Laboratory Detection and Reporting of Carbapenem-Resistant Enterobacteriaceae (CRE)

CLSI Outreach Working Group

Spring, 2016

After review of this program, you will be able to:

- List current CLSI recommendations for antimicrobial susceptibility testing (AST) and reporting of CRE.
- Describe the differences between CRE (Carbapenem-resistant Enterobacteriaceae) and CPE (Carbapenemase-producing Enterobacteriaceae).
- Explain the significance of CRE and CPE from clinical and epidemiological perspectives.
- Describe when and where to go for help when physicians need more information than your laboratory can provide for a potential CRE isolate.

Acronyms Used in this Presentation

- **CRE = Carbapenem-R Enterobacteriaceae**
- **CPE = Carbapenemase-Producing Enterobacteriaceae**
- **Also CRO, CRKP, CPKP, CPO**
  - **"O" = organism**
  - **"KP"= *Klebsiella pneumoniae***

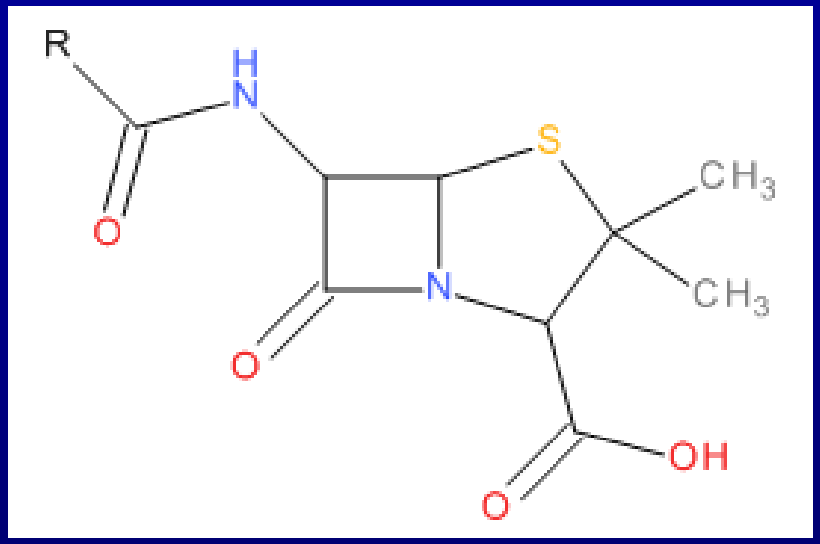
β-Lactams and GNRs

Basic Concepts

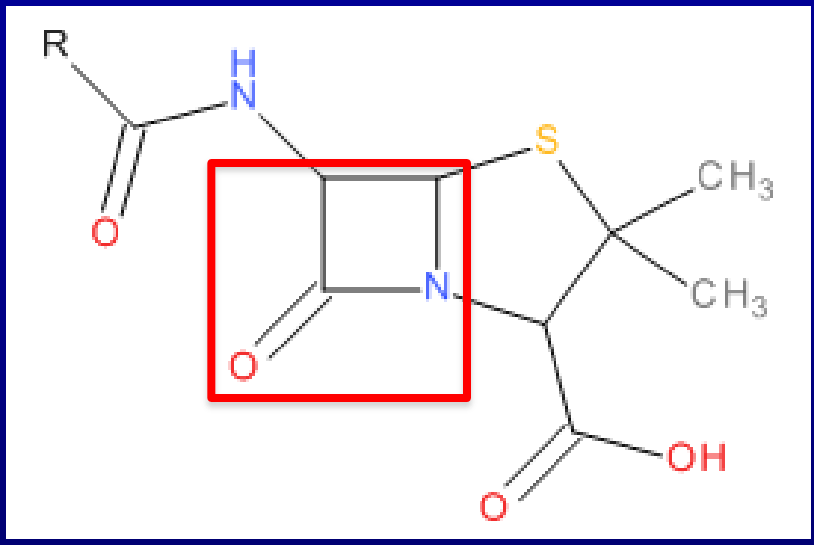
Common β-Lactam Agents Active Against Gram-Negative Rods (GNR)

Penicillins	β-lactam Inhibitor Combos	Cephalosporins	Cephamycin	Carbapenems
Amoxicillin	Amoxicillin-clav	Cefazolin (1)	Cefoxitin	Doripenem
Ampicillin	Ampicillin- sulb	Cefuroxime (2)	Cefotetan	Ertapenem
Piperacillin	Piperacillin-tazo	Cefotaxime (3)	Monobactam	Imipenem
Ticarcillin	Ticarcillin-clav	Ceftazidime (3)	Aztreonam	Meropenem
	Ceftolozane-tazo	Ceftriaxone (3)		
	Ceftaz-avibactam	Cefepime (4)		

β-Lactams



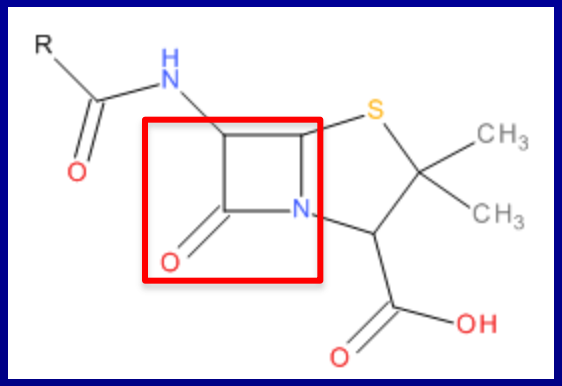
β-Lactam
(Penicillin)



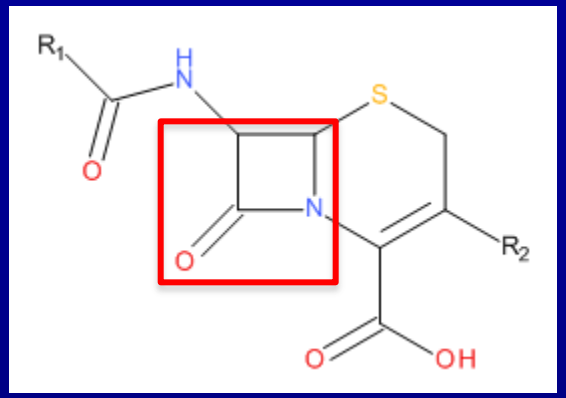
β-Lactam
(Penicillin)

