

PEARLS OF LABORATORY MEDICINE

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TITLE: “Laboratory detection of carbapenem resistance in Gram-negative bacteria”

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Slide 1:

Hello, my name is Holly Huse. I am a post-doctoral fellow at the University of California Los Angeles. Welcome to this Pearl of Laboratory Medicine on “Laboratory detection of carbapenem resistance in Gram-negative bacteria.”

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Here are the objectives for today’s presentation. I will review the mode of action of β -lactam antibiotics, describe different classes and subclasses of β -lactam antibiotics, review β -lactam resistance mechanisms and classes of β -lactamases, discuss methods for detecting carbapenem resistance and indications for carbapenemase testing in Gram-negative bacteria, and explain methods for detecting carbapenemases.

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Pathogenic Gram-negative rods are broadly divided into the *Enterobacteriaceae* and glucose non-fermenters. The *Enterobacteriaceae* are colonizers of human and animal gastrointestinal tracts or are environmental. Genera within this family include *Escherichia*, *Shigella*, *Citrobacter*, *Salmonella*, *Edwardsiella*, *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, *Providencia*, *Morganella*, and *Yersinia*. Glucose non-fermenters are environmental and cause opportunistic infections.

The most commonly encountered non-fermenters are *Pseudomonas aeruginosa*, *Acinetobacter* spp., and *Stenotrophomonas maltophilia*. Both groups cause diverse infections, including pneumonia, wound or surgical site infections, meningitis, gastrointestinal infections, and bloodstream infections.

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β -lactam antibiotics are often used to treat infections caused by Gram-negative rods. β -lactams share a common core structure, the β -lactam ring, and are grouped based on structural similarities. Penicillins include amoxicillin and ampicillin and have the narrowest spectrum of activity. β -lactam inhibitor combinations have a β -lactam and β -lactamase inhibitor and include amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, ceftolozane-tazobactam, and ceftazidime-avibactam. Cephalosporins are further divided into generations, with higher generations having greater activity against *Enterobacteriaceae*. The cephamycins, cefoxitin and cefotetan, are similar to cephalosporins. Aztreonam is the only available monobactam and has activity against aerobic Gram-negative bacteria. The carbapenems, doripenem, ertapenem, imipenem, and meropenem, are some of the most important β -lactams because they have the broadest spectrum of activity. Often considered last resort antibiotics, they are reserved for serious infections. Carbapenem-resistant *Enterobacteriaceae*, or CRE, are usually resistant to all β -lactams, severely limiting treatment options for patients with these infections.

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β -lactam antibiotics target bacterial cell wall biosynthesis. They bind to and inhibit penicillin binding proteins, or PBPs, which are required for building peptidoglycan, an essential cell wall component. In Gram-negative bacteria, β -lactams enter the periplasm through outer membrane porin channels and bind to PBPs, causing cell death. β -lactam resistance can be conferred by β -lactamases, enzymes that hydrolyze β -lactams. There are many different types of β -lactamases with varying capacities to hydrolyze β -lactams.

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Carbapenem resistance can arise via two mechanisms. Bacteria with an extended spectrum β -lactamase, or ESBL, or an AmpC β -lactamase can poorly hydrolyze carbapenems. However, in combination with porin loss or obstruction, these enzymes can confer carbapenem resistance. These organisms are “non-carbapenemase producers,” or non-CP. A bacterium can also acquire a carbapenemase, which efficiently hydrolyzes carbapenems and confers resistance to carbapenems. These organisms are “carbapenemase producing organisms” or CPOs. *Enterobacteriaceae* are “carbapenemase producing CRE, or CP-CRE.” Differentiating between these mechanisms is important because carbapenemases are transmissible and therefore important for infection control. CP-CRE can hydrolyze penicillins, cephalosporins, monobactams, and carbapenems.

