM, to test for suspected metallo-β-lactamase production (*Enterobacteriaceae* only)
- **Principle and Method**:
  - Detection of meropenem disk inactivation in the presence of EDTA
  - **Results are valid only for *Enterobacteriaceae* that are positive by mCIM
  - Disk diffusion

eCIM Method

- Add 0.5 M EDTA to the eCIM TSB tube (final 5mM EDTA)
- Make a broth suspension of the isolate to be tested in 2 mL TSB
- Add 10µg meropenem disk to tube & incubate at 35^{o}C 4hrs
- Remove disk from tube (below) an place on plate inoculated with a 0.5 McFarland lawn of meropenem-susceptible *E. coli* (on the same plate as the mCIM)
- Incubate O/N at 35^{o}C. Measure zones of inhibition after 18-24 hrs

**mCIM and eCIM tubes should be processed in parallel

**eCIM can only be interpreted why the mCIM test is positive

eCIM Interpretation

Interpret only when mCIM is positive

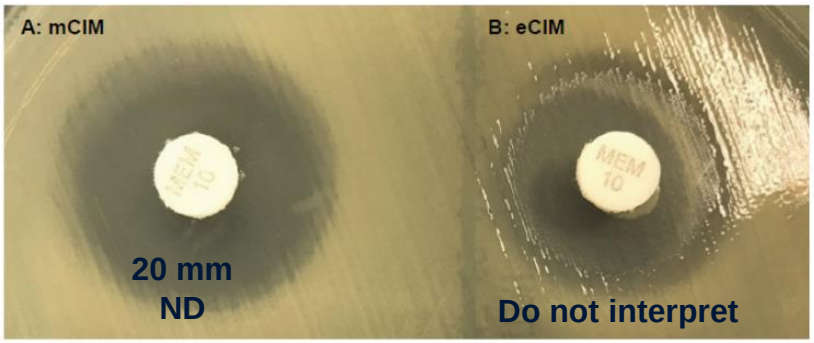
- **Metallo-β-lactamase positive**:
  - ≥ 5 mm increase in zone diameter for eCIM vs mCIM
    - Ex: mCIM = 6 mm; eCIM = 15 mm; 9 mm zone diameter difference
  - For eCIM only, ignore pinpoint colonies within zone of inhibition

  *EDTA inhibits the activity of metallo-β-lactamases. For eCIM the meropenem will not be hydrolyzed as efficiently

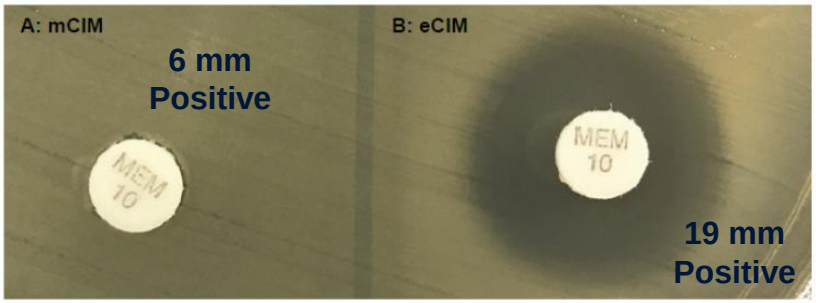
- **Metallo-β-lactamase negative**:
  - ≤ 4 mm increase in zone diameter compared to mCIM
  - Ignore pinpoint colonies

  *If the test isolate produces a serine carbapenemase, EDTA will not affect the enzyme activity. NO or very little increase in zone diameter in presence of EDTA

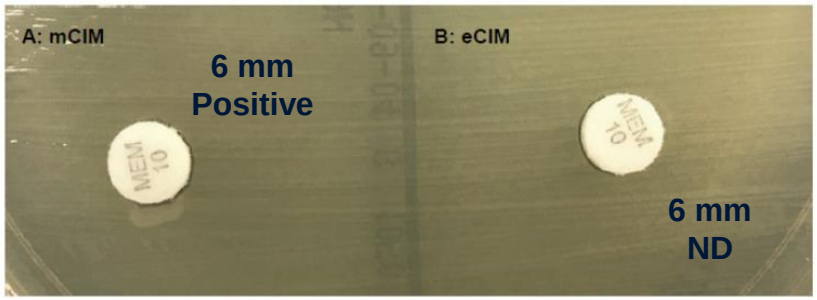
eCIM Interpretation



Carbapenemase not detected



Metallo-β-lactamase detected



Serine carbapenemase detected

**Interpret ONLY when mCIM is positive

eCIM

ADVANTAGES

- High sensitivity and specificity for distinguishing MBLs from serine carbapenemases among *Enterobacteriaceae*

