PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Carbapenemase Screening & Confirmation

Department of Microbiology

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1.0 Clinical Significance

Carbapenems, including imipenem, meropenem, and ertapenem, are often used to treat infections caused by multi-drug resistant *Enterobacteriaceae*. An emerging mechanism of carbapenem resistance is due to the production of carbapenemase enzyme. Carbapenemase production occurs most commonly in *K. pneumoniae*, but it has also been reported in other species of *Enterobacteriaceae*. The enzyme confers resistance to all β-lactam agents including penicillins, cephalosporins, monobactams, and carbapenems.

2.0 Principle

Detection of carbapenemase producing *Enterobacteriaceae* (CPE) is complicated because some isolates demonstrate elevated but susceptible, carbapenem MICs. CLSI has published guidelines for detection of isolates producing carbapenemases (CLSI document M100). Ertapenem has proven to be the most sensitive screening agent for detecting carbapenemase-producing isolates. Meropenem may also be used. CPE may test intermediate, resistant, or susceptible to a carbapenem but demonstrate reduced susceptibility, either by disk diffusion or MIC testing. In addition to carbapenem susceptibility, screening also includes resistance to one or more third generation cephalosporins (ceftazidime or ceftriaxone). The BD Phoenix has been configured to screen for CPE based on an elevated ertapenem MIC (≥ 2) and resistance to either ceftazidime or ceftriaxone. Isolates that are screen-positive should be confirmed by performing a phenotypic test for carbapenemase activity, the Modified Hodge Test (MHT). Carbapenemase production is detected by the MHT when the isolate produces the enzyme and allows growth of a carbapenem susceptible strain (*E. coli* ATCC 25922) towards a carbapenem disk.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiar with and trained to perform and interpret automated and manual susceptibility testing. Testing includes but is not limited to: instrument maintenance, performance checks, basic troubleshooting, Quality Control testing, and record keeping.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

This procedure may expose you to:

- Multi-drug-resistant organisms

To perform this procedure, you must use:

- Laboratory Coat – must be worn when handling cultures.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

5.0 Specimen Requirements

Inoculum should be prepared from overnight growth of four or five isolated colonies of similar colony morphology.

6.0 Materials

6.1 Equipment and Testing System

- BD Phoenix™ , BD Phoenix™ AP, and associated consumables
- Epicenter™ software

- Incubator set at 35 ± 2°C with ambient air

6.2 General

- Sterile swabs
- BBL™ Prompt™ Inoculation System
- Inoculating loop

6.3 Media

- Mueller Hinton Agar
- TSA II with 5% blood agar

6.4 Disks

- Ceftriaxone, 30 µg
- Ceftazidime, 30 µg
- Meropenem 10 µg disks (10-µg ertapenem disks may also be used)

6.5 Control Strains

1. *E. coli* ATCC 25922
2. *K. pneumoniae* ATCC BAA-1705
3. *K. pneumoniae* ATCC BAA-1706

7.0 Procedure

7.1 Automated Screening Using the Phoenix Instrument

Refer to the Phoenix Test Procedure for instructions for performing isolate testing on the Phoenix system. Isolates that yield an ertapenem MIC value ≥ 2 µg/mL and are resistant to a third generation cephalosporin are screen-positive for carbapenemase. Epicenter rules will suppress any susceptible penicillin cephalosporin, and carbapenem results until the confirmatory test results are available and the user manually enters the results in Epicenter (refer to the Epicenter AST Management Procedure). Before proceeding with confirmatory testing, the growth on the Phoenix purity plate should be stained to confirm the purity of the inoculum.

7.2 Manual Screening by Disk Diffusion

1. Inoculate 0.5 McFarland suspension of the isolate on Mueller Hinton agar, and apply the meropenem disk. Incubate the plate overnight at 35 ± 2°C, as outlined in the Kirby Bauer susceptibility testing procedure.
2. On the following day, examine the zone size for the meropenem 10 µg disk. This zone size may be used to initially screen for possible CPE.
3. If the meropenem zone measures ≤ 21 mm and the isolate is resistant to a third generation cephalosporin, confirmatory phenotypic testing should be performed to rule out CPE. Do not report any beta-lactam results with a value of susceptible or intermediate.

