

Laboratory Detection and Reporting of Carbapenem-Resistant Enterobacteriaceae (CRE)
CLSI Outreach Working Group
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The following script or explanations are provided to accompany the PowerPoint slides.

Slide #1-5 – no script

Slide #6

- The four-membered, nitrogen-containing beta-lactam ring at the core of the structure is key to the mode of action of beta-lactam antimicrobial agents.
- Beta-lactam antimicrobial agents target the penicillin-binding proteins (PBPs), a group of enzymes in the cell membrane which are involved in the cross-linking of the bacterial cell wall.
- The agents bind to these different PBPs and prevent the PBPs from performing their role in cell wall synthesis. With breakdown of the bacterial cell wall, the cell then dies due to osmotic instability or autolysis.

Slide #7

- There are 5 different classes of beta-lactam antimicrobial agents shown on this slide.
- The monobactam (aztreonam) does not have the bicyclic structure of the rest of the beta-lactams but still contains the four-membered beta-lactam ring highlighted in red. Aztreonam has no gram-positive activity, unlike the other beta-lactam agents listed.

Slide #8

- The gram-negative bacterial cell is composed of an outer membrane and a thin peptidoglycan cell wall separated by periplasmic space in-between, as well as an inner cell membrane.
- Gram-negative bacteria contain chromosomal and usually also plasmid DNA. Ribosomes are also present in the cytoplasm.
- The dotted blue box indicates the cross-section close-up which is outlined on the following slide.

Slide #9

- This slide represents a close-up of the bacterial cell wall, with the periplasmic space sandwiched between the thin peptidoglycan layer and the outer membrane.
- The penicillin-binding proteins (PBPs) which are involved in the cross-linking of the bacterial cell wall are marked in blue on the slide.
- Porins are present in the outer membrane and act like pores for passage of particular molecules, such as beta-lactam antimicrobial agents, to cross into the periplasmic space.

Slide #10

- When the beta-lactam antimicrobial agents pass into the periplasmic space, they have access to the PBPs which they target.
- The integrity of the cell wall then breaks down due to inability of the PBPs to be involved in maintenance and synthesis of the cell wall.

Slide #11

- Beta-lactamases are enzymes produced by bacteria that hydrolyze or inactivate the ring of the beta-lactam antimicrobial agent.

- Beta-lactamases are present in the periplasmic space. When the beta-lactam antimicrobial agents pass through the porins into the periplasmic space, before they reach the PBPs to exert their action, the beta-lactamases will cleave them.
- The bacteria have then acquired resistance to beta-lactams, the degree of which depends on the specificity of certain beta-lactamases for specific beta-lactam antimicrobial agents.
- Thus, the bacterial cell wall remains intact.

Slide #12

- Resistance to beta-lactam antimicrobial agents in gram-negatives may also be due to obstruction, or plugging, of the porin channel.
- Obstruction may be due to porin mutations or modifications that structurally do not allow certain molecules to pass through the porin.
- If the beta-lactams cannot pass through the porin channels, they cannot exert their effect on the PBPs; thus, the bacterial cell wall remains intact.
- Resistance may also develop through modification of an efflux pump. An efflux pump is essentially a channel that actively exports antimicrobial agents and other compounds out of the cell. By actively pumping out antimicrobial agents, the efflux pumps prevent the intracellular accumulation of antimicrobial agents necessary to exert their activity inside the cell.

Slide #13

- Beta-lactamases are enzymes produced by bacteria that hydrolyze, or inactivate, one or more beta-lactam antimicrobial agents.
- ESBLs are one category of beta-lactamase enzymes produced by gram-negative bacteria that hydrolyze, or inactivate, one or more of the extended-spectrum cephalosporins (also referred to as third- or fourth-generation cephalosporins) and/or the monobactam aztreonam.
- Carbapenemases are another category of beta-lactamase enzymes produced by gram-negative bacteria that hydrolyze, or inactivate, one or more carbapenem antimicrobial agents.

Slide #14

- Carbapenemases attack or hydrolyze the beta-lactam ring of the carbapenem molecule. Once this occurs, the carbapenem no longer has antibacterial activity.

Slide #15 - no script

Slide #16 - no script

Slide #17

- Beta-lactamases are divided into molecular classes based on their amino acid sequence and similarity to each other. This molecular classification is called the Ambler classification.
- Ambler Class A includes the ESBLs, of which there are hundreds. ESBLs are mostly inhibited by beta-lactamase inhibitors such as clavulanic acid, which has been a property utilized in the laboratory to confirm the presence of an ESBL. ESBLs are plasmid-mediated and therefore are easily transferrable among bacteria. Carbapenem resistance can occur if other resistance mechanisms co-exist with an ESBL, such as a porin modification.
- Ambler Class C includes AmpC beta-lactamases which unlike ESBLs are not inhibited by clavulanic acid. Carbapenem resistance can also occur when a porin modification coexists with an AmpC.