DISADVANTAGES

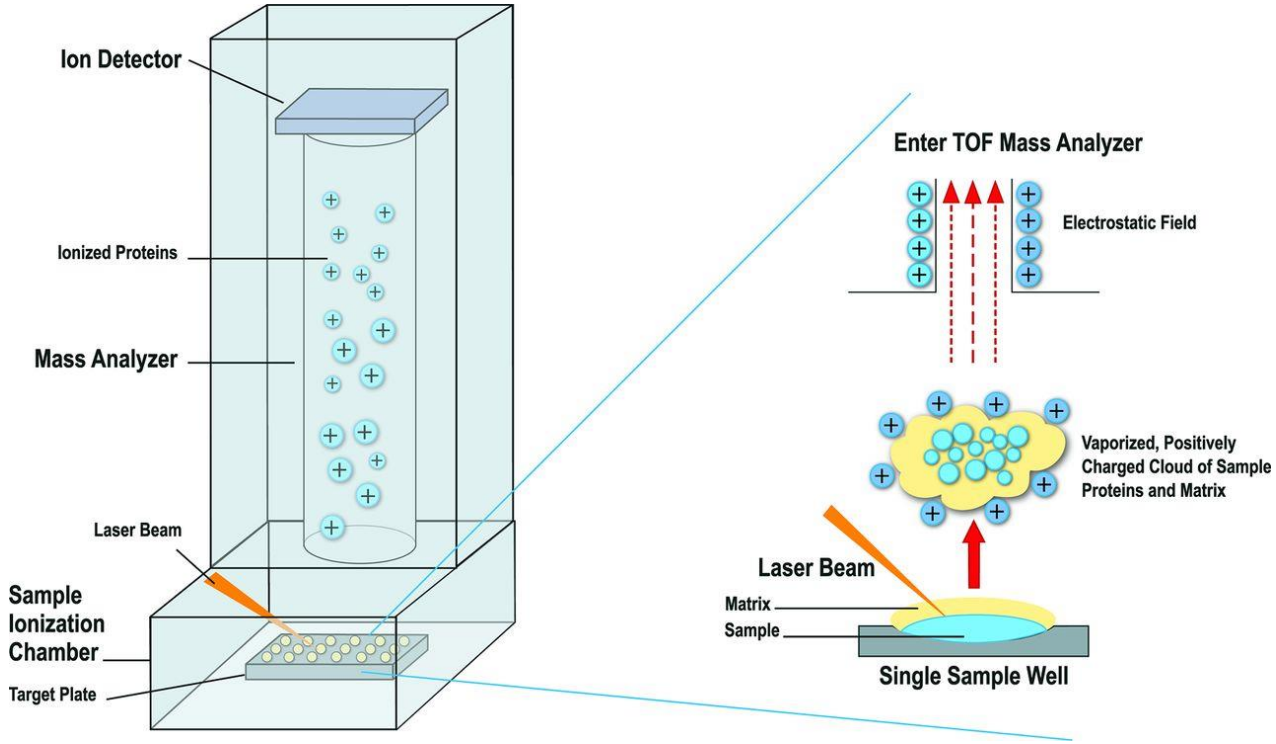
- Requires overnight incubation
- Potential for indeterminate results
- Presence of both a serine carbapenemase and MBL in one organism can lead to false negative eCIM
- Guidelines only available for *Enterobacteriaceae*

MALDI-TOF

- **Purpose**:
  - Detection of carbapenemase-mediated resistance to carbapenems in *Enerobacteriaceae*
- **Principle and Method**:
  - Determination of presence or absence of peaks corresponding to intact meropenem and its sodium salt
  - Absence indicates hydrolysis of meropenem by a carbapenemase
  - MALDI-TOF MS