All β-lactams contain a 4-membered β-lactam ring that is essential for activity

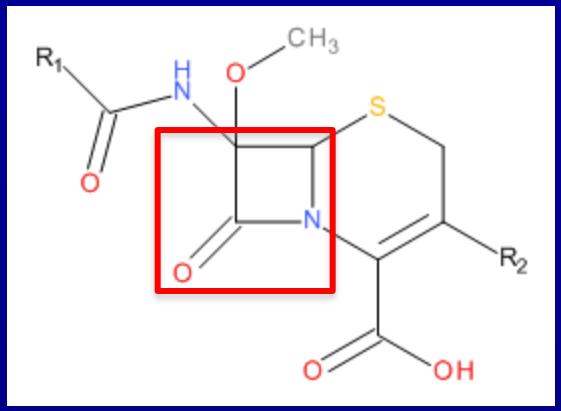
Different β-Lactams



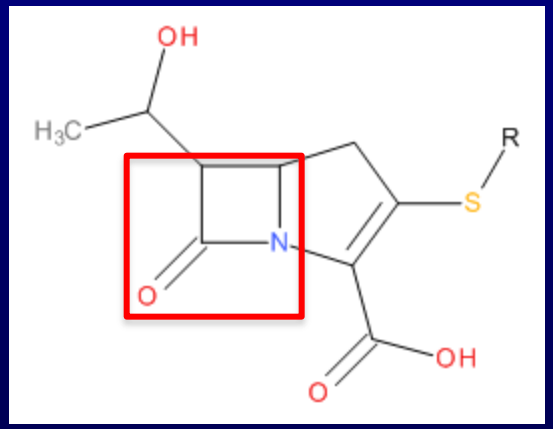
Penicillins



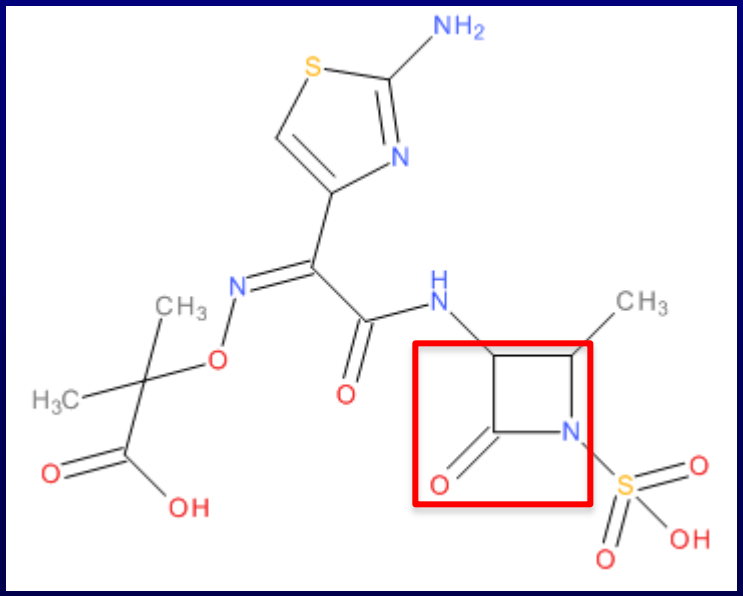
Cephalosporins



Cephamycins

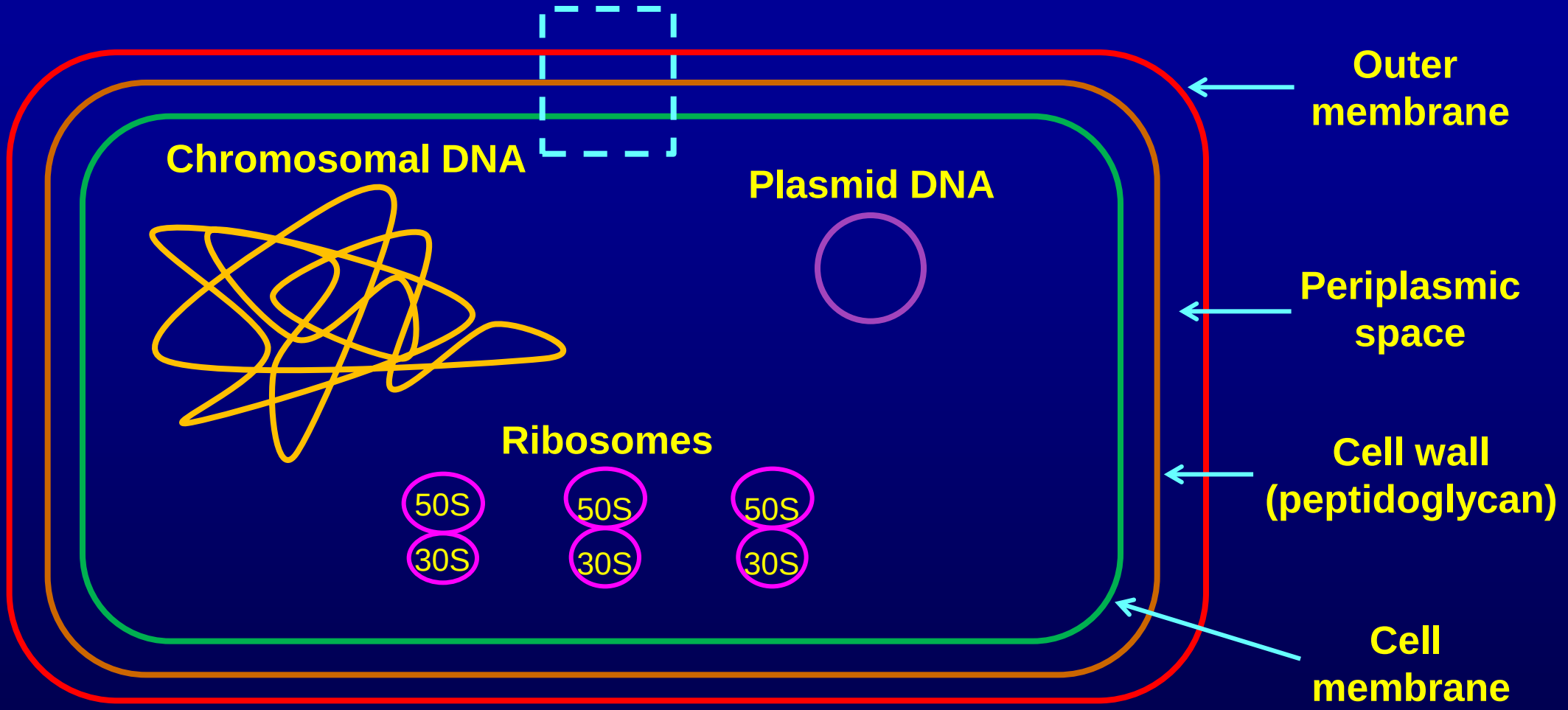


Carbapenems



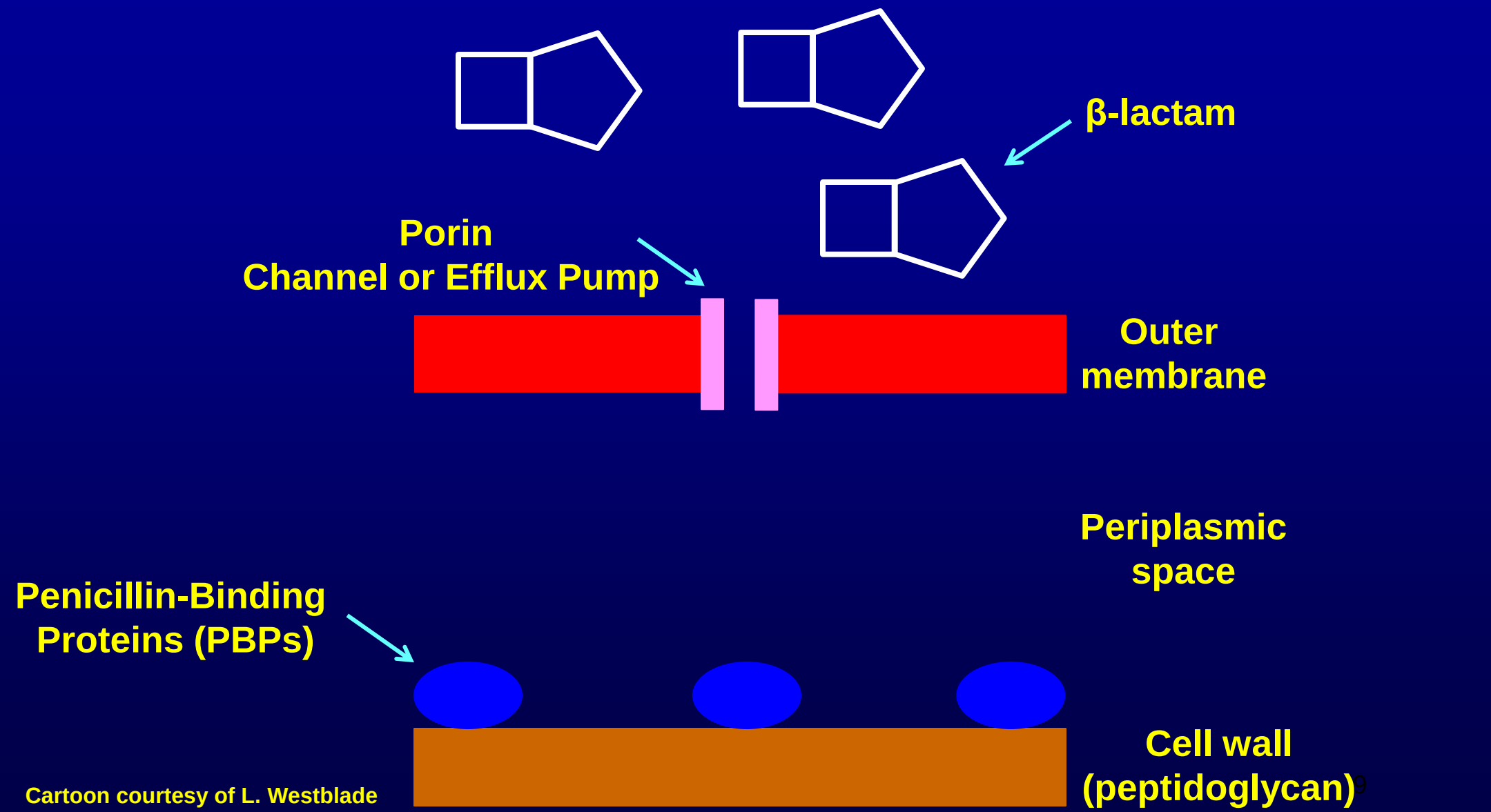
Monobactam

Gram-Negative Bacterial Cell



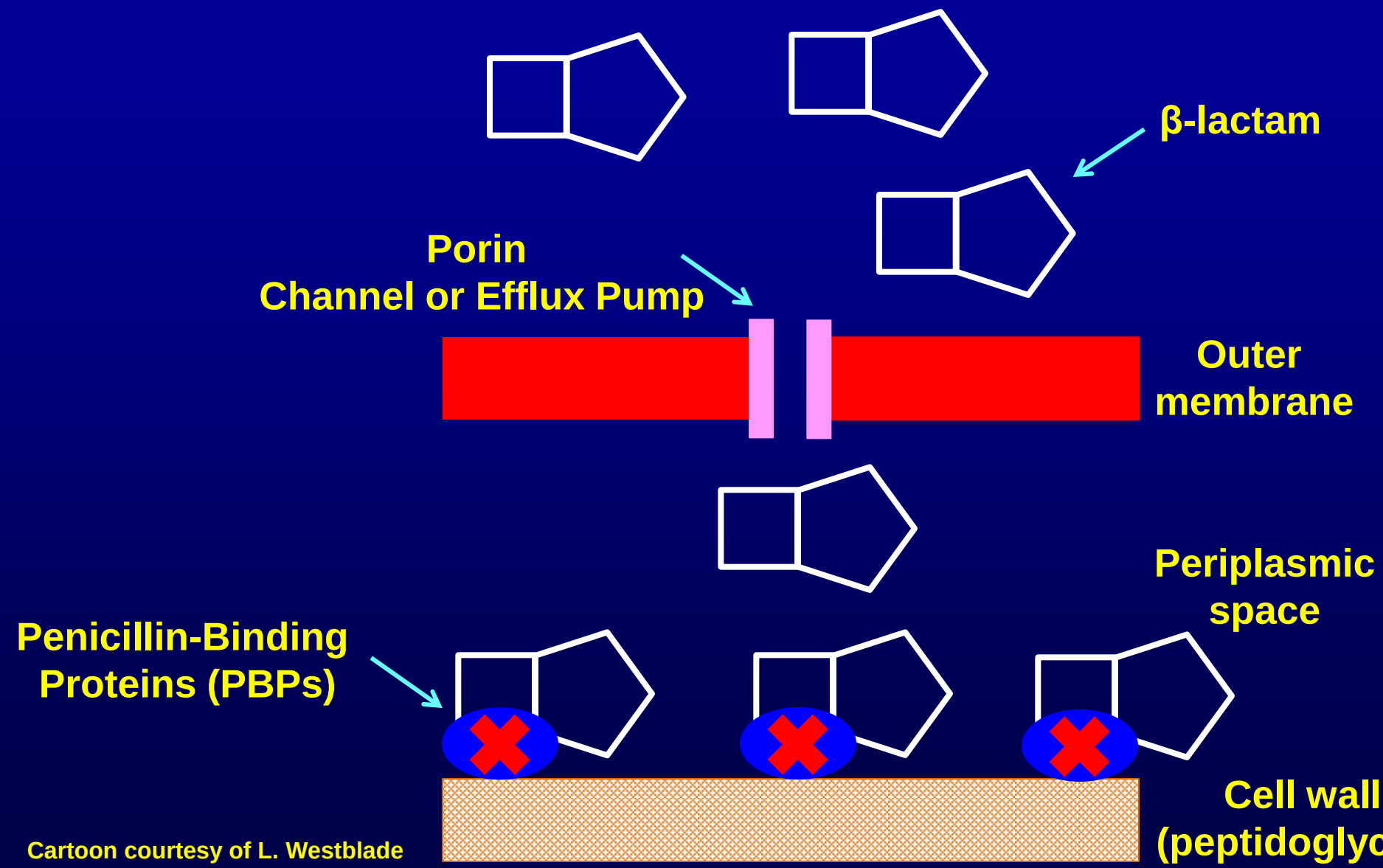
Cartoon courtesy of L. Westblade

β-Lactam Activity Against Gram-Negatives



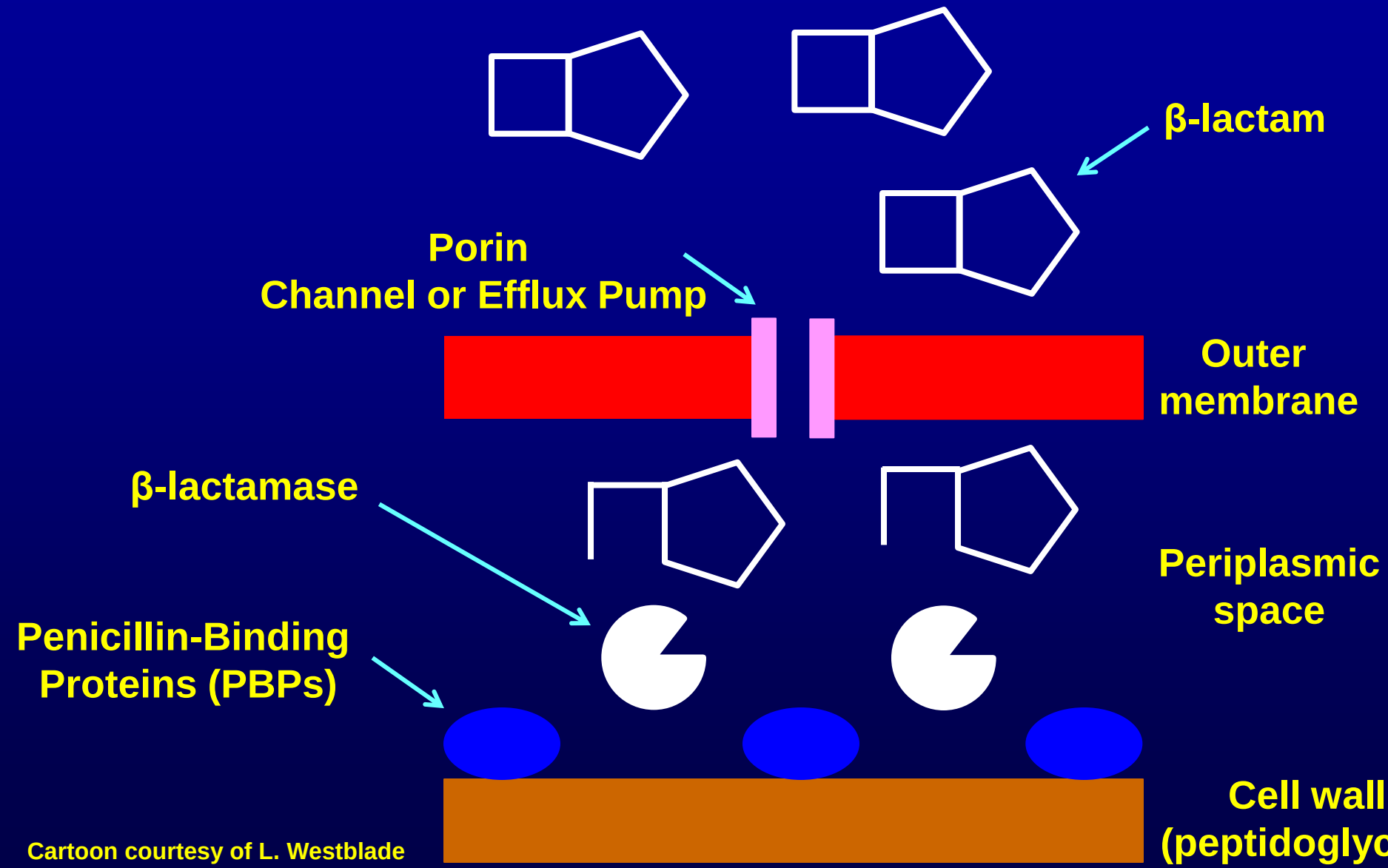
Cartoon courtesy of L. Westblade

β-Lactam Activity Against Gram-Negatives



