Detection of Carbapenem-Resistant or Carbepenemase-Producing Enterobacterales from Rectal Swabs [Microbiology Manual]		Page **1** of **5**
Doc#: MIC 990	**Section: MICROBIOLOGY**	**Effective Date:** September 9, 2019

SCOPE: This policy applies to UPMC Hanover.

KEYWORDS: Rectal swabs, Screen for CRE

PURPOSE:
To identify patients colonized with carbapenem-resistant or carbapenamase-producing Enterobacterales
in the intestinal tract. Patients who grow these organisms should be placed on Contact Precautions to prevent transmission of the resistant bacteria.
Carbapenem-resistant Enterobacterales (CRE) are usually resistant to all β-lactam agents as well as most other classes of antimicrobial agents. The treatment options for patients infected with CRE are very limited. Healthcare-associated outbreaks of CRE have been reported. Patients colonized with CRE are thought to be a source of transmission in the healthcare setting. Identifying patients who are colonized with CRE and placing these patients in isolation precautions may be an important step in preventing transmission.
Carbapenem resistance in Enterobacterales occurs when an isolate acquires a carbapenemase or when an isolate produces an Extended-spectrum cephalosporinase, such as an AmpC-type β-lactamase, in combination with porin loss. In the US, the most common mechanism of carbapenem resistance is the *Klebsiella pneumoniae* carbapenemase (KPC). Carbapenem resistance and carbapenemase-production in any species of Enterobacterales is an infection control concern.

POLICY:
It is at the discretion of the Infection Prevention Department to perform this screen, as required.

EQUIPMENT/MATERIALS:
Vortex, 35±2ºC, ambient air incubator, 100 µL Calibrated pipettes, Sterile loops, Forceps, BSC Level 2, Ertapenem disks, MAC agar plates, calipers.

SPECIMEN: Nursing will collect a rectal swab from the perianal area from the patient to be screened. Single white or double yellow swabs with liquid Stuart's are acceptable.

QUALITY CONTROL:

Carbepenem disks used in this procedure are quality control tested with the patient specimens. The 10 µg/mL ertapenem disk is tested against E.coli ATCC 25922.

PROCEDURE:

1	Aseptically, place one 10-µg ertapenem disc in 5 ml trypticase soy broth (TSB). .Place rectal swab into tube. Immediately incubate the broth with the swab. Incubate overnight at 35 + 2 ºC, ambient air.
2	Vortex and subculture 100 µl of the incubated broth culture onto a MAC agar plate. (It may be helpful to place a 10-µg ertapenem in first quadrant of the MAC plate and use the CLSI disk diffusion screening criteria to identify potential carbapenemase producing isolates (i.e., an ertapenem disk zone < 21 mm). These will need a mCIM or identification. Streak for isolation onto another MAC plate. Incubate overnight at 35 +2 °C, ambient air.
3	Examine the MacConkey agar for lactose-fermenting (pink-red) colonies. More than one colony morphology may represent different species of Enterobacterales (see procedure note #1). It may be necessary to subculture representative colonies of each morphology type to a non-selective media for isolation and/or for susceptibility testing. Screen representative isolated colonies using a phenotypic test for carbapenemase production, such as MCIM, or test for carbapenem susceptibility using a standardized method and follow the CLSI guidelines for identification of carbapenemase-producing Enterobacterales (see procedure note 2).
4	For CRE and/or MCIM isolates, perform species-level identification.

INTERPRETATION: Report all cultures that are positive for CRE or carbapenemase-producing Enterobacterales to Infection Prevention. Contact Precautions should be implemented for all patients with positive cultures for CRE or carbapenemase-producing Enterobacterales.

QUALITY ASSURANCE:

The ability to recover CRE using this procedure could be assessed as follows: Inoculate 5 mL of TSB containing the 10-µg carbapenem disk with a swab that was used to sample a known CRE-negative stool

specimen. Also inoculate the TSB with a 0.5 mL of a 1 x 10^5 CFU/mL suspension of a known carbapenemase-producing isolate (e.g., *Klebsiella pneumoniae* ATCC BAA-1705), (See procedural note 3 for suspension preparation). Proceed with Step 2 of the procedure. The carbapenemase-producing *Klebsiella pneumonia* should be recovered on the MacConkey agar. To test for specificity of the procedure, use a carbapenem susceptible *Klebsiella pneumoniae*, (e.g., ATCC 700603 or BAA-1706) and follow the same steps. The carbapenem susceptible *Klebsiella pneumoniae* isolate should not grow on the MacConkey agar.