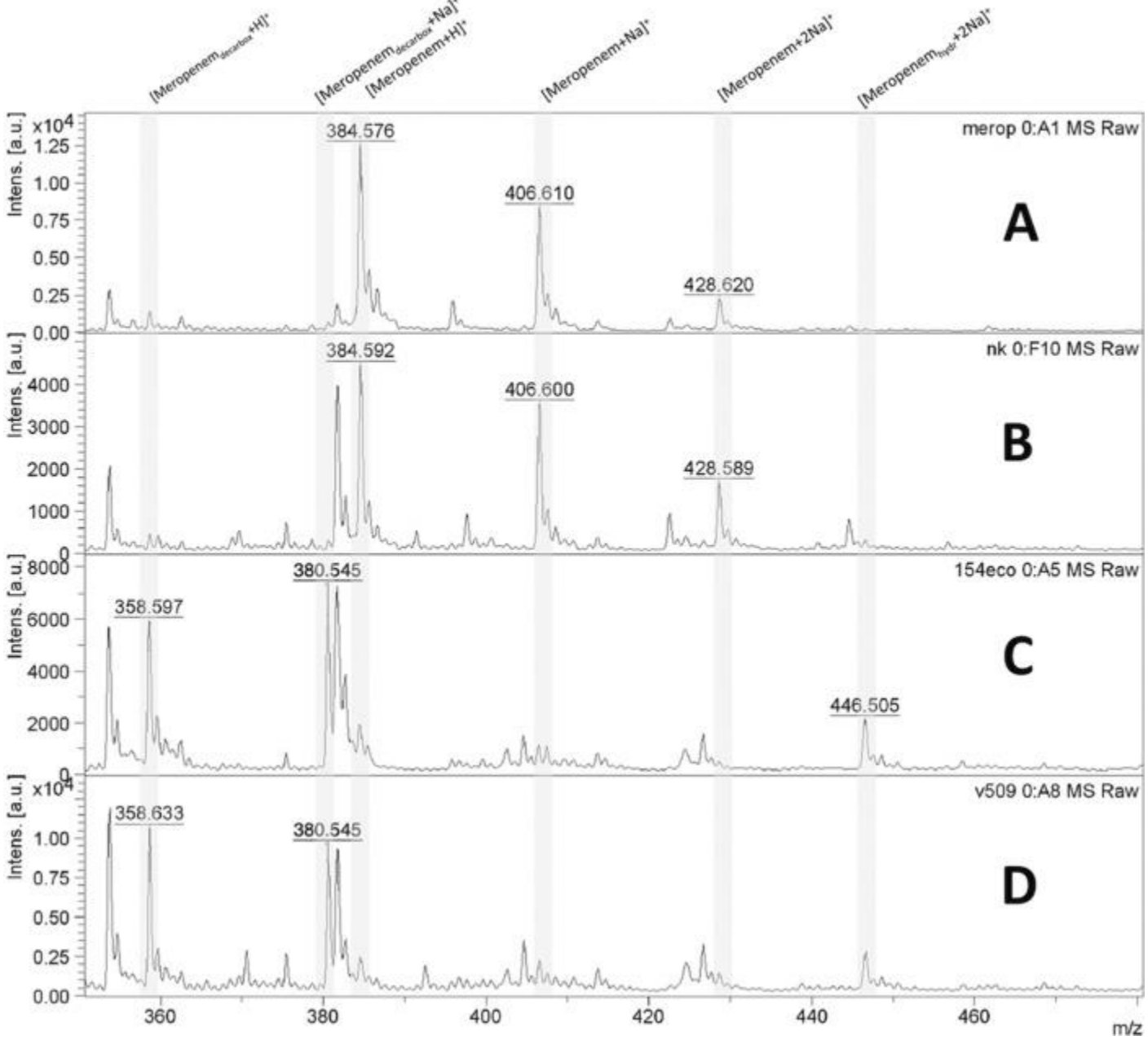
Hrabák, Jaroslav et al. "Detection of NDM-1, VIM-1, KPC, OXA-48, and OXA-162 carbapenemases by matrix-assisted laser desorption ionization-time of flight mass spectrometry." Journal of clinical microbiology vol. 50,7 (2012): 2441-3. doi:10.1128/JCM.01002-12

Hrabák, Jaroslav et al. "Carbapenemase activity detection by matrix-assisted laser desorption ionization-time of flight mass spectrometry." Journal of clinical microbiology vol. 49,9 (2011): 3222-7. doi:10.1128/JCM.00984-11



- Buffered meropenem solution incubated at 35°C with standardized bacterial culture for 2 hr
- Centrifuged, and supernatant analyzed by MALDI-TOF MS
- Determination of presence or absence of peaks corresponding to meropenem and its sodium salts
- Lack of intact meropenem and sodium salt peaks corresponds to carbapenemase presence

MALDI-TOF MS for the Diagnosis of Infectious Diseases. Robin Patel. Clinical Chemistry Jan 2015, 61 (1) 100-111; ***DOI:*** *10.1373/clinchem.2014.221770*
Hrabák, Jaroslav et al. "Carbapenemase activity detection by matrix-assisted laser desorption ionization-time of flight mass spectrometry." Journal of clinical microbiology vol. 49,9 (2011): 3222-7. doi:10.1128/JCM.00984-11



Meropenem Solution

Non-carbapenemase-producing *K. pneumoniae*

NDM-1-producing *E. coli*

NDM-1-producing *A. baumanii*

Hrabák, Jaroslav et al. "Detection of NDM-1, VIM-1, KPC, OXA-48, and OXA-162 carbapenemases by matrix-assisted laser desorption ionization-time of flight mass spectrometry." Journal of clinical microbiology vol. 50,7 (2012): 2441-3. doi:10.1128/JCM.01002-12

MALDI-TOF

Advantages

- Detection of multiple carbapenemases (KPC, NDM-1, VIM, OXA-48, OXA-162)
- Rapid: ~2-4 hours

Disadvantages

- Distinguishing carbapenemases may be challenging
- Potential for false negatives with low carbapenemase expression

Genotypic Tests

FilmArray blood culture ID panel

Verigene GN blood culture ID

GenXpert Carba-R

Molecular Testing

"*Enterobacteriaceae* and *P. aeruginosa* that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by CarbaNP or mCIM"

FilmArray Blood Culture ID

- **Purpose**:
  - "Detect and identify the most common causes of bloodstream infection" and select resistance genes
  - Directly from a positive blood culture bottle
- **Principle and Method**:
  - Targeted detection of amplified nucleic acid from common bloodstream pathogens
  - Nested Polymerase Chain Reaction (PCR)

FilmArray BCID Panel CE IVD Instruction Booklet. BioFire Diagnostics, LLC. July 2018.