Cartoon courtesy of L. Westblade

β-Lactam Resistance in Gram-negatives: β-Lactamases



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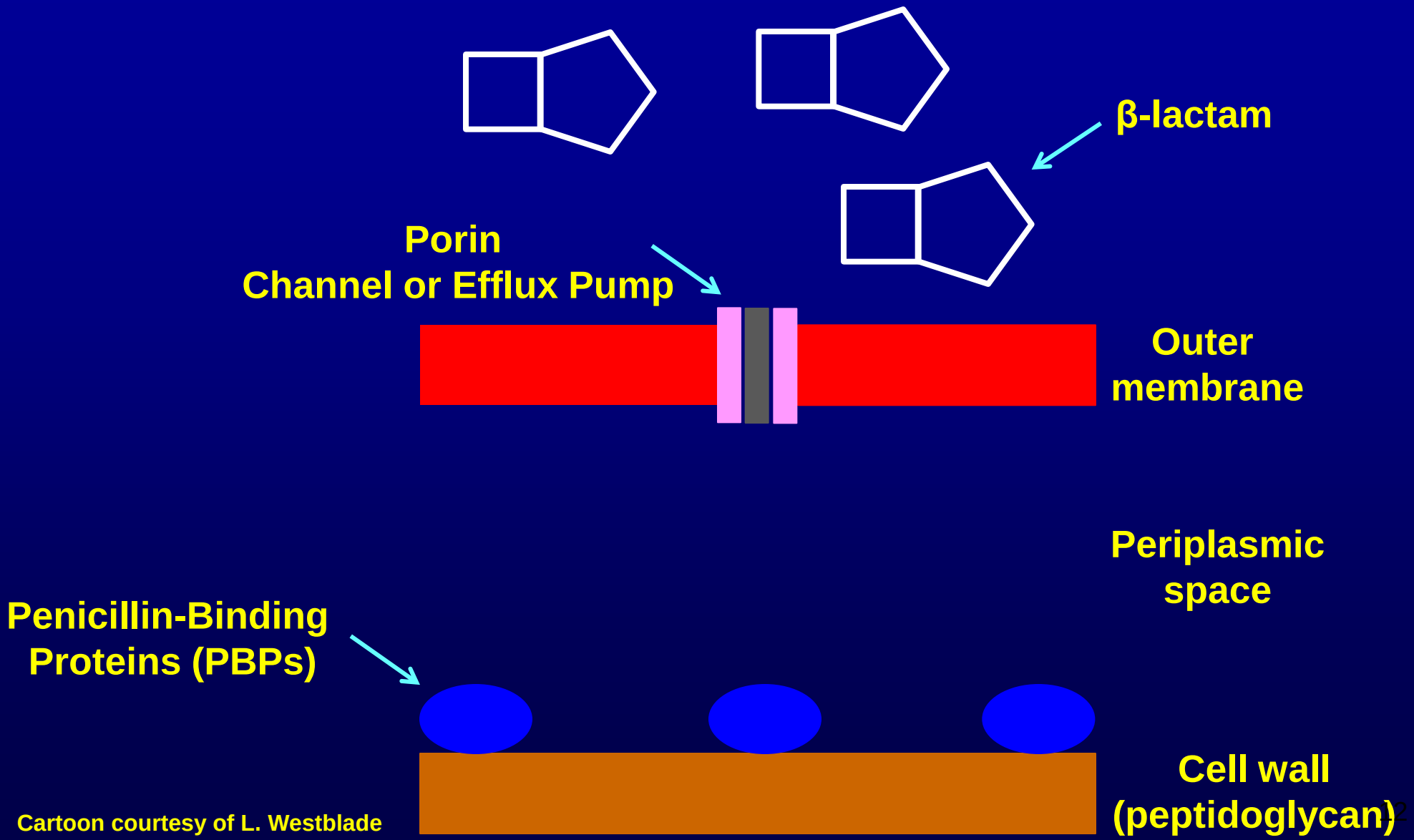
β-Lactam Resistance in Gram-negatives: Porin Channel Obstructed or Efflux Pump Altered

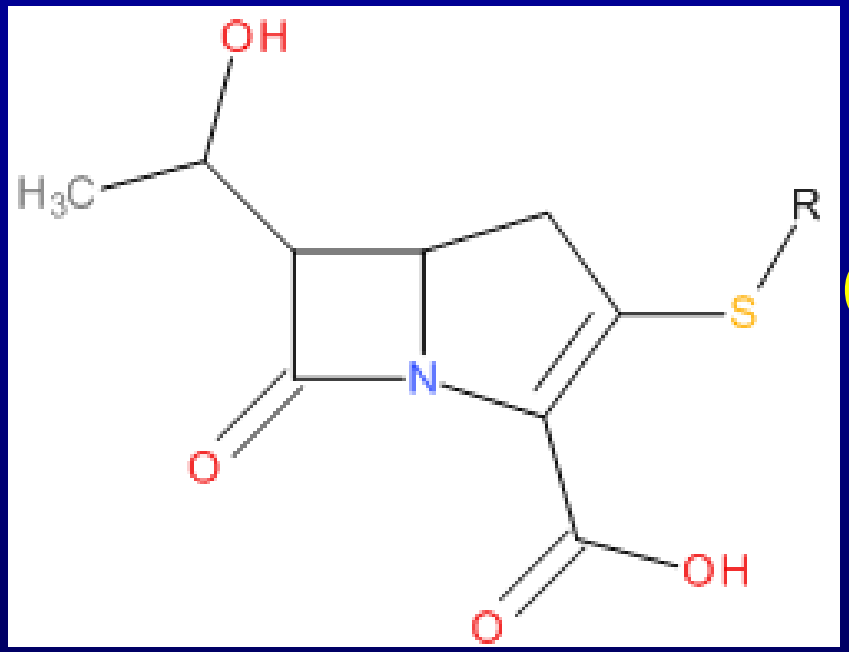


Cartoon courtesy of L. Westblade

β-Lactamases Produced by Gram-negatives

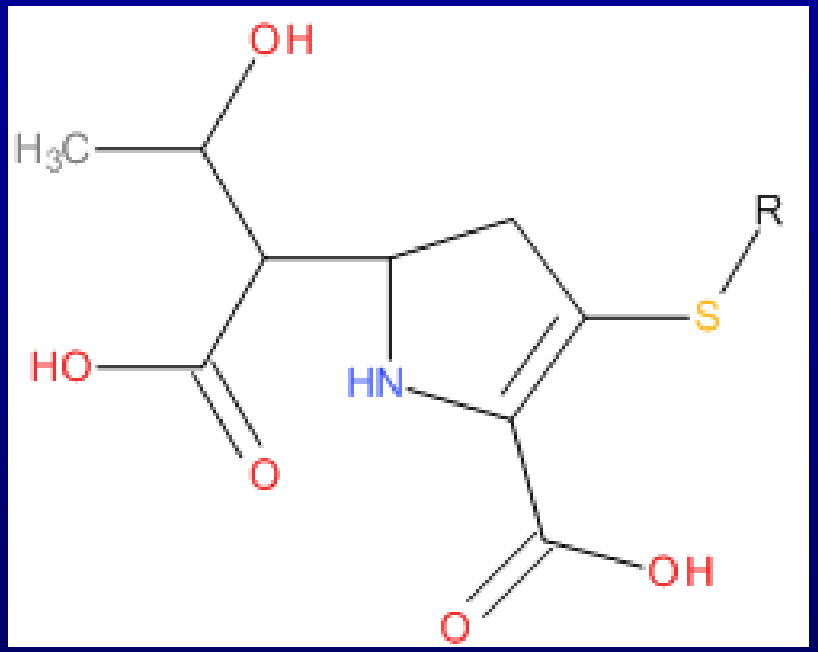
- Enzymes that hydrolyze the β-lactam ring, inactivating the β-lactam
- Hundreds of different types including:
  - Penicillinases
  - ESBLs
  - Carbapenemases
  - AmpCs
- Selectively inactivate various β-lactam antimicrobial agents