Slide #18

- Ambler Class A carbapenemases include KPCs and SMEs and are serine carbapenemases (so called because these enzymes utilize serine at their active sites). KPCs are found in *K. pneumoniae*, other Enterobacteriaceae, and non-Enterobacteriaceae and are plasmid-mediated.
- Ambler Class B carbapenemases are metallo-beta-lactamases, so named because these enzymes require zinc (a metal) for their activity. They are found in a variety of GNRs. MBLs include the New Delhi metallo-beta-lactamase (NDM) and others. They are inhibited by EDTA because EDTA chelates (binds) zinc, thus preventing the beta-lactamase from inactivating the beta-lactam.
- Ambler Class D carbapenemases are the OXA class. They are found in a variety of GNRs and only weakly hydrolyze carbapenems.

Slide #19 – no script

Slide #20

- KPCs are the most common carbapenemase in the US.
- KPCs generally produce a high level of enzyme, thus usually leading to high MICs to carbapenems in the resistant range.
- KPCs are found in a variety of bacterial species.
- The plasmid that carries the KPC gene often carries other beta-lactamase genes (such as ESBLs) and genes that confer resistance to other antimicrobial agent classes (such as aminoglycosides).

Slide #21

- NDMs are MBLs which are frequently encountered in many parts of the world now but originated in India and Pakistan.
- As an MBL, the NDMs require zinc for activity.
- NDMs are mostly found in *K. pneumoniae* and *E. coli* but are spreading to other GNRs. The bla_{NDM} gene is highly mobile and easily transferable between organisms even of different genera.
- The MBL group also includes IMPs and VIMs.
- Refer to Dortet L et al. Biomed Res Int. 2014; Article ID 249856 for a recent review of NDMs.

Slide #22

- OXAs were first described in *Acinetobacter baumannii* but are now carried by many GNRs including Enterobacteriaceae.
- OXA-48 was first reported in Turkey in *K. pneumoniae* and *E. coli* and is now spreading across the globe.
- Some OXA-48-like variants recently described include OXA-181 and OXA-232.
- In general, OXAs are weak hydrolyzers of carbapenems and cephalosporins, but OXA-48 has been shown to have greater ability to hydrolyze carbapenems as compared to the other OXAs.

Slide #23

- The number of MBL-producing GNRs in the U.S. reported by the CDC from 2007 to 2015 is shown on the graph on this slide (as of April 27, 2016).
- The majority of MBLs are NDM (blue bars).
- There were high numbers of NDMs reported in 2012 and 2013, driven by small and large outbreaks, respectively.
- Reporting to the CDC is voluntary, so this likely represents an underestimation of true numbers of NDM isolates.

Slide #24

- As evidence of the propensity of many carbapenemases to spread easily, reports have demonstrated several different species of bacteria containing the KPC gene in a single patient, as well as a single patient with isolates that produce several different carbapenemases.

Slide #25 – no script

Slide #26

CLSI and FDA set and revise breakpoints in the U.S.

- Carbapenem breakpoints for Enterobacteriaceae were updated within the past few years by both the CLSI and FDA.
- Current FDA breakpoints may not be included on the commercial system that a specific laboratory uses.
- It is thus essential that each laboratory check with the manufacturer of their system to ensure that the laboratory is aware which breakpoints are being used.
- If the laboratory wishes to implement breakpoints other than those that are FDA-cleared for that particular system, they must perform a verification for those breakpoints.

Slide #27

- The current CLSI breakpoints for Enterobacteriaceae are listed in Table 2A of the M100S 26th ed published in January 2016.

Slide #28

- The current FDA breakpoints can be found by searching the link on this slide (or follow the Google search criteria above).
- The following slides direct you as to how to complete the search for FDA breakpoints.

Slide #29 – no script

Slide #30 – no script

Slide #31

- The FDA breakpoints are listed in the Microbiology subsection of the antimicrobial agent label (also known as prescribing information or package insert).

Slide #32

- There are several references that discuss recommendations for verification of ASTs. A recent addition is CLSI M52: Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems, 1st Edition, 2015.

Slide #33

- AST verification requirements are regulated by CLIA.
- Laboratories must perform verification studies when using disk diffusion or commercial systems prior to use for testing patient isolates.
- If a commercial system is not FDA cleared for the current CLSI/FDA breakpoints and a laboratory wishes to implement the current CLSI/FDA breakpoints, a verification study must be performed even if the test system was verified in their laboratory previously with the old breakpoints.