METHOD LIMITATIONS:

1. Patients may be colonized with CRE or carbapenemase-producing Enterobacterales at a concentration that is not detectable by this method. Studies described by Landman et al. and studies performed at CDC suggest that the lower limit of detection is between ranges from 1 x 10^2 CFU/mL to 1x10^6 CFU/mL.

2. Non-fermenting gram-negative bacilli with intrinsic mechanisms of carbapenem-resistance, such as *Acinetobacter* species and *Pseudomonas aeruginosa*, will be detected on the MacConkey agar. These isolates should be identified as non-lactose fermenters on the MacConkey agar and therefore would not be picked for characterization. If carbapenem-resistant non-fermenters are present at high concentration, they could overgrow CRE or carbapenemase-producing Enterobacterales on the media and prevent detection of colonization.

3. Enterobacterales can be resistant to carbapenems by mechanisms other than carbapenemase, the most common of which is expression of an extended-spectrum cephalosporinase, such as an AmpC-type enzyme or an ESBL, combined with porin loss. These isolates will also grow on the MacConkey agar and be identified as carbapenem-intermediate or resistant by standard susceptibility testing but these isolates are negative by MCIM. For isolates that test intermediate or resistant to carbapenems, it may not be necessary to distinguish between those mechanisms of resistance because all carbapenem-non-susceptible Enterobacterales produce a broad-spectrum β-lactamase, and are therefore an infection control concern.

PROCEDURE NOTES:

1.Carbapenemases are known to exist in several different species of gram-negative bacilli including

species of Enterobacterales and *Pseudomonas aeruginosa.* However, carbapenemases are more common in lactose-fermenting species of Enterobacterales (e.g., *Klebsiella pneumoniae* and *E. coli*) than in non-lactose fermenting Enterobacterales (e.g., *Serratia marcescens* and some *Enterobacter* species) and *P. aeruginosa*. In this procedure, it is suggested that laboratories focus their efforts on detection of resistant lactose-fermenting bacteria to reduce workload. Healthcare facilities that have identified clinical infections with carbapenemase-producing non-lactose fermenting gram-negative species should consider altering this procedure to include characterization of colonies with a morphology that is consistent with those species.

2. The exact procedure for confirmation of CRE or carbapenemase-production should be laboratory specific and chosen based upon laboratory workflow and the types of isolates causing clinical infections in the patient population served. It may be helpful to refer to CLSI guidelines for identification of carbapenemase production in isolates that test susceptible to carbapenems in document M100.

3. A 1 x 10^4 CFU/mL suspension of the known carbapenem-resistant or the carbapenem-susceptible isolates could be prepared as follows: Dilute 0.1 mL of a 0.5 McFarland standard suspension (equals approximately 1 x 10^8 CFU/mL) in 9.9 mL sterile water or saline for a 1:100 dilution. From the 1:100 dilution, dilute 1.0 mL in 9.0 mL water or saline for a 1:1000 dilution. Add 0.5 mL of the 1:1000 dilution (equals approximately 1 x 10^5 CFU/mL), suspension to the 5 mL TSB for a final concentration of approximately 1 x 10^6/mL.

REFERENCE

Department of Health and Human Resources, Centers for Disease Control and Prevention, *Laboratory Protocol for Detection of Carbapenem-Resistant of Carbapenemase-Producing Klebsiella spp. and E. coli from Rectal Swab.*www.cdc.gov/hai/pdfs/labsettings/klebsiella_or_ecoli.pdf

Clinical and Laboratory Standards institute/CLSI. 2013. *Performance Standards for Antimicrobial Susceptibility Testing.* Twenty-third Informational Suplement. M100-S23. CLSI, Wayne, PA

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