Mechanism of Action of β-Lactamases



β-Lactam
(Carbapenem)

β-lactamase
(Carbapenemase)



Hydrolyzed
β-Lactam
(Carbapenem)

Concepts Related to AST

- When an enzyme produced by a bacterium hydrolyzes an antimicrobial agent, the enzyme inactivates that agent
- Low level activity of an enzyme implies that the MICs are reduced to a lesser extent than occurs with enzymes that exhibit higher level activity
  - MICs may be in the resistant, intermediate or even the high end of the susceptible range
  - Carbapenemases can exhibit low level or higher level activity

Carbapenem-Resistant Enterobacteriaceae

- Two mechanisms may result in carbapenem MICs or zone diameters in the "I" or "R" range among Enterobacteriaceae
  - Carbapenemase production
  - Cephalosporinase or ESBL together with porin loss
    - Some AmpC β-lactamases and ESBLs have low-level carbapenem-hydrolyzing activity
    - Porin loss limits entry of the carbapenem into the cell

GNR β-Lactamases (Non-carbapenemase)

Class	Examples	Produced by:	Notes
A	ESBLs [TEM, SHV, CTX-M]	*K. pneumoniae* and other Enterobacteriaceae	Most inhibited by β-lactamase inhibitors Usually plasmid-mediated; Can confer carbapenem resistance if other "R" mechanisms are present (e.g., porin modification)
B	- - -	- - -	- - -
C	AmpC	Enterobacteriaceae and some non-fermenters	Inducible in some genera (SPACE/SPICE organisms); Not inhibited by clavulanic acid
D	- - -	- - -	- - -

Adapted from Queenan & Bush. 2007. Clin Microbiol Rev. 20:440.
Bush & Jacoby. 2010. AAC. 54:969; Bush, K. 2013. Ann NY Acad Sci 1277:84.

GNR Carbapenemases

Class	Examples	Produced by:	Notes
A	Serine carbapenemases: KPC, SME	*K. pneumoniae* and other Enterobacteriaceae *S. marcescens*	Usually plasmid-mediated (not SME)
B	MBL carbapenemases: e.g. NDM, VIM, IMP, GIM, SPM	*P. aeruginosa* Enterobacteriaceae *Acinetobacter* *S. maltophilia*	Inhibited by EDTA Do not hydrolyze aztreonam
C	- - -	- - -	- - -
D	OXA carbapenemases	*Acinetobacter baumannii* Pseudomonads Enterobacteriaceae	Weakly hydrolyze carbapenems

Adapted from Queenan & Bush. 2007. Clin Microbiol Rev. 20:440.
Bush & Jacoby. 2010. AAC. 54:969; Bush, K. 2013. Ann NY Acad Sci 1277:84.

Most Common
Carbapenemases

KPC
(*Klebsiella pneumoniae* Carbapenemase)

- Most common carbapenemase in USA
- First report 1996 from North Carolina
- Usually a high level of enzyme can be produced
- Mostly *K. pneumoniae*, also *K. oxytoca, E. coli, C. freundii, Enterobacter* spp., *Salmonella, Serratia* spp., *P. aeruginosa* and other GNRs
- Plasmid with KPC gene generally has other R genes including genes for ESBLs

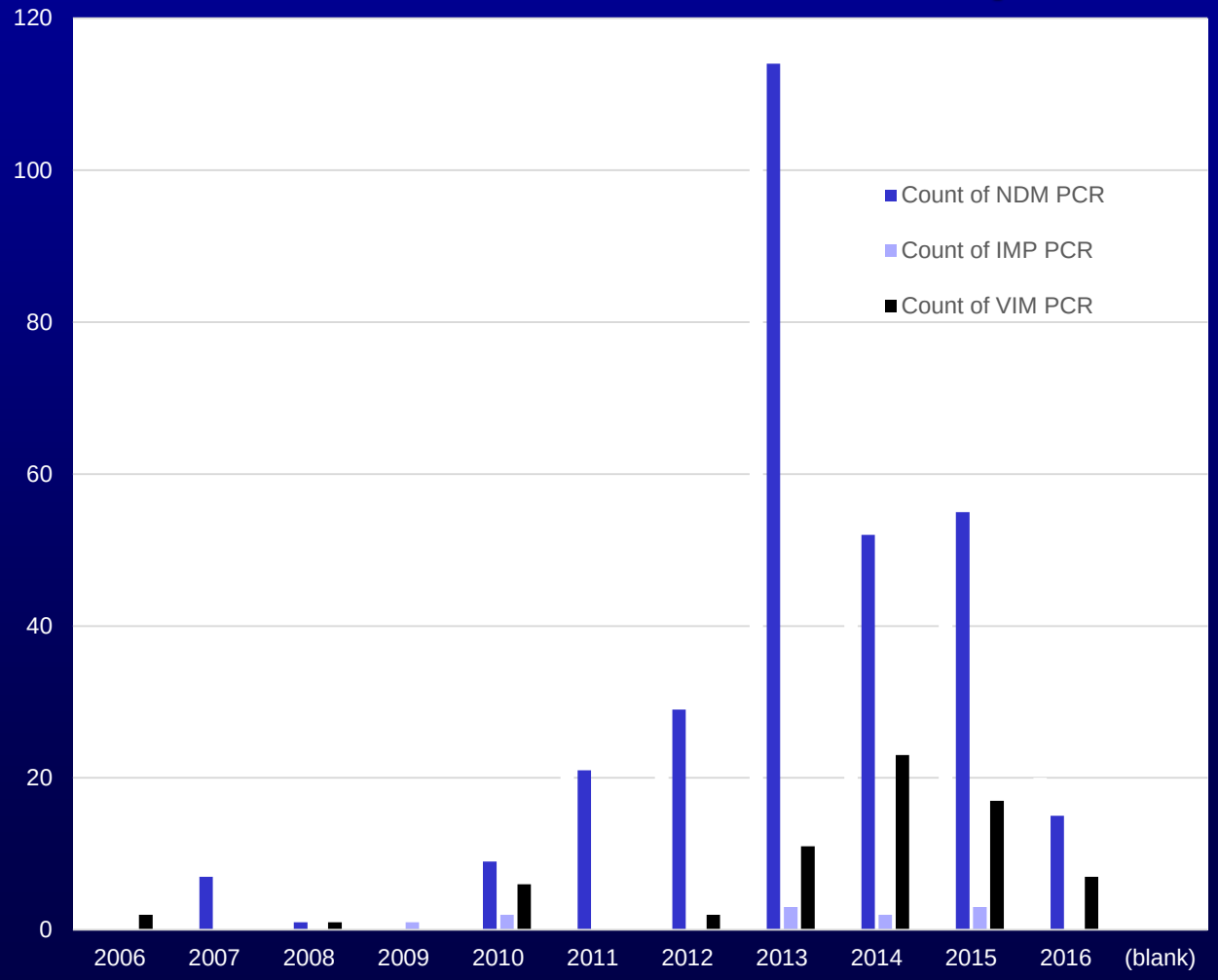
Metallo β-Lactamase (MBL) Carbapenemase

- **NDM (New Delhi MBL) is the most common MBL worldwide; frequently encountered in India and Pakistan**
- **First report 2008 in a Swedish patient who was hospitalized in India**
- **Called MBL because zinc is required for activity**
- **Mostly *K. pneumoniae* and *E. coli***
- ***bla*$_{NDM}$ gene is highly mobile**
- **Also includes *bla*$_{IMP}$, *bla*$_{VIM}$**

OXA Carbapenemase

- First described in *Acinetobacter baumannii* in 1985
- OXA-48
  - Commonly found in Europe and Africa; relatively rare in USA
  - First reported in 2008 in Turkey
  - Mostly *K. pneumoniae, E. coli*
- Many OXA-48-like variants described to date (OXA-181, OXA-232)
- Weakly hydrolyze carbapenems and cephalosporins (OXA-48 has greater hydrolytic properties than some other OXAs against carbapenems)

Number of MBL-producing Gram-Negative Rods Reported in the U.S. to the CDC (as of 4/27/2016)



Slide courtesy of B. Limbago, CDC

Carbapenemase Spread and Diversity

- A single patient may harbor several species containing *bla*$_{KPC}$
  - One patient had KPC-producing *K. pneumoniae, E. coli,* and *Serratia marcescens*

    Sidjabat et al. 2009. Clin Infect Dis. 49:1736.
- A single patient may harbor several species with different carbapenemases
  - One UCLA patient
    - KPC (*K. pneumoniae*)
    - SME (*S. marcescens*)

    Pollett et al. 2014. J Clin Microbiol. 52:4003.

Breakpoints

Breakpoint Reminders

- CLSI and FDA set / revise breakpoints in USA
- Carbapenem breakpoints for Enterobacteriaceae were updated by:
  - CLSI in 2010
  - FDA in 2012 (ertapenem, imipenem); 2013 (meropenem)
  - Note: for doripenem, FDA lists "S" only breakpoint of ≤0.5 µg/ml (zone ≥23 mm); 2012
    - Current FDA breakpoints may not be updated on commercial systems
  - Clinical laboratory must perform a verification for a commercial system if using breakpoints other than those that are FDA-cleared on that system

Check with manufacturer of your commercial system for breakpoint status.

