Laboratory Protocol for Detection of Carbapenem-Resistant or Carbapenemase-Producing, *Klebsiella* spp. and *E. coli* from Rectal Swabs

Purpose

To identify patients colonized with carbapenem-resistant or carbapenamase-producing Enterobacteriaceae in the intestinal tract. Patients who grow these organisms should be placed on Contact Precautions (5) to prevent transmission of the resistant bacteria. The procedure described below is a modification of the procedure described by Landman et al. (4). See the procedural notes for steps in the procedure which can be modified.

Background

Carbapenem-resistant Enterobacteriaceae (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 (1). Identifying patients who are colonized with CRE and placing these patients in isolation precautions may be an important step in preventing transmission (6).

Carbapenem resistance in Enterobacteriaceae 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 United States, the most common mechanism of carbapenem resistance is the *Klebsiella pneumoniae* carbapenemase (KPC).

Detection of carbapenemase production is complicated because some carbapenemase-producing isolates demonstrate elevated but susceptible, carbapenem MICs. CLSI has published guidelines for detection of isolates producing carbapenemases (CLSI document M100) (2). For isolates that test susceptible to a carbapenem but demonstrate reduced susceptibility either by disk diffusion or MIC testing, performing a phenotypic test for carbapenemase activity, the Modified Hodge Test (MHT), is recommended.

Carbapenem resistance and carbapenemase-production in any species of Enterobacteriaceae is an infection control concern. However, the methodology described here focuses on the detection of carbapenem-resistant or carbapenemase-producing *Klebsiella spp* and *E. coli* as this facilitates performance of the test in the microbiology laboratory and, more importantly, because these organisms, especially *Klebsiella* spp. represent the vast majority of CRE encountered in the United States (3).

Reagents
5 ml Trypticase Soy Broth
10-µg carbapenem disks
MacConkey agar

Equipment
Vortex
35 ± ºC, ambient air

Supplies
100 µl calibrated pipettes
Forceps
Sterile loops

Specimen
Rectal swab or perianal swab specimen in suitable transport media

Special safety precautions
Biosaftey Level 2

Quality Control (QC)
The carbapenem disks that are used in this procedure should be quality control tested using disk diffusion methods and quality control strains as described in the CLSI guideline documents M2 and M100 (2,(2). For example, if the10-µg/mL meropenem disk is used in this procedure, test *E. coli* ATCC 25922 by the disk diffusion method using meropenem disks from the same lot. An acceptable control test will yield a zone size between 28-34 mm. Follow CLSI guidelines for frequency of disk QC testing and corrective action if results are out of range.

Procedure

Step 1 Day One	Aseptically, place one 10-µg ertapenem or meropenem disc in 5 ml trypticase soy broth (TSB) (see procedure note 1) Immediately inoculate the broth with the rectal culture swab Incubate overnight at 35 ± 2ºC, ambient air
Step 2 Day Two	Vortex and subculture 100 µl of the incubated broth culture onto a MacConkey agar plate (see procedure note 2) Streak for isolation Incubate overnight at 35 ± 2ºC, ambient air
Step3 Day Three	Examine the MacConkey agar for lactose-fermenting (pink-red) colonies. More than one colony morphology may represent different species of Enterobacteriaceae (see procedure note 3). 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 the Modified Hodge Test (MHT) or test for carbapenem susceptibility using a standardized method and follow the CLSI guidelines for identification of carbapenemase-producing Enterobacteriaceae (see procedure note 4).
Step 4 Day Four	For CRE and/or MHT-positive isolates, perform species-level identification.

Interpretation/Results

Report all cultures that are positive for CRE or carbapenemase-producing Enterobacteriaceae to the appropriate infection control personnel. Contact Precautions should be implemented for all patients with positive cultures for CRE or carbapenemase-producing Enterobacteriaceae.

Quality assurance

The ability to recover CRE using this procedure could be assessed as follows: Inoculate 5mL of TSB containing the 10-ug carbapenem disk with a swab that was used to sample a known CRE-negative stool specimen. In addition, inoculate the TSB with 0.5 mL of a 1 x 10^5 CFU/mL suspension of a known carbapenemase-producing isolate (e.g., *K. pneumoniae* ATCC BAA-1705), (see procedural note 5 for suspension preparation) Proceed with Step 2 of the procedure. The carbapenemase-producing *K. pneumoniae* should be recovered on the MacConkey agar.

To test for specificity of the procedure, use a carbapenem susceptible *Klebsiella pneumoniae,* (e.g. ATCC 700603) and follow the same steps. The carbapenem susceptible *K. pneumoniae* isolate should not grow on the MacConkey agar.

Method limitations

1. Patients may be colonized with CRE or carbapenemase-producing Enterobacteriaceae at a concentration that is not detectable by this method. Studies described by Landman et al. and studies performed at the CDC suggest that the lower limit of detection is between ranges from 1 x 10^2 CFU/ mL to 1 x 10^6 CFU/ mL (4).

2. Non-fermenting gram-negative bacilli with intrinsic mechanisms of carbapenem-resistance, such as *Acinetobacter* spp. and *P. 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 Enterobacteriaceae on the media and prevent detection of colonization.

3. Enterobacteriaceae can be resistant to carbapenems by mechanisms other than a 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 the MHT. For isolates that test intermediate or resistant to carbapenems, it may not be necessary to distinguish between these mechanisms of resistance because all carbapenem-nonsusceptible Enterobacteriaceae produce a broad-spectrum β-lactamase, and are therefore an infection control concern. Implementing Contact Precautions for patients colonized with these bacteria would be appropriate. Laboratories may choose to test carbapenem-intermediate or resistant isolates with the MHT to identify carbapenemase-production for epidemiological purposes.

Procedure notes

1. The procedure described by Landman et al. (4) describes using a 10-µg imipenem disk for step 1. However, there are species of Enterobacteriaceae which have intrinsic mechanisms of resistance to imipenem other than a carbapenemase (See CLSI document M100, Appendix G)(2). Therefore, ertapenem or meropenem may provide more specific selection for acquired carbapenem resistance in Enterobacteriaceae.

2. Some laboratories performing cultures for isolation of CRE from rectal specimens place a 10-µg carbapenem disk in the first quadrant of the MacConkey plate. Only colonies growing "close" to the carbapenem disk are picked for characterization. No clear criteria for "close" have been established. However, it may be helpful to use either a meropenem or ertapenem disk and then apply the CLSI disk diffusion screening criteria to identify potential carbapenemase-producing isolates (i.e., an ertapenem or meropenem disk zone ≤ 21 mm). Note: These zone size criteria

were validated for standardized disk diffusion testing methods as described in CLSI document M2.

3. Carbapenemases are known to exist in several different species of gram-negative bacilli including species of Enterobacteriaceae and *Pseudomonas aeruginosa*. However, carbapenemases are more common in lactose-fermenting species of Enterobacteriaceae (e.g., *K. pneumoniae* and *E. coli*) than in non-lactose fermenting Enterobacteriaceae (e.g. *Serratia marcescens* and some *Enterobacter* spp.) 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.

4. 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 the CLSI guidelines for identification of carbapenemase production in isolates that test susceptible to carbapenems in document M100 (2).

5. A 1 x 10^4 CFU/mL suspension of the known carbapenem-resistant or 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, 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 10^4 CFU/mL.

References

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