Slide #34

- This schematic reflects an option for the verification process. There are no explicit rules for how to perform the verification study; however, the new method or new breakpoints must be verified by evaluating both accuracy and reproducibility. Review of materials available from the manufacturer and others is often useful to learn more about performance of the new method.

Slide #35

- This slide lists one option for the in-house testing of clinical isolates as part of the verification of the current CLSI/FDA breakpoints on a commercial automated AST. The manufacturer of the AST system has not yet updated their system with the current CLSI/FDA breakpoints.
- Laboratory A obtains a “reference set” of 30 isolates from laboratory B.
- Laboratory B provides meropenem MIC and S, I, and R results for each isolate sent to Laboratory A. Laboratory B’s AST system is FDA cleared with the current meropenem breakpoints and was previously verified by Laboratory B.
- Laboratory A tests the 30 isolates on their automated AST system. The meropenem MICs and S, I, R results obtained on each isolate are compared to those provided from Laboratory B.
- As part of the in-house verification study, run to run and within run reproducibility must also be demonstrated. This can be done by testing the routine ATCC QC strains.

Slide #36

- This example shows a summary of results from the verification study described on the previous slide.
- Essential, Categorical, and Major Error rates are all within acceptable limits. However, the Very Major Error (VME) or false susceptible error rate for all isolates is 11% which is unacceptable, despite the fact that only 9 meropenem resistant isolates were tested.
- The laboratory should repeat testing of the problem isolate and 2 isolates that did not show problems. They may test several additional meropenem-R *K. pneumoniae* isolates. Since the results for the “reference set” were not obtained from a CLSI reference method, another option would be to send the isolate in question to a reference laboratory that utilizes the CLSI MIC reference broth microdilution method to arbitrate the discordant result.
- One possibility for the error is that the isolate may have lost the resistance plasmid during repeat sub culturing; therefore, laboratories should avoid excessive sub culturing of isolates to avoid plasmid loss.

Slide #37 – no script

Slide #38

- The current CLSI MIC and disk diffusion breakpoints for Enterobacteriaceae are listed in M100S 26th ed Table 2A.

Slide #39

- Table 2A also states the drug dosage regimens upon which breakpoints are currently based.
- Although these are fairly common antimicrobial agent dosages, each laboratory should check with their institution to determine whether these are the standard dosing regimens used at their institution.

- If this is not the case, the laboratory should discuss breakpoints and corresponding dosing regimens on which breakpoints are based with the institution's pharmacists and infection control to determine appropriate utilization of breakpoints.

Slide #40

- There are limited treatment options for patients with infections due to CREs. Therefore, there is a statement in Table 2A of M100 specifying that clinicians may wish to use maximum recommended doses of carbapenems or possibly prolonged intravenous infusion regimens for patients who have infections due to organisms with carbapenem MICs in the intermediate range. The pharmacokinetic principle behind this is that, through the use of these alternative regimens, higher antimicrobial agent levels may be maintained in the patients for longer periods of time.
- References for this suggestion (which are also listed in M100) include:
 1. Perrott J et al. *Ann Pharmacother.* 2010;44:557-564.
 2. Cirillo I et al. *J Clin Pharmacol.* 2009;49:798-806.
 3. Sakka SG et al. *Antimicrob Agents Chemother.* 2007;51:3304-3310.
 4. Peleg AY and Hooper DC. *N Engl J Med.* 2010;362:1804-1813.

Slide #41

- Current and old CLSI MIC breakpoints are compared in this table.
- The current breakpoints for ertapenem, imipenem, and meropenem are lower than the old breakpoints.

Slide #42 – no script

Slide #43

- Some suggest that all CRE should be tested for carbapenemase production since carbapenemase-producing CRE are much more worrisome than non-carbapenemase-producing CRE and present more challenges to both patient care and infection control.

Slide #44

- This table is modified from Tables 3B and 3C in M100S 26th ed. It provides guidance on the appropriateness and use of various tests which can be used to detect carbapenemases.

Slide #45

- Table 3B in M100S 26th ed outlines the procedure for MHT testing and reporting.

Slide #46

- Table 3C in M100S 26th ed outlines the procedure for CarbaNP testing and reporting.
- The principle of the test is based on hydrolysis of imipenem to an acidic byproduct in the presence of a carbapenemase. Hydrolysis is detected by a change in a pH indicator (phenol red) from red to yellow or yellow-orange.
- The test is rapid with results (both negative and positive) available in two hours.
- The CLSI-described method is microtube-based and requires in-house preparation of several reagents. There are variations of this method currently under evaluation and a commercial test based on the principle of the CarbaNP test may be available in the near future.