Where are the current CLSI breakpoints?

M100S 26th ed Table 2A (January 2016)

Table 2A-1. Zone Diameter and Minimal Inhibitory Concentration Interpretive Standards for *Enterobacteriaceae*

Testing Conditions	
Medium:	Disk diffusion: MHA Broth dilution: CAMHB Agar dilution: MHA
Inoculum:	Growth method or direct colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	35°C±2°C; ambient air Disk diffusion: 16–18 hours Dilution methods: 16–20 hours

Routine QC Recommendations (See Tables 4A and 5A for acceptable QC ranges.)

Escherichia coli ATCC®* 25922
Pseudomonas aeruginosa ATCC® 27853 (for carbapenems)
Escherichia coli ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

* ATCC® is a registered trademark of the American Type Culture Collection.

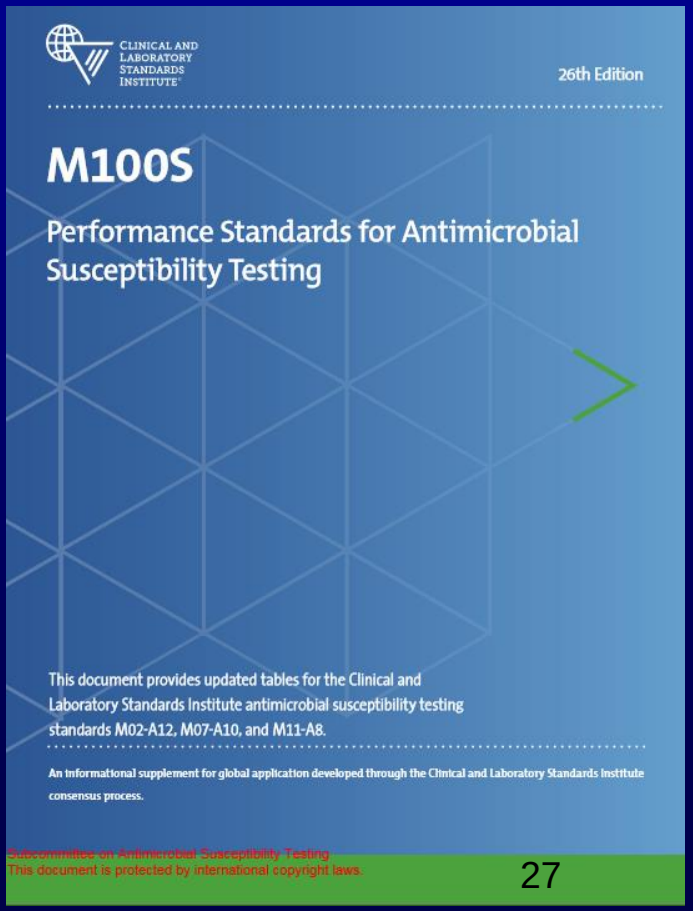
Refer to Tables 3A, 3B, and 3C for additional testing recommendations, reporting suggestions, and QC.

General Comments

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02-A12, Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., ignore the thin veil of swarming growth in an otherwise obvious zone of growth inhibition. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(2) When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. In addition, for extraintestinal isolates of *Salmonella* spp., a third-generation cephalosporin should be tested and reported, and chloramphenicol may be tested and reported if requested. Susceptibility testing is indicated for typhoidal *Salmonella* (*S.* Typhi and *Salmonella* Paratyphi A–C) isolated from extraintestinal and intestinal sources. Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. **In contrast, susceptibility testing is indicated for all *Shigella* isolates.**

(3) The dosage regimens shown in the comments column below are those needed to achieve plasma drug exposures (in adults with normal renal and hepatic functions) on which breakpoints were based. When implementing new breakpoints, it is strongly recommended that laboratories share this information with infectious diseases practitioners, pharmacists, pharmacy and therapeutics committees, and infection control committees.



Where are the current FDA breakpoints?

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Antibacterial and Antifungal Product Labeling: Microbiology Susceptibility Interpretive Criteria (Breakpoints) and Quality Control Parameter Updates

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Table Last Updated: January 21, 2016
New or Changes are in bold.

This table lists approved Antibacterial and Antifungal products whose Microbiology subsection, Susceptibility Interpretive Criteria and Quality Control Parameter information in the labeling, has been reviewed and is current. The labels for these products are posted on the Drugs@FDA or Daily Med websites.

Active Ingredient	Product Name	Company Name	Type of Appli-cation	Application Number	Date of Most Recent FDA Review of Microbiology Susceptibility Interpretive Criteria*
Amoxicillin	Amoxil (amoxicillin) Chewable Tablets	Dr. Reddy's Laboratories	NDA	50-542	**09/24/15**

Updated 1/21/16

http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm275763.htm

Google: "FDA Interpretive Criteria Updates" and you will see list of dates of updated breakpoints

To view the FDA breakpoints for a specific antimicrobial agent, click on: "Dailymed" or "Drugs@FDA"

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Antibacterial and Antifungal Product Labeling: Microbiology Susceptibility Interpretive Criteria (Breakpoints) and Quality Control Parameter Updates

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Table Last Updated: January 21, 2016
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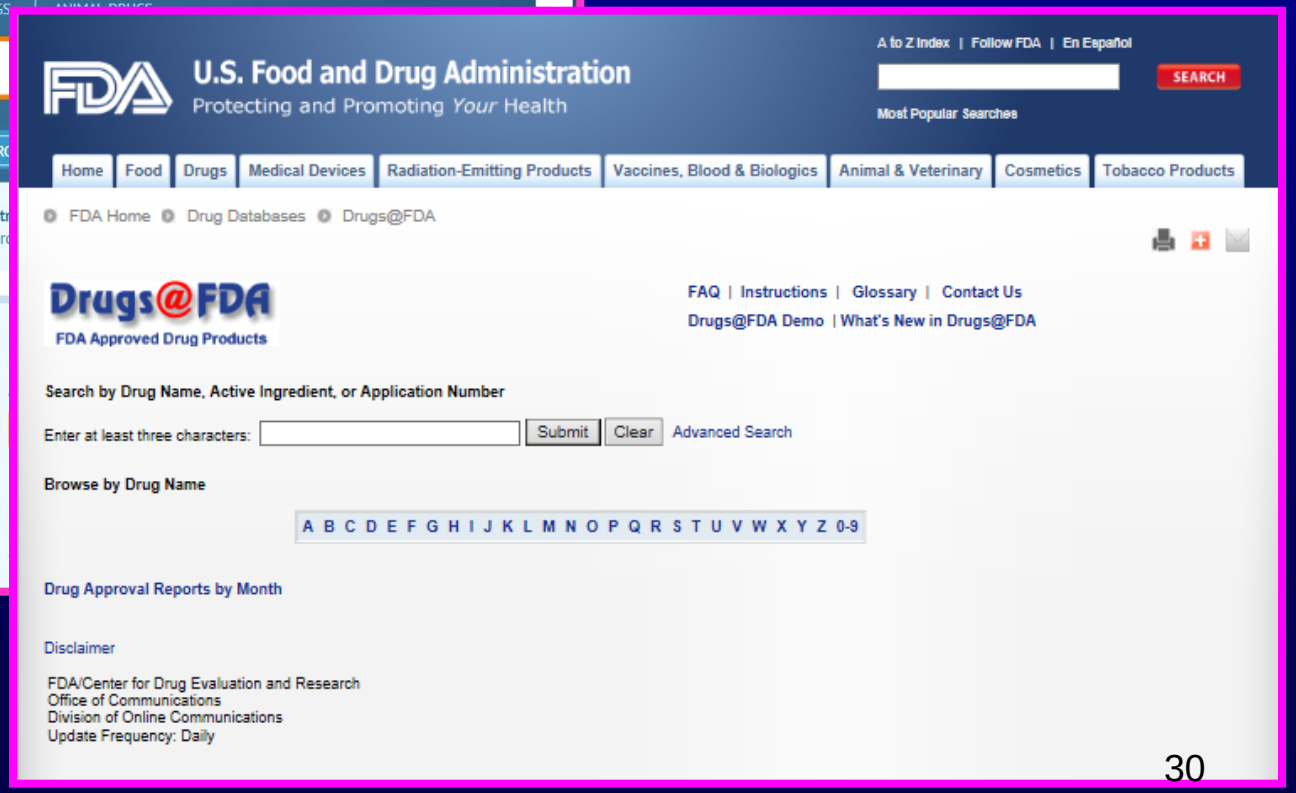
Amoxicillin	Amoxil (amoxicillin) Chewable Tablets	Dr. Reddy's Laboratories	NDA	50-542	09/24/15

Type in antimicrobial agent name, then click on "Label Information" for the antimicrobial agent, which will open up a pdf of the package insert.



Then Go to "Clinical Pharmacology section" in the package insert (subsection Microbiology) for FDA BPs

Table 6: Susceptibility Test Result Interpretive Criteria for Doripenem

	Minimum Inhibitory Concentrations (µg/mL)	Disk Diffusion (zone diameters in mm)
Pathogen	Susceptible*	Susceptible*
Enterobacteriaceae	≤ 0.5	≥ 23
Pseudomonas aeruginosa	≤ 2	≥ 24
Acinetobacter baumannii	≤ 1	≥ 17
Streptococcus anginosus group (*S. constellatus* and *S. intermedius*)	≤ 0.12	≥ 24
Anaerobes	≤ 1	n/a

* The current absence of resistant isolates precludes defining any results other than "Susceptible". Isolates yielding MIC or disk diffusion results suggestive of "Nonsusceptible" should be subjected to additional testing.
n/a = not applicable

FDA Breakpoints for Doripenem

Where can I find information about "verification" of ASTs?

ASMPress

Browse by Category

ASMPRESS Categories

- Applied and Industrial Microbiology
- ASM Meetings - Abstract CDRoms
- Bacterial Pathogenesis
- Catalog
- Clinical Microbiology
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109 *Case Report:* Fatal Pulmonary *Mycobacterium abscessus* Infection in an Immunosuppressed Patient

Verification of Antimicrobial Susceptibili Testing Methods: a Practical Approach

Jean B. Patel,[1] Susan Sharp,[2] and Susan Novak-Weekley,[3] [1]Division of Healthcare Quality Pr for Disease Control and Prevention, Atlanta, Georgia, [2]Northwest Permanente Physicians and Su Oregon, and [3]SCPMG Regional Reference Laboratories, North Hollywood, California

Abstract

The process of verifying an antimicrobial susceptibility testing (AST) system can be very c are different AST methods, such as MIC methods and disk diffusion testing. In addition eral different reasons why verification might be necessary, such as implementing a new method in the laboratory or implementing non-FDA interpretive criteria or breakpoints on an FDA-cleared AST system. The Clinical Laboratory Improvement Amendment (CLIA) provides some general guidance, but ultimately, it is the responsibility of a laboratory director to decide the composition of a verification study protocol. Variables to consider are what methods should be compared, what and how many isolates should be tested, how the results will be compared, and what study results will result in an acceptable study outcome. This article provides some general guidelines for developing and conducting a verification study of an AST system.