FilmArray Blood Culture Identification Panel

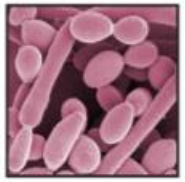
1 Test. 27 Targets. All in about an hour.

Gram-Positive Bacteria

Enterococcus
Listeria monocytogenes
Staphylococcus
Staphylococcus aureus
Streptococcus
Streptococcus agalactiae
Streptococcus pyogenes
Streptococcus pneumoniae

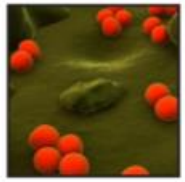
Gram-Negative Bacteria

Acinetobacter baumannii
Haemophilus influenzae
Neisseria meningitidis
Pseudomonas aeruginosa
Enterobacteriaceae
Enterobacter cloacae complex
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumoniae
Proteus
Serratia marcescens



Yeast

Candida albicans
Candida glabrata
Candida krusei
Candida parapsilosis
Candida tropicalis



Antibiotic Resistance Genes

mecA - methicillin resistant
vanA/B - vancomycin resistant
KPC - carbapenem resistant

The FilmArray Pouch

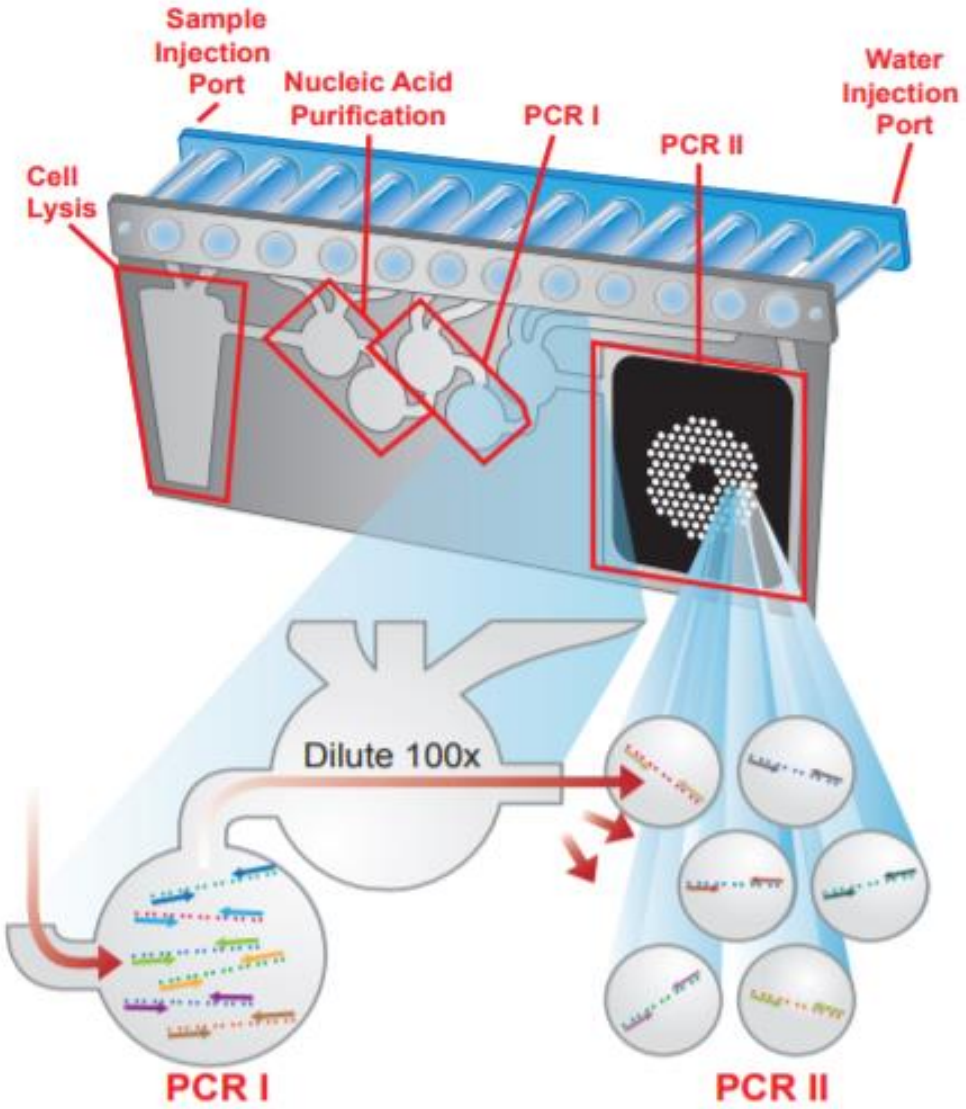
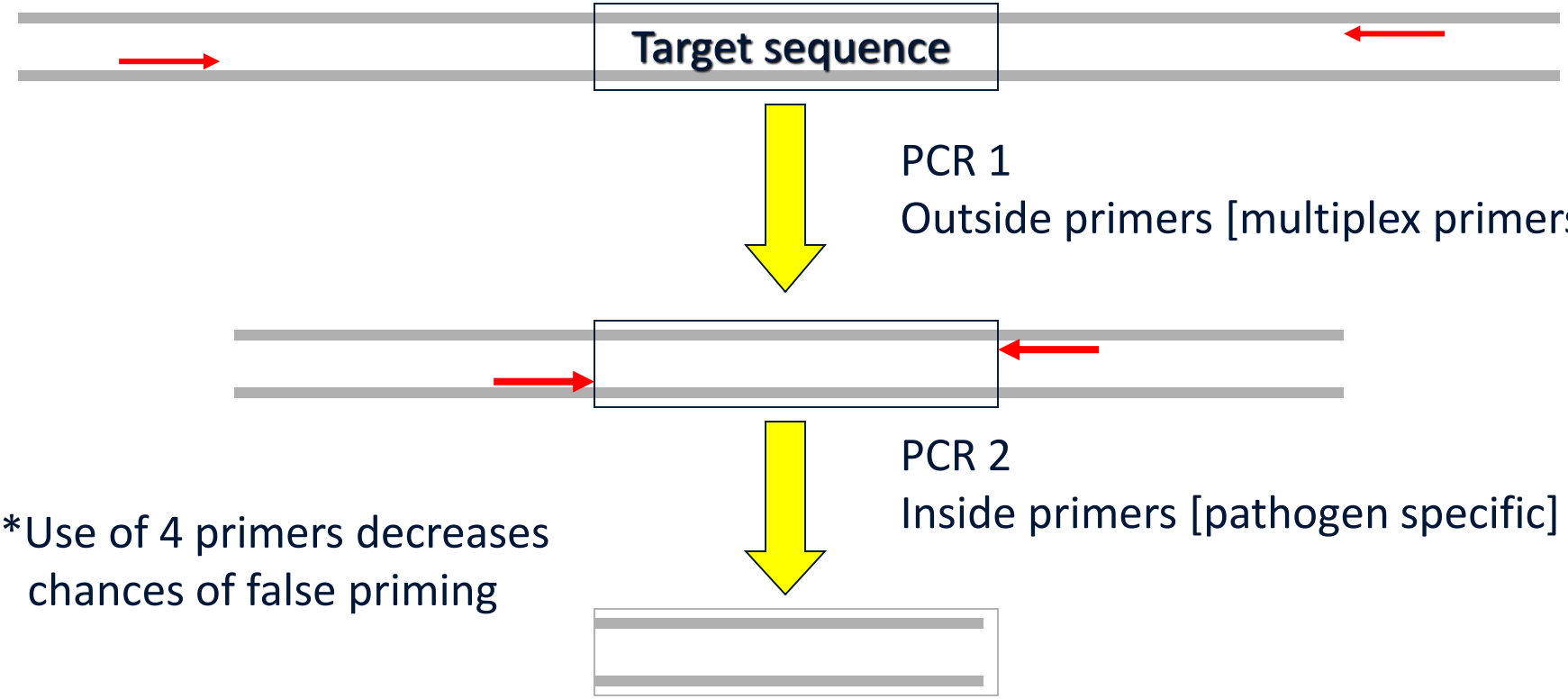