7.3 Confirmatory Testing with the Modified Hodge Test

1. Prepare a 0.5 McFarland suspension of *E. coli* ATCC 25922.
2. Dilute the suspension of *E. coli* ATCC 25922 1:10 with sterile saline.
3. Using a sterile swab, streak the diluted suspension on the surface of a MHA plate for confluent growth.
4. Allow the agar surface to dry briefly, and place a 10-µg meropenem disk in the center of the plate.
5. Using a sterile 10-µl inoculation loop, pick 3-5 colonies of the test or QC organism grown overnight on a blood agar plate, and inoculate in a straight line out from the edge of the disk. The streak should be at least 20-25 mm in length. Three organisms may be tested with each disk simultaneously on a small MHA plate. Be sure to label the plate carefully so that the test and QC isolates may be correctly identified later.
6. Incubate the plate for 16-20 h at 35 ± 2°C; ambient air.

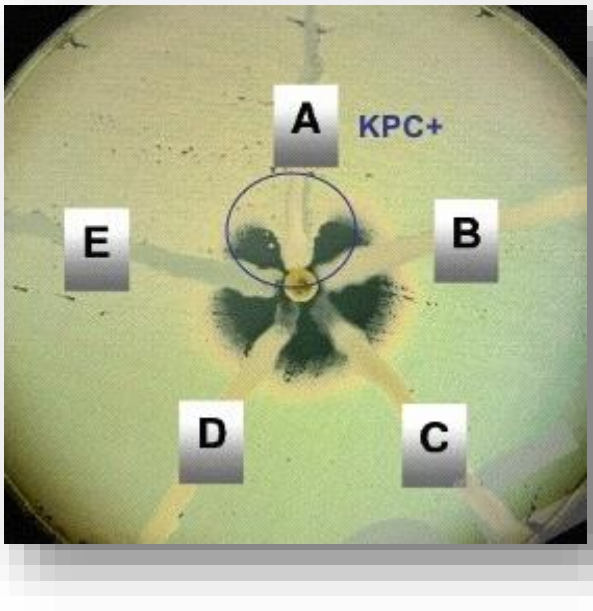
8.0 Interpretation & Reporting of Results

8.1 Positive Screen

If the screen test is positive, and the inoculum purity has been confirmed, attach comment **"Isolate may be resistant to all carbapenems, confirmatory testing pending." [KPC]**

8.2 Positive MHT

After overnight incubation, examine the MHT. If the patient isolate produces carbapenemase, there will be a distortion at the intersection of the isolate streak and the zone of inhibition for the *E. coli* ATCC 25922. This is due to the inactivation of meropenem by the carbapenemase enzyme produced by the test isolate which allows the *E. coli* ATCC 25922 strain to grow along the streak of the test isolate (see isolate A in the figure below).



Confirm all positive MHT results on Rounds. Report all beta-lactam agents as resistant and attach comment, **"This organism produces carbapenemase. Resistant microorganism. Contact precautions required." [KPCPOS]**

8.3 Negative MHT

If the patient isolate does not produce carbapenemase, the zone of inhibition will not be distorted (see examples B, C, D, and E in the figure above). Consult Rounds if result looks ambiguous. Interpret and report carbapenem and other β-lactam MICs using current CLSI interpretive criteria.

9.0 Quality Control & Quality Assurance

Test positive and negative QC organisms each day of testing. Enter QC results into LIS.

- Positive MHT: *K. pneumoniae* ATCC BAA-1705
- Negative MHT: *K. pneumoniae* ATCC BAA-1706

10.0 Limitations

1. Some isolates show a slight indentation but do not produce carbapenemase.

11.0 References

1. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement.* CLSI document M100-S23. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
2. Anderson, K. F., D. R. Lonsway, J. K. Rasheed, J. Biddle, B. Jensen, L. K. McDougal, R. B. Carey, A. Thompson, S. Stocker, B. Limbago, and J. B. Patel. 2007. Evaluation of Methods to Identify the *Klebsiella pneumoniae* Carbapenemase in *Enterobacteriaceae*. J. Clin. Microbiol. 45:2723-2725.
3. Moland, E. S., K. Soo-Young, S. G. Hong, K. S. Thomson. 2008. Newer β-Lactamases: Clinical and Laboratory Implications, Part II. Clin. Microbiol. Newsletter. Vol. 30, No. 11.

12.0 Document Control History

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Updates and Revisions: 05/01/2014 Updated to include screening with the Phoenix system. 11/14/14 The KPCP comment was updated (shortened per AR).