Introduction

The Clinical Laboratory Improvement Amendment (CLIA) regulation requires laboratories to implement revised breakpoints face a significant obstacle, because only FDA breakpoints can be used on FDA-approved devices. Use of alterna-

CLINICAL AND LABORATORY STANDARDS INSTITUTE

1st Edition

M52

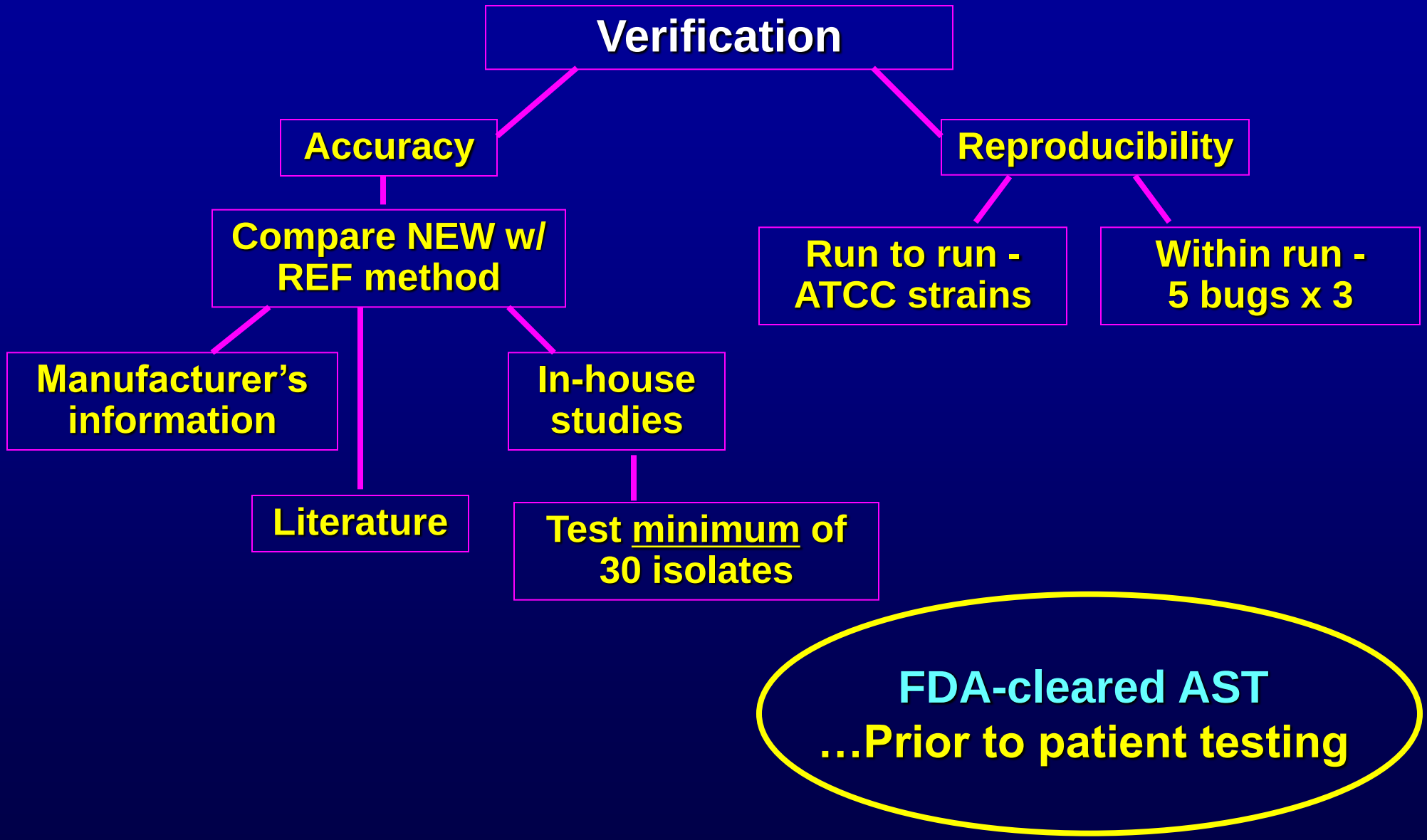
Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems

This guideline includes recommendations for verification of commercial US Food and Drug Administration–cleared microbial identification and antimicrobial susceptibility testing systems by clinical laboratory professionals to fulfill regulatory or quality assurance requirements for the use of these systems for diagnostic testing.

A guideline for US application developed through the Clinical and Laboratory Standards Institute consensus process.

AST Verification Requirements

- In USA, laboratories must perform verification study when implementing a new test or new breakpoints
  - CLIA requirement
- Must perform if using:
  - Disk diffusion
  - Commercial system that is FDA-cleared for the new breakpoints
  - Commercial system that is not FDA-cleared for the new breakpoints


Verification
Accuracy
Reproducibility
Compare NEW w/ REF method
Run to run - ATCC strains
Within run - 5 bugs x 3
Manufacturer's information
In-house studies
Literature
Test minimum of 30 isolates
FDA-cleared AST
…Prior to patient testing

AST In-House Verification Study - Example*

Step	Details
Obtain Isolates N ≈ 30	• Obtain isolates from a reference laboratory or other source ("reference set"); "Reference set": – is provided with meropenem MIC and S, I, R results that were obtained from method verified with current CLSI/FDA BPs – includes both "S" and "R" phenotypes with variety of MICs
Test by Automated MIC	• Evaluate carbapenems routinely tested in your laboratory • Interpret MICs (M100S 26th ed)
Review MIC and S, I, R results	• Compare MIC results from automated AST system to "reference" MIC results (essential agreement) • Compare S, I, R results from automated AST system to "reference" S, I, R results (categoric agreement) • Some results may not agree; need plan to arbitrate (e.g., send to another lab that uses CLSI reference MIC method)
Write verification report	• Submit for laboratory director approval

* verify current CLSI/FDA meropenem BPs on commercial automated AST system using set of organisms obtained from a reference laboratory

Example: Meropenem In House Verification Study
Test Method: Automated AST System
Reference (Comparator): "Reference Set" from Lab B

Organism	Isolates No. (No. R)	EA		CA		VME		ME	
		#	%	#	%	#	%	#	%
E. coli 2 CR (KPC)	10 (2)	10	100	10	100	0	0	0	0
K. pneumoniae 5 CR (4 KPC, 1 NDM)	10 (5)	9	90	9	90	1	20*	0	0
Other Enterobacteriaceae (1 SME, 1 NDM)	10 (2)	9	90	10	100	0	0	0	0
Total	30 (9)	28	93.3	29	97	1	11*	0	0

CR = carbapenem resistant; VME = very major error; ME = major error

* Unacceptable result → repeat, send to second reference lab for additional testing. Isolates may have lost plasmid. Avoid excessive subbing to avoid plasmid loss.

Enterobacteriaceae

Interpreting Carbapenem Results

The best way to detect CRE is to use current breakpoints!

CLSI M100S 26th ed Table 2A Enterobacteriaceae and Carbapenems

											regimen of 1 g every 8 h. See comment (7).
CARBAPENEMS **(25)** Following evaluation of PK-PD properties, limited clinical data, and MIC distributions that include recently described carbapenemase-producing strains, revised interpretive criteria for carbapenems were first published in June 2010 (M100-S20-U) and are listed below. Because of limited treatment options for infections caused by organisms with carbapenem MICs or zone diameters in the intermediate range, clinicians may wish to design carbapenem dosage regimens that use maximum recommended doses and possibly prolonged intravenous infusion regimens, as has been reported in the literature.[1-4] Consultation with an infectious diseases practitioner is recommended for isolates for which the carbapenem MICs or zone diameter results from disk diffusion testing are in the intermediate or resistant ranges. Laboratories using *Enterobacteriaceae* MIC interpretive criteria for carbapenems described in M100-S20 (January 2010) should perform the MHT, the Carba NP test, and/or a molecular assay when isolates of *Enterobacteriaceae* are suspicious for carbapenemase production based on imipenem or meropenem MICs of 2–4 µg/mL or ertapenem MIC of 2 µg/mL (refer to Tables 3B and 3C). After implementation of the current interpretive criteria, the MHT does not need to be performed other than for epidemiological or infection control purposes (refer to Table 3B). The following information is provided as background on carbapenemases in *Enterobacteriaceae* that are largely responsible for MICs and zone diameters in the intermediate and resistant ranges, and thus the rationale for setting revised carbapenem breakpoints: • The clinical effectiveness of carbapenem treatment of infections produced by isolates for which the carbapenem MIC or disk diffusion test results are within the intermediate range is uncertain due to lack of controlled clinical studies. • Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (eg, MICs in the intermediate or resistant range) than meropenem or doripenem MICs. These isolates may have elevated imipenem MICs by mechanisms other than production of carbapenemases.											
B	Doripenem	10 µg	≥23	–	20–22	≤19	≤1	–	2	≥4	**(26)** Interpretive criteria are based on a dosage regimen of 500 mg every 8 h.
B	Ertapenem	10 µg	≥22	–	19–21	≤18	≤0.5	–	1	≥2	**(27)** Interpretive criteria are based on a dosage regimen of 1 g every 24 h.
B	Imipenem	10 µg	≥23	–	20–22	≤19	≤1	–	2	≥4	**(28)** Interpretive criteria are based on a dosage regimen of 500 mg every 6 h or 1 g every 8 h.
B	Meropenem	10 µg	≥23	–	20–22	≤19	≤1	–	2	≥4	**(29)** Interpretive criteria are based on a dosage regimen of 1 g every 8 h.