Image modified from www.biofiredx.com

Nested PCR

- Two pairs of primers: Inside and outside
  - Outside primers amplify the first product; the product of this PCR is transferred to a second reaction containing the inside primers



**Use of 4 primers decreases chances of false priming

Slide courtesy of Dr. K. Landis-Piwowar

FilmArray Blood Culture ID Panel

ADVANTAGES

- FDA cleared
- Rapid: ~1 hr from blood culture positivity
- Direct from specimen

DISADVANTAGES

- Only detects KPC gene
- Only use blood as a sample
- Mutations and polymorphisms may affect detection
- Will not detect intrinsic resistance mechanisms
- One sample per instrument

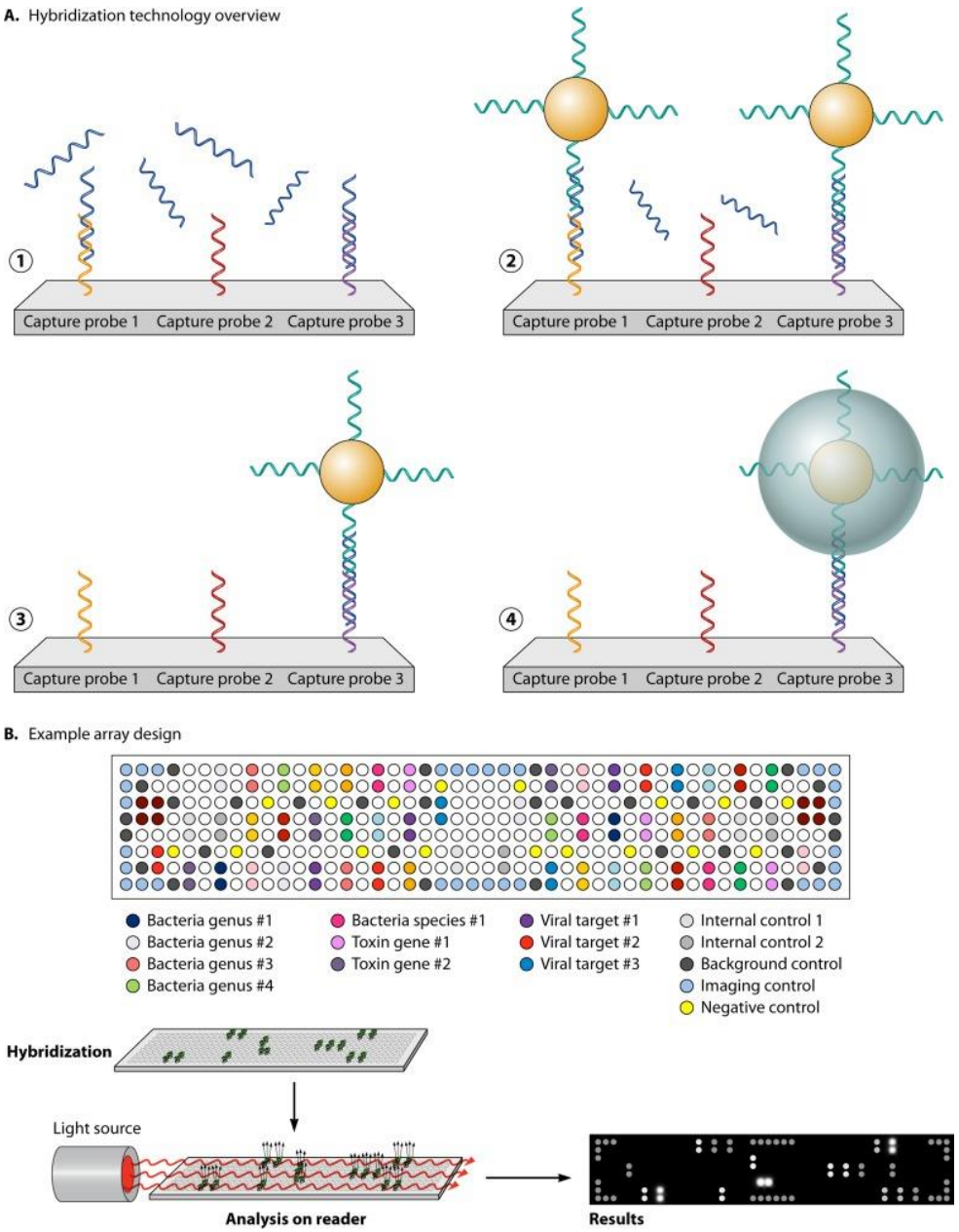
Verigene Gram-negative Blood Culture Tests

- **Purpose**:
  - Detect and identify common Gram-negative bloodstream pathogens and select resistance genes
- **Principle and Method**:
  - Detection of bacterial DNA and resistance genes using target-specific capture probes
  - Solid phase microarray; multiplexed nucleic acid assay (with no amplification)

Verigene Gram-negative Blood Culture Tests

Verigene BC-GN
SPECIES
Escherichia coli[a]
Klebsiella oxytoca
Klebsiella pneumoniae
Pseudomonas aeruginosa
GENUS
Acinetobacter spp.
Citrobacter spp.
Enterobacter spp.
Proteus spp.
RESISTANCE
CTX-M (ESBL)
IMP (carbapenemase)
KPC (carbapenemase)
NDM (carbapenemase)
OXA (carbapenemase)
VIM (carbapenemase)

- 8 bacterial targets in the United States
- 6 resistance genes
  - 5 carbapenemases targets



Buchan BW, Ledeboer NA. Emerging technologies for the clinical microbiology laboratory. Clin Microbiol Rev. 2014;27(4):783–822. doi:10.1128/CMR.00003-14

Verigene Gram-negative Blood Culture Tests

ADVANTAGES

- Quick setup time (<5min)
- Rapid detection (~2 hours)
- Direct from sample
- Ability to identify multiple resistance determinants (KPC, NDM, IMP, VIM, OXA)
- Separate panels for GN and GP organisms

DISADVANTAGES

- Only use blood as a sample
- Mutations and polymorphisms may affect detection
- Will not detect intrinsic resistance mechanisms
- One sample per instrument
- *Serratia marcesans* not FDA-cleared in the US

GeneXpert CarbaR

- **Purpose**:
  - To rapidly identify carbapenemase-producing organisms associated with non-susceptibility in *Enterobacteriaceae, Pseudomonas aeruginosa,* and *Acinetobacter baumanii*
    - Clinicians or infection control
- **Principle and Method**:
  - Detection and differentiation of genes encoding multiple carbapenemase-encoding genes
  - Real-time PCR

GeneXpert Carba-R

- Rectal swab: add 500µL of swab transport media to 5mL of sample reagent
- Isolate: Make 0.5 McFarland and add 10µL to 5mL of sample reagent
- Vortex 10 seconds
- Using provided transfer pipette, transfer prepared sample to cartridge chamber

- Close cartridge and place on the instrument within 30 min
- Principle: real-time PCR