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Ambler classification divides β -lactamases into four classes via amino acid sequence homology. In this table, carbapenemases are shown in red. Class A β -lactamases include ESBLs and KPC and are found in the *Enterobacteriaceae*. KPC is the most common carbapenemase and was discovered in *Klebsiella pneumoniae*. These enzymes are endemic in the United States. Metallo- β -lactamases are Class B enzymes and include the NDM, VIM, and IMP carbapenemases. Unlike class A, C, and D enzymes, which contain active-site serines, class B enzymes contain zinc. Class B enzymes are found in *Enterobacteriaceae*, *P. aeruginosa*, *A. baumannii*, and *S. maltophilia*. These enzymes are endemic in India and Asia and are becoming more common in the United States. AmpC β -lactamases are class C enzymes. They are found in some *Enterobacteriaceae* and non-fermenters. Expression of AmpC β -lactamases is inducible or constitutive. Class D contains the OXA carbapenemases, which are found in *A. baumannii* and the *Enterobacteriaceae*. They hydrolyze carbapenems and are endemic in Asia and Europe.

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The Clinical Laboratory Standards Institute, or CLSI, outlines methods for laboratory detection of carbapenem resistance in their M100-S27 document, “Performance Standards for

Antimicrobial Susceptibility Testing.” Methods include disk diffusion and broth microdilution. In June 2010, CLSI lowered the *Enterobacteriaceae* minimum inhibitory concentration, or MIC, breakpoints for carbapenems. The MIC is the minimum concentration of antibiotic required to inhibit growth of an organism. The current interpretive categories and breakpoints for *Enterobacteriaceae* are shown. Isolates are susceptible to doripenem, imipenem, or meropenem at an MIC less than or equal to 1, intermediate at an MIC of 2, and resistant at an MIC greater than or equal to 4. For ertapenem, isolates are susceptible at an MIC less than or equal to 0.5, intermediate at an MIC of 1, and resistant at an MIC greater than or equal to 2. MIC breakpoints were revised based on evaluation of pharmacokinetic/pharmacodynamic properties, clinical data, and MIC data that included the evaluation of CPOs. When using the current MIC breakpoints, routine carbapenemase testing is not required. Disk diffusion breakpoints are not shown.

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When using the current breakpoints, perform antibiotic susceptibility testing on isolates of *Enterobacteriaceae*, and interpret the results based on the current breakpoints. Report the results for treatment purposes, and perform carbapenemase testing only for infection control or epidemiological purposes. Perform carbapenemase testing on isolates that test intermediate or resistant to one or more carbapenems. When using the old MIC breakpoints, perform carbapenemase testing on isolates of *Enterobacteriaceae* with an ertapenem MIC of 2 µg/ml or imipenem or meropenem MICs of 2-4 µg/ml.

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CLSI recommends using the Carba NP, the modified carbapenem inactivation method, or mCIM, or molecular testing for detecting carbapenemases. An asterisk appears next to the Modified Hodge Test because while current recommendations describe using the Modified Hodge Test for carbapenemase detection, this recommendation is changing, and CLSI will no longer recommend this test. Use any of these methods to test for carbapenemases in *Enterobacteriaceae*. Use the Carba NP or molecular methods for testing *Pseudomonas aeruginosa* and *Acinetobacter* spp. For phenotypic tests, report positive results as

carbapenemase positive or carbapenemase producer and negative results as carbapenemase not detected.

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While the Modified Hodge Test will no longer be recommended by CLSI, I will describe it here. This test measures growth of a susceptible *E. coli* strain in the presence of a carbapenem disk and a potential carbapenemase producer. A lawn of susceptible indicator *E. coli* is spread on a Mueller Hinton Agar plate, and a 10 microgram disk of ertapenem or meropenem is placed on the lawn. The test isolate is inoculated in a straight line approximately 20-25 mm in length from the disk edge, and the plate is incubated 16-20 hours. If the test isolate is a carbapenemase-producer, the carbapenemase will diffuse into the agar and hydrolyze the antibiotic, allowing growth of susceptible *E. coli* around the test organism and towards the carbapenem disk, as shown in the bottom panel for isolates 5-8. This results in an indentation of growth toward the carbapenem disk due to carbapenemase production by the test organism. If the organism does not produce a carbapenemase, *E. coli* growth is not indented toward the carbapenem disk, as shown in the top panel for isolates 1-4.