CLSI Carbapenem Breakpoints / Dosage Comments

			S		I	R	S		I	R	
B	Doripenem	10 µg	≥23	–	20–22	≤19	≤1	–	2	≥4	**(26)** Interpretive criteria are based on a dosage regimen of 500 mg every 8 h.
B	Ertapenem	10 µg	≥22	–	19–21	≤18	≤0.5	–	1	≥2	**(27)** Interpretive criteria are based on a dosage regimen of 1 g every 24 h.
B	Imipenem	10 µg	≥23	–	20–22	≤19	≤1	–	2	≥4	**(28)** Interpretive criteria are based on a dosage regimen of 500 mg every 6 h or 1 g every 8 h.
B	Meropenem	10 µg	≥23	–	20–22	≤19					

(26) Interpretive criteria are based on a dosage regimen of 500 mg every 8 h.

(27) Interpretive criteria are based on a dosage regimen of 1 g every 24 h.

(28) Interpretive criteria are based on a dosage regimen of 500 mg every 6 h or 1 g every 8 h.

(29) Interpretive criteria are based on a dosage regimen of 1 g every 8 h.

M100S 26th ed Table 2A.

CLSI Carbapenem Dosage Comment

"Because of limited treatment options for infections caused by organisms with carbapenem MICs or zone diameters in the intermediate range, clinicians may wish to design carbapenem dosage regimens that use maximum recommended doses and possibly prolonged intravenous infusion regimens as has been reported in the literature."

Maintain higher antimicrobial agent levels in patients for longer periods of time.

CLSI M100S 26th ed.
Daikos et al. 2011. Clin Microbiol Infect. 17:1135.

Enterobacteriaceae - Carbapenem Breakpoints (MIC µg/ml)[1]

Agent	Current CLSI & FDA Breakpoints			Old CLSI Breakpoints		
	Susc	Int	Res	Susc	Int	Res
Ertapenem	≤0.5	1	≥2	≤2	4	≥8
Imipenem	≤1	2	≥4	≤4	8	≥16
Meropenem	≤ 1	2	≥4	≤4	8	≥16

Agent	Current CLSI Breakpoints			Old CLSI Breakpoints
Doripenem[2]	≤1	2	≥4	none

[1]CLSI M100 26th ed; also lists corresponding disk diffusion breakpoints
[2]FDA breakpoint "S" only = ≤0.5

Carbapenemase

Testing for the Enzymes

Do we need to determine if a carbapenemase is present in CRE?

- **Patient care**
  - No, when using the "current" CLSI breakpoints
  - Yes, if using "old" CLSI breakpoints
- **Infection control**
  - Yes, if outbreak suspected; possibly in other settings
- **Epidemiology / research**
  - Yes, to better understand emerging resistance and plan for "challenges"

Some say MUST identify resistance mechanism in all CRE as carbapenemase-producers are more worrisome than non-carbapenemase-producing CRE.

Introduction to Tables 3B and 3C. Tests for Carbapenemases in Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter* spp.

	MHT	Carba NP	Molecular
Use	Enterobacteriaceae	Enterobacteriaceae *P. aeruginosa* *Acinetobacter*	Enterobacteriaceae *P. aeruginosa* *Acinetobacter*
Strengths	Simple	Rapid	Determines type of carbapenemase
Limitations	Some false pos (eg, ESBL/AmpC + porin) Some false neg (eg NDM) Enterobacteriaceae only	Special "fresh" reagents Some invalid results False neg for OXA-type carbapenemase	Special reagents Specific to targeted gene Difficult for some micro labs to implement

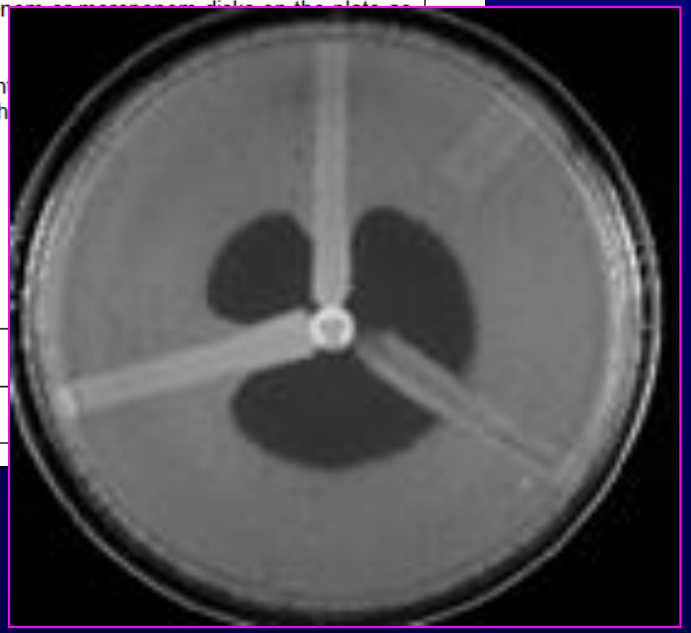
CLSI M100S 26th ed.

Modified Hodge Test for Carbapenemases

Table 3B. The Modified Hodge Confirmatory Test for Suspected Carbapenemase Production in *Enterobacteriaceae*

NOTE: If using FORMER minimal inhibitory concentration (MIC) interpretive criteria for carbapenems described in M100-S20 (January 2010), please refer to modifications in Table 3B-1 below.

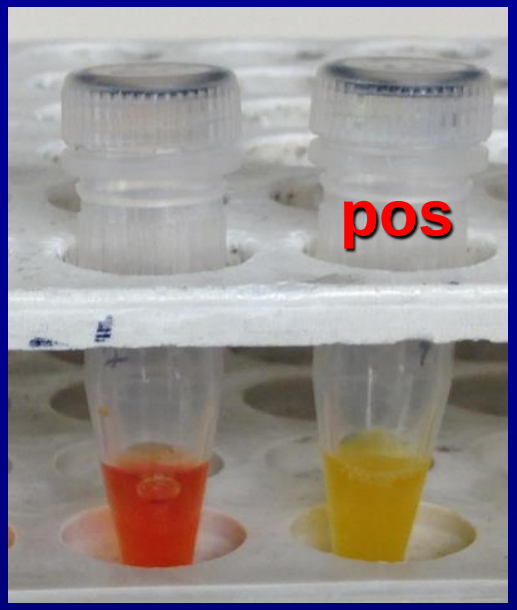
Test	Confirmatory Test
When to Do This Test:	**For epidemiological or infection control purposes. NOTE: No change in the interpretation of carbapenem susceptibility test results is required for carbapenemase-positive isolates.**
Test Method	MHT
Medium	**MHA**
Antimicrobial Concentration	Ertapenem disk 10 µg or Meropenem disk 10 µg
Inoculum	(1) Prepare a 0.5 McFarland standard suspension (using either direct colony suspension or growth method) of *E. coli* ATCC® 25922 (the indicator organism) in broth or saline, and dilute 1:10 in saline or broth. Inoculate an MHA plate as for the routine disk diffusion procedure. Allow the plate to dry 3 to 10 minutes. Place the appropriate number of ertapen[illegible] noted below and shown in Figures 1 and 2. (2) Using a 10-µL loop or swab, pick 3 to 5 colonies of test or QC organism grown overnigh[illegible] straight line out from the edge of the disk. The streak should be at least 20–25 mm in length[illegible] noted below and shown in Figures 1 and 2. Capacity of small and large MHA plates (100-mm or 150-mm diameter, respectively): Small / Large Disks: 1 / 1–4 Test isolates: 1 / 1–6 QC isolates: 2 / 2
Incubation Conditions	35°C ± 2°C; ambient air
Incubation Length	16–20 hours



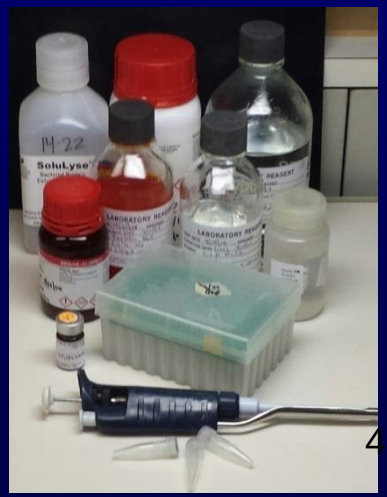
M100S 26th ed. Table 3B.

Carba NP Test for Carbapenemase Production

- Isolated colonies (lyse)
- Hydrolysis of imipenem
- Detected by change in pH of indicator (red changes to yellow/orange)
- Rapid <2h
- Microtube method

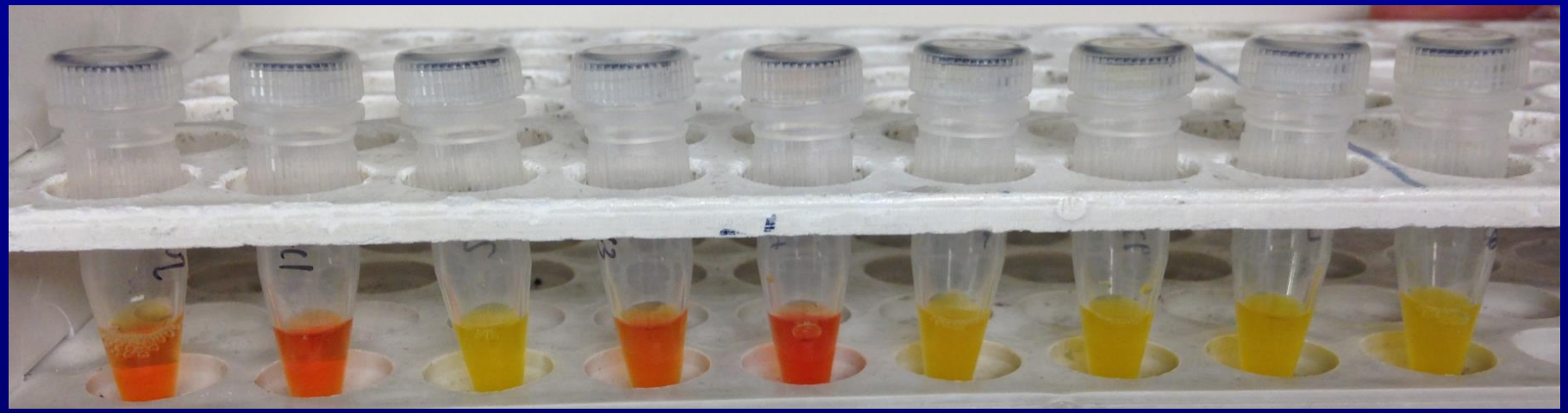


NO imipenem + imipenem



Nordmann et al. 2012. Emerg Infect Dis. 18:1503.
Tijet et al. 2013. Antimicrob Agents Chemother. 57:4578.
Vasoo et al. 2013. J Clin Microbiol. 51:3092.
Dortet et al. 2014. J Med Microbiol. 63:772.
Dortet et al. 2014. Antimicrob Agents Chemother. 58:2441.