Images modified from Cepheid.com

GeneXpert Carba-R

ADVANTAGES

- Rapid: ~1 hr
- FDA cleared
- Sample types: isolate or rectal swab
- Isolates of *Enterobacteriaceae, P. aeruginosa,* and *Acinetobacter baumanii*
- Detection and differentiation of KPC, NDM, VIM, IMP-1, and OXA-48

DISADVANTAGES

- Use of isolate still requires traditional culture methods
- Mutations and polymorphisms may affect detection
- Will not detect intrinsic resistance mechanisms
- May miss other OXA-types, SPM, SME, and IMI

Immunoassay

Carba 5

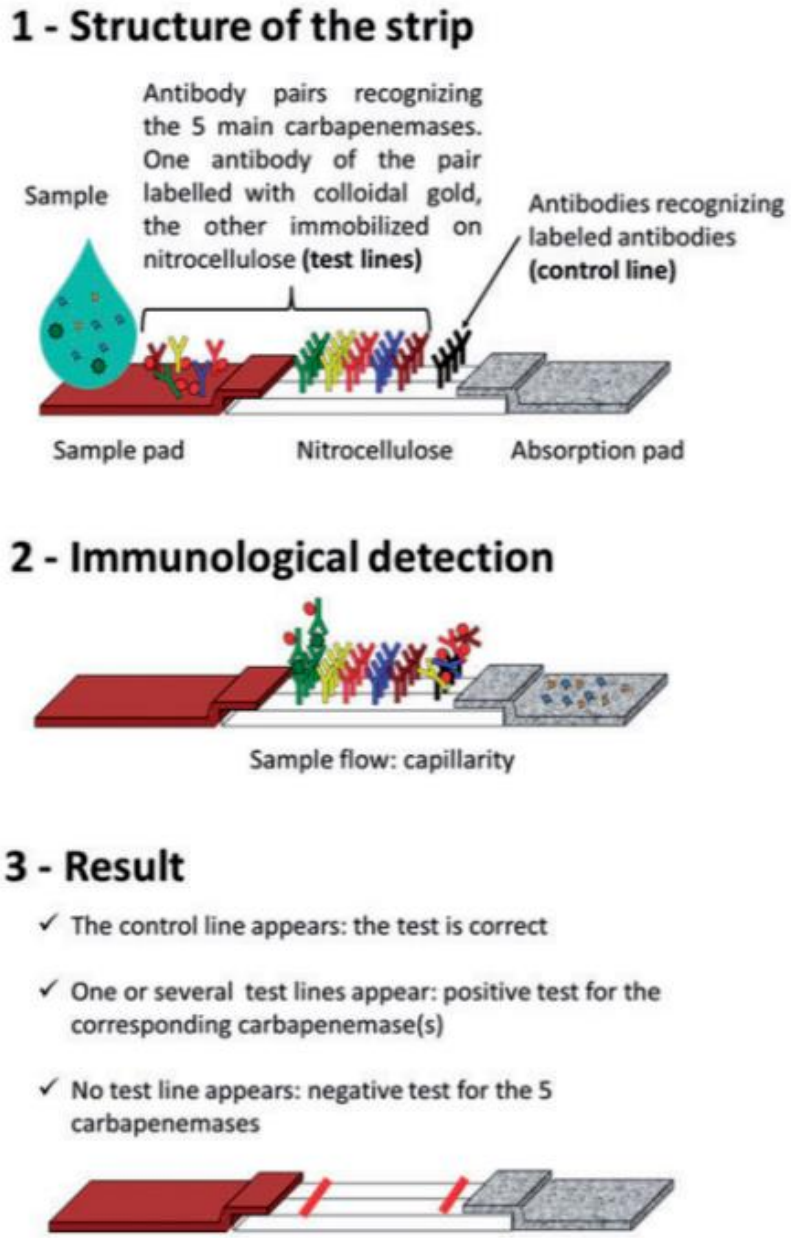
Carba 5

- **Purpose**:
  - Rapidly detect and distinguish between carbapenemases produced by *Enterobacteriaceae*
  - Suggested for confirmation of strains with decreased susceptibility to at least one carbapenem
- **Principle and Method**:
  - Detect and distinguish multiple analytes (carbapenemases) potentially present in an isolate, which are able to bind to capture antibodies
  - Lateral flow immunoassay (LFIA)

Boutal H, Vogel A, Bernabeu S, et al. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother. 2018;73(4):909–915. doi:10.1093/jac/dkx521

