Modified Carbapenemase Inactivation Method (mCIM) for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*

Purpose

To determine the Enterobacterales and *P. aeruginosa* isolates that are suspicious for carbapenemase production based on not susceptible to one or more carbapenems using the current breakpoints.

Materials

1. Overnight culture of bacterial isolate on non-selective media (Tryptic soy agar (TSA), Sheep blood agar (SBA))
2. Tryptic soy broth (TSB) (2 mL aliquots)
3. 1-µL and 10-µL loops
4. Mueller-Hinton agar (MHA)
5. Mueller-Hinton broth (MHB)
6. Vortex mixer
7. Sterile cotton swab
8. Sterile forcep
9. 0.5 McFarland standard
10. Nephelometer
11. 35°C ambient-air incubator
12. Meropenem disks (10 µg)
13. Indicator strain: Meropenem-susceptible indicator strain – *E. coli* (ATCC 25922)
14. Quality control strains
    - *K. pneumoniae* ATCC BAA-1705™ KPC positive, mCIM positive
    - *K. pneumoniae* ATCC BAA-1706™ Carbapenemase negative, mCIM negative

Procedure

1. For each isolate to be tested, emulsify a **1-µL loopful of bacteria for Enterobacterales or 10-µL loopful of bacteria for *P. aeruginosa*** from an overnight blood agar plate in 2 mL TSB.

2. Vortex for 10–15 secs.

3. Add a 10-µg meropenem disk to each tube using sterile forceps or a single disk dispenser. Ensure the entire disk is immersed in the suspension.

4. Incubate at 35°C ± 2°C in ambient air for 4 hrs ± 15 mins.

5. **Just before or immediately following completion of the TSB-meropenem disk suspension incubation**, prepare a 0.5 McFarland suspension (using the colony suspension method) of *E. coli* ATCC 25922 in MHB.

6. Inoculate an MHA plate with *E. coli* ATCC 25922 as for the routine disk diffusion procedure making sure the inoculum suspension preparation and MHA plate inoculation steps are each completed within 15 mins. Allow the plates to dry for 3–10 mins before adding the meropenem disks.

7. Remove the meropenem disk from each TSB-meropenem disk suspension using a 10-µL loop by placing the flat side of the loop against the flat edge of the disk and using surface tension to pull the disk out of the liquid. Carefully drag and press the loop along the inside edge of the tube to expel excess liquid from the disk. Continue using the loop to remove the disk from the tube and then place it on the MHA plate previously inoculated with the meropenem-susceptible *E. coli* ATCC 25922 indicator strain. **Disk capacity: 4 disks on a 100 mm MHA plate; 8 disks on a 150 mm MHA plate**.

8. Invert and incubate the MHA plates at 35°C ± 2°C in ambient air for 18–24 hrs.

9. Following incubation, measure the zones of inhibition as for the routine disk diffusion method.

Interpretation of results

- Carbapenemase positive (see Figures 1B and 1C):
  - Zone diameter of 6–15 mm or presence of pinpoint colonies within a 16–18 mm zone
  - If the test isolate produces a carbapenemase, the meropenem in the disk will be hydrolyzed and there will be no inhibition or limited growth inhibition of the meropenem-susceptible *E. coli* ATCC 25922.
- Carbapenemase negative (see Figure 1A):
  - Zone diameter of ≥ 19 mm (clear zone)
  - If the test isolate does not produce carbapenemase, the meropenem in the disk will not be hydrolyzed and will inhibit growth of the meropenem-susceptible *E. coli* ATCC 25922.
- Carbapenemase indeterminate:
  - Zone diameter of 16–18 mm
  - Zone diameter of ≥ 19 mm and the presence of pinpoint colonies within the zone
  - The presence or absence of a carbapenemase cannot be confirmed.