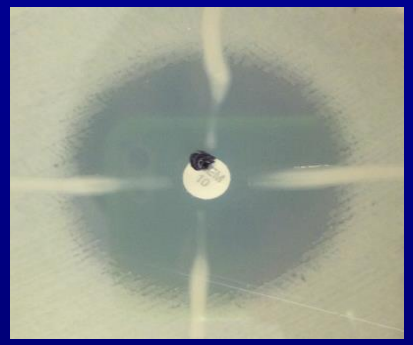
Carba NP Test - Examples



Blank	Neg	KPC	OXA48	OXA181	NDM	IMP	VIM	SME
		Pos	Invalid	Neg	Pos	Pos	Pos	Pos

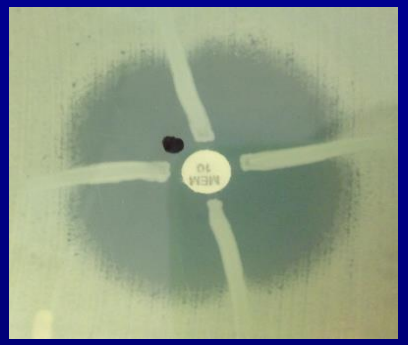
Courtesy of Shaun Yang, P. Hemarajata

Modified Hodge Test - Examples

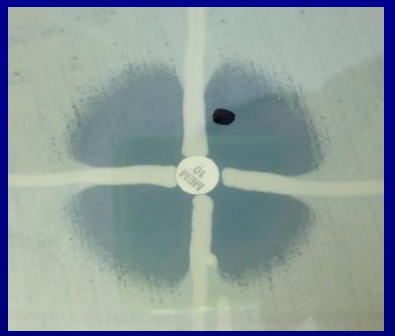


Neg Control

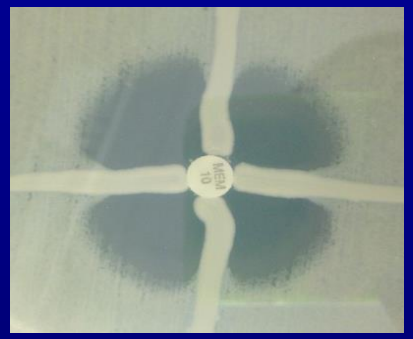
Pos Control
KPC



NDM
False neg



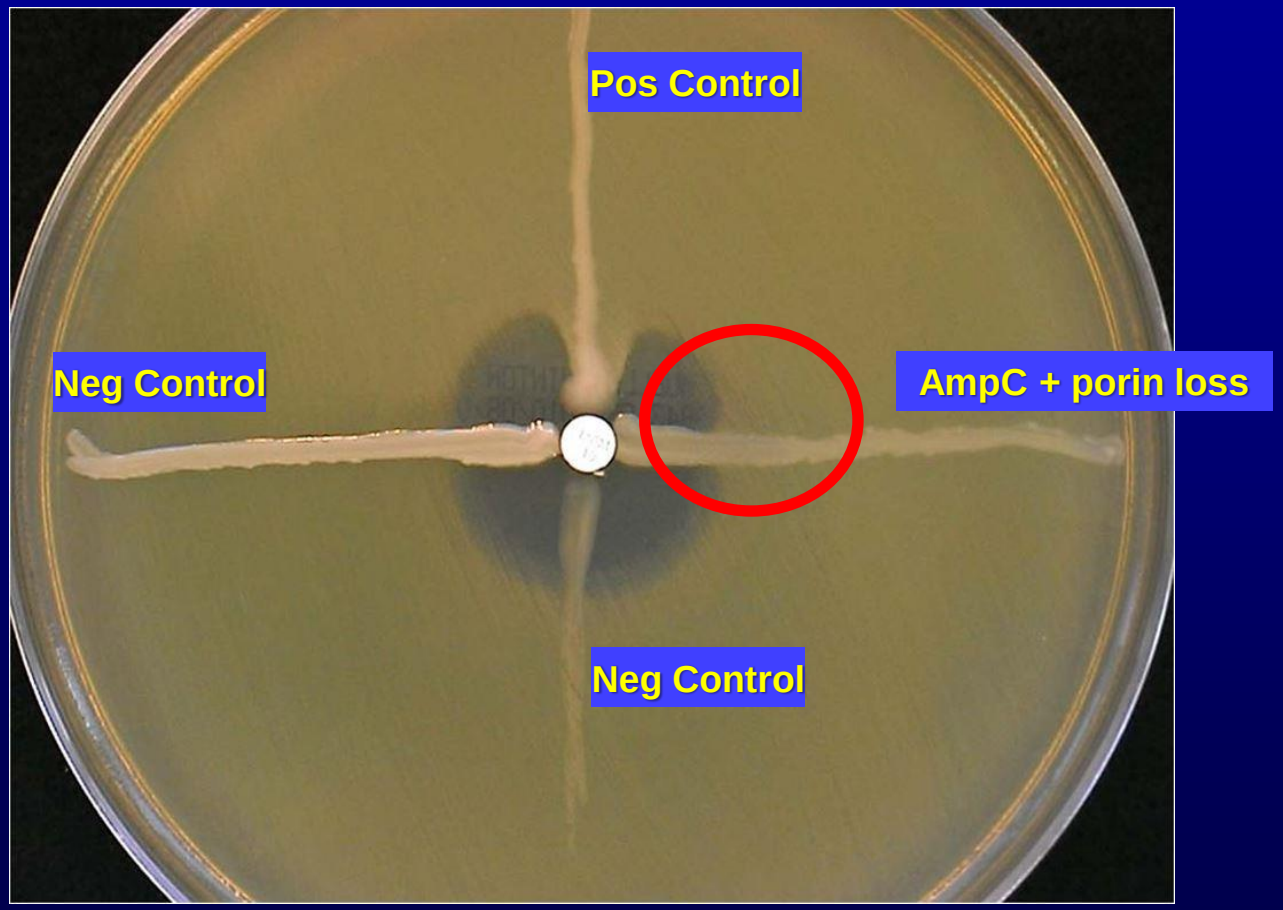
OXA 232
Positive



SME
Positive

Courtesy of Shaun Yang, P. Hemarajata

Modified Hodge Test False Positive



Courtesy of Paul Schreckenberger

CIM Test – for Carbapenemase (Carbapenem Inactivation Method)

- **Principle:**
  - A suspension of bacteria is incubated with a standard meropenem disk.
  - If the organism produces carbapenemase, it will inactivate the meropenem in the disk.
  - Following 2 h incubation, the meropenem disk is removed from the suspension and placed on a lawn of *E. coli* ATCC 25922.
  - Following overnight incubation, carbapenemase activity is demonstrated by a loss of meropenem activity in the disk.

#1 carbapenemase positive
#2 carbapenemase negative

van der Zwaluw K, et al. 2015. PLoS One. 10(3):e0123690

Neo-Rapid CARB Screen Kit

- Commercial kit; similar to Carba NP
- Enterobacteriaceae and *P. aeruginosa*
- Tablets
  - Imipenem + indicator
  - Negative control
- ≤2 hours
- Research use only

www.keyscientific.com

Molecular Tests for Carbapenemases[1]

- Biofire [2]
  - KPC
- Nanosphere [2]
  - KPC, NDM, OXA, IMP, VIM
- Cepheid [3]
  - KPC, NDM, OXA-48, IMP-1, VIM
- BD Max [4]
  - KPC, NDM, OXA-48
- NucliSENS EasyQ KPC [4]
  - KPC
- Check-Points CPE [4]
  - KPC, NDM, OXA-48, OXA-181, IMP, VIM

[1] Not all inclusive
[2] FDA-cleared; for use with positive blood culture broth
[3] FDA-cleared; for use with isolated colonies
[4] RUO (Research Use Only)

CRE

Reporting Results

Testing and Reporting Additional Agents

Specimen: Blood
Diagnosis: Pneumonia
Klebsiella pneumoniae

Final Report with Optional Comment

	MIC (µg/ml)
amikacin	>32 R
cefepime	>32 R
ceftriaxone	>32 R
ciprofloxacin	>2 R
ertapenem	>4 R
gentamicin	>10 R
meropenem	4 R
piperacillin-tazo	>128/4 R
tobramycin	>10 R
trimeth-sulfa	>4/76 R

"This *Klebsiella pneumoniae* has unusual carbapenem results and is considered carbapenem-resistant Enterobacteriaceae (CRE); Infectious Diseases Consult suggested"

Treatment Options for CRE

- Polymyxins
  - Colistin, polymyxin B
- Tigecycline
- Minocycline
- Aminoglycosides (not tobramycin)
- Fosfomycin
- Ceftazidime-avibactam (class A producers only)

Usually a combination of antimicrobial agents (often with a polymyxin) are used

Supplemental Antimicrobial Agents to Consider for AST of CRE

Antimicrobial Agent	Enterobacteriaceae (CRE)
Minocycline	Yes
Tigecycline [1]	Yes (excluding *Pro/Prov/Morg*)
Colistin (or polymyxin B)	Yes [2]
Fosfomycin	Yes [3]
Ceftazidime-avibactam	Yes

[1] Not on urine isolates. Breakpoints (µg/ml): No CLSI; FDA ≤2 S, 4 I, ≥8 R; EUCAST ≤1 S, >2 R

[2] Breakpoints (µg/ml): No CLSI; EUCAST ≤2 S, >2 R

[3] Breakpoints (µg/ml): CLSI ≤64 S, ≥256 R for *E. coli* only; EUCAST ≤32 S, >32 R for all Enterobacteriaceae

Availability of ASTs for Supplemental Drugs for CRE

Antimicrobial	Automated Systems	Disk Diffusion	Etest	CLSI Reference MIC method [1]
Minocycline	Some	Yes	Yes	Yes
Tigecycline	Yes	Yes	Yes	Yes
Colistin (or polymyxin B) [2]	No	Yes, but poor performance, RUO	Poor performance, RUO	Yes
Fosfomycin [3,4]	No	Yes	Yes	Yes
Ceftaz-avibactam	Some	Yes, RUO	Yes, RUO	Yes

[1] performed by few laboratories
[2] no FDA-cleared commercial methods
[3] only test reliably by agar methods
[4] FDA-cleared for *E. coli* and *E. faecalis* only

Thank you for your attention!

Please check this website again for additional materials on CRE and other topics in AST!

Brought to you by the Outreach Working Group of the CLSI Subcommittee on Antimicrobial Susceptibility Testing