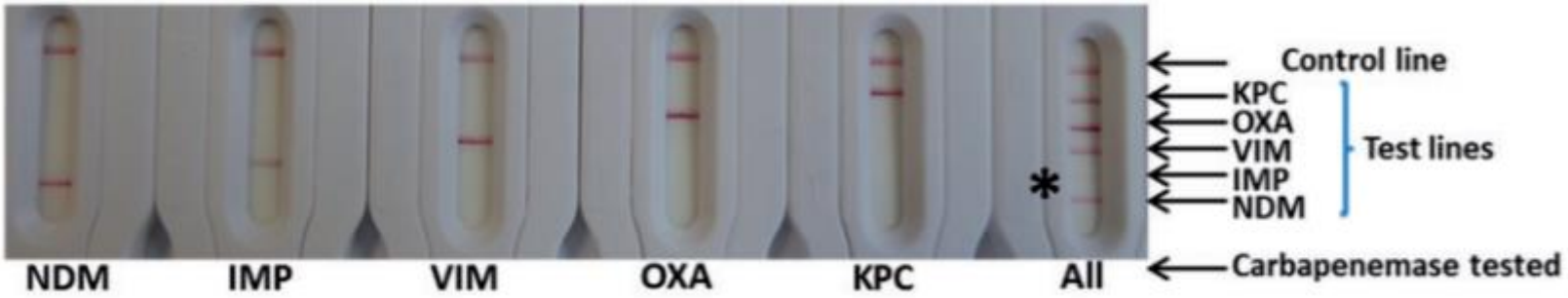
Carba 5

- Isolated colony from overnight growth inoculated in lysis buffer (150 µL)
- Sample to be tested for analyte presence is added to the sample pad (100 µL)
- Capillary action moves the sample and analytes across the nitrocellulose
- Analyte binds to specific paired antibodies
    1. Flow along the nitrocellulose and a sandwich is formed
    2. Bound by control line antibodies
- Interpretation and reporting



Boutal et al (2018) J Antimicrob Chemother

Carba 5



Boutal et al (2018) J Antimicrob Chemother

Carba 5

Advantages

- Able to detect the 5 main carbapenemases (KPC, NDM, IMP, VIM, OXA-48 like) with high sensitivity and specificity
- Easy and rapid: ~15 min
- Potential for use with isolates from CRO screening media

Disadvantages

- Only for use on *Enterobacteriaceae*
- Requires an isolate (overnight growth)
- Not FDA-cleared

Boutal et al (2018) J Antimicrob Chemother;
Takissian J, Bonnin RA, Naas T, Dortet L. 2019. NG-Test Carba 5 for rapid detection of carbapenemase-producing Enterobacterales from positive blood cultures. Antimicrob Agents Chemother 63:e00011-19. https://doi .org/10.1128/AAC.00011-19.

Significance

- Guidance for more effective therapy
  - Each class of enzymes has slightly different activity and inhibition
- MBLs in particular are more difficult to treat

Drug	Drug Class	Activity		
		KPC	MBL	OXA-48-like
Ceftazidime-avibactam	β-lactam/β-lactamase inhibitor	+	-	+
Ceftolozane-tazobactam	β-lactam/β-lactamase inhibitor	-	-	-
Meropenem-vaborbactam	Carbapenem/β-lactamase inhibitor	+	-	-
Eravacycline	Tetracycline	+	+	+
Plazomycin	Aminoglycoside	+	-	+
Cefiderocol	Siderophore β-lactam	+	+	+
Imipenem-relebactam	Carbapenem/β-lactamase inhibitor	+	-	+
Aztreonam-avibactam	β-lactam/β-lactamase inhibitor	+	+	+

References for Antibiotics Chart

- *[1] Bonomo RA, Burd EM, Conly J, et al. Carbapenemase-Producing Organisms: A Global Scourge. Clin Infect Dis. 2018;66(8):1290–1297. doi:10.1093/cid/cix893*
- *[2] Sheu CC, Chang YT, Lin SY, Chen YH, Hsueh PR. Infections Caused by Carbapenem-Resistant Enterobacteriaceae: An Update on Therapeutic Options. Front Microbiol. 2019;10:80. Published 2019 Jan 30. doi:10.3389/fmicb.2019.00080*
- *[3] Livermore DM, Mushtaq S, Warner M, Woodford N. 2016. In vitro activity of eravacycline against carbapenem-resistant Enterobacteriaceae and Acinetobacter baumannii. Antimicrob Agents Chemother 60:3840–3844. doi:10.1128/AAC.00436-16*
- *[4] Yunliang Zhang, Xiaoyan Lin, and Karen Bush. "In Vitro Susceptibility of β-Lactamase-Producing Carbapenem-Resistant Enterobacteriaceae (CRE) to Eravacycline." The Journal of Antibiotics 69.8 (2016): 600–604.*
- *[5] Petty LA, Henig O, Patel TS, Pogue JM, Kaye KS. Overview of meropenem-vaborbactam and newer antimicrobial agents for the treatment of carbapenem-resistant Enterobacteriaceae. Infect Drug Resist. 2018;11:1461–1472. Published 2018 Sep 12. doi:10.2147/IDR.S150447*
- *[6] van Duin D, Bonomo RA. Ceftazidime/Avibactam and Ceftolozane/Tazobactam: Second-generation β-Lactam/β-Lactamase Inhibitor Combinations. Clin Infect Dis. 2016;63(2):234–241. doi:10.1093/cid/ciw243*

Summary

- Carbapenemases are β-lactamase enzymes capably of hydrolyzing and inactivating a number of antibiotic classes
- The emergence and spread of carbapenem resistance represents an urgent threat to public health in the United States
- Multiple methods are available for detection of carbapenemases
- Detection and differentiation of carbapenemases is important for implementing effective antibiotic therapy