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The advantages to the MHT are that it is simple to perform and no special reagents or media are required. However, false-positive results can occur in isolates that produce an ESBL or AmpC coupled with a porin mutation, the test has poor sensitivity for the New Delhi metallo- β -lactamase, as shown.

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The Carba-NP is a colorimetric test for carbapenemases. The test isolate is emulsified in a microcentrifuge tube containing extraction reagent. Solutions containing imipenem and a pH indicator are added, and the reaction is incubated for up to 2 hours. If a carbapenemase is present, the imipenem is hydrolyzed, decreasing the reaction pH and leading to a color change from red or red-orange to light orange, dark yellow, or yellow. A negative reaction remains red-

orange. An invalid reaction changes from red or red-orange to orange. The image shows a negative reaction and a positive reaction for a KPC producer.

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One advantage to the CarbaNP test is that results are obtained in two hours or less. Positive tests are reported as soon as a color change occurs. This test can be performed on *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* spp. Limitations to this test are that some reagents require in-house preparation and have short shelf-lives; colors can be hard to interpret, invalidating results; and this test has low sensitivity for OXA-type carbapenemases, as shown.

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The mCIM test is a carbapenem inactivation test. The test isolate is suspended in Tryptic Soy Broth in a microcentrifuge tube, and a 10 microgram disk of meropenem is added to the suspension. The tube is incubated for 4 hours. A Mueller Hinton Agar plate is inoculated with a lawn of indicator *E. coli*, the meropenem disk is removed from the reaction tube and placed on the lawn, and the plate is incubated for 18-24 hours. If the test isolate is a carbapenemase producer, the enzyme will hydrolyze the meropenem within the disk, and there will be no or limited inhibition of *E. coli* growth. The reaction is positive if the zone of inhibition is 6-15 mm or colonies are present within a 16-18 mm zone. In the figure, the positive test shows no growth inhibition of *E. coli*. A negative reaction has a zone size that is greater than or equal to 19 mm, as shown. The test is indeterminate if the zone is 16-18 mm.

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The advantages to the mCIM test are that no special reagents or media are required, and it is highly sensitive and specific. Additionally, CLSI now endorses this test for *P. aeruginosa*. However, the mCIM requires overnight incubation, increasing time to results, and not all carbapenemase-producing *Enterobacteriaceae* are mCIM positive. For example, one isolate of OXA-232 producing *K. pneumoniae* was negative by this assay at some validation sites.

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Molecular testing can be performed on *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* spp. It determines the presence or type of carbapenemase. The only FDA-cleared test is the Cepheid Xpert Carba-R, which detects KPC, NDM, VIM, IMP-1 and OXA-48. Advantages to molecular testing are that it is rapid and can distinguish different carbapenemase types. However, false-negatives can occur if the test isolate has a carbapenemase not targeted by the assay, and it is more expensive because special reagents and equipment are required.

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If your lab is using the current carbapenem breakpoints, perform carbapenemase testing if requested by infection control or for epidemiological purposes. If your lab is using the old carbapenem breakpoints, carbapenemase testing is required in isolates that have an ertapenem MIC of 2 µg/ml or imipenem or meropenem MICs of 2-4 µg/ml. Tests endorsed by CLSI for carbapenemase testing are the Carba-NP, mCIM, and molecular testing. Remember that while the MHT is in current guidelines, it will no longer be recommended by CLSI. Challenges to carbapenemase testing are that ertapenem resistance is often due to AmpC or ESBL; *Proteus*, *Providencia*, and *Morganella* have intrinsically elevated imipenem MICs; and no phenotypic test detects all carbapenemase resistance mechanisms.

Slide 19: References

The references for this talk are listed here.

Slide 20: Disclosures

I have nothing to disclose.

Slide 21: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Laboratory detection of carbapenem resistance in Gram-negative bacteria.”