Slide #47

- Here are examples of CarbaNP testing for various isolates with the carbapenemase type indicated.
- Yellow, dark yellow, or light orange colors are considered positive for carbapenemase production, while red or red-orange are considered negative. An orange color is considered invalid.
- In the examples above, the positive reactions are for the carbapenemases KPC, NDM, IMP, VIM, and SME. The OXA-181 (an OXA-48-like variant) is negative. The OXA-48 tube would likely be considered invalid.
- For isolates with invalid results, labs should check the reagents and repeat the test. If the test is continued invalid, molecular assays should be performed.

Slide #48

- Here are examples of the MHT for various isolates with the carbapenemase type indicated.
- Enhanced growth of the test organism or QC strain at the intersection of the streak and the zone of inhibition is considered a positive result.
- In the examples above, the positive isolates include KPC, OXA-232 and SME. The NDM is considered a false negative, because NDM is a carbapenemase but is not detected by the MHT.
- NDMs are known to be missed (falsely negative) by the MHT (reference Mochon AB et al. J Clin Microbiol 2011;49:1667-1670).

Slide #49

- The MHT has poor specificity, in that *Enterobacter* spp. which harbor AmpC plus porin losses can also lead to false positive results. Here you can see a slight indentation at the intersection of the isolate streak and zone of inhibition circled in red.

Slide #50

- CIM is a newer phenotypic test for carbapenemase activity.
- The isolate suspicious for carbapenemase production is incubated with a meropenem disk in a small amount of water or broth. If the isolate produces carbapenemase, it will hydrolyze meropenem in the disk.
- When the disk is removed from the reaction tube and used for a routine disk diffusion test with the standard *E. coli* ATCC 25922 QC strain, there will be no zone or a small zone around the disk.
- If no carbapenemase is produced, the size of the meropenem zone will be similar to that of a control meropenem disk that was not exposed to any test isolates.
- CLSI is evaluating the CIM test as it is easy to perform and appears to have some advantages over the other phenotypic tests for carbapenemases that have been described (e.g., MHT, CarbaNP tests).

Slide #51

- The Neo-Rapid CARB screen kit from Key Scientific is a phenotypic carbapenemase detection kit which is research use only at this time.
- The kit can be used with Enterobacteriaceae and *Pseudomonas aeruginosa*. The principle is similar to the CarbaNP assay and the test reagents are contained in tablets. Test results are available within 2 hours.

Slide #52

- There are a variety of molecular assays currently available for testing for carbapenemases.

- BioFire and Nanosphere can test for the presence of certain carbapenemases from blood culture broths positive for Enterobacteriaceae.
- Cepheid is FDA cleared for use with isolated colonies.
- Check with the manufacturers to determine which products can be used for direct detection from primary specimens, positive blood cultures and/or isolated colonies.
- Many of these are RUO at this time.

Slide #53 – no script

Slide #54

- In this example of an isolate of *K. pneumoniae* from blood, both ertapenem and meropenem are resistant.
- This is an example of a CRE based on carbapenem MICs in the resistant range. When results such as these are obtained and confirmed to be accurate, they can be reported.
- Again, carbapenemase testing is not necessary when reporting results to guide patient treatment decisions.

Slide #55

- There are limited treatment options for CRE, including polymyxins (colistin and polymyxin B), tigecycline, minocycline, aminoglycosides (excluding tobramycin), fosfomycin (only for urinary tract infections), and the recently FDA-approved ceftazidime-avibactam (only for Ambler Molecular Class A carbapenemases which include KPCs).
- Usually a combination of the above antimicrobial agents (most commonly a polymyxin with another antimicrobial agent) is used.

Slide #56

- Tigecycline generally is not used clinically for urinary tract infections due to poor excretion in the urine but may be used as an option in some difficult-to-treat cases. Tigecycline should not be reported for *Proteus/Providencia/Morganella* since isolates belonging to these genera are intrinsically resistant to this antimicrobial agent.
- Although there are no CLSI breakpoints for polymyxins and Enterobacteriaceae, there are EUCAST breakpoints as shown on this slide.
- A parenteral form of fosfomycin is available in some parts of the world, but only oral fosfomycin is currently available in the USA and the FDA clinical indication is only for urinary tract infections. Although CLSI breakpoints are only for *E. coli*, EUCAST breakpoints apply to all Enterobacteriaceae. Fosfomycin is sometimes considered in combination therapy for infections due to CRE.
- Ceftazidime-avibactam is a relatively new antimicrobial agent and active against some CRE, particularly KPC producers. This agent has no activity against isolates that produce metallo beta-lactamase such as NDM. There are currently no CLSI breakpoints for ceftazidime-avibactam; however, there are FDA breakpoints for this antimicrobial agent.

Slide #57

- There is limited availability of methods to test several supplemental agents that might be useful for CRE. Just because a test is “available” does not mean it is FDA cleared.
- Laboratories should follow their institution’s protocols when using diagnostic tests that are not FDA cleared.

Slide #58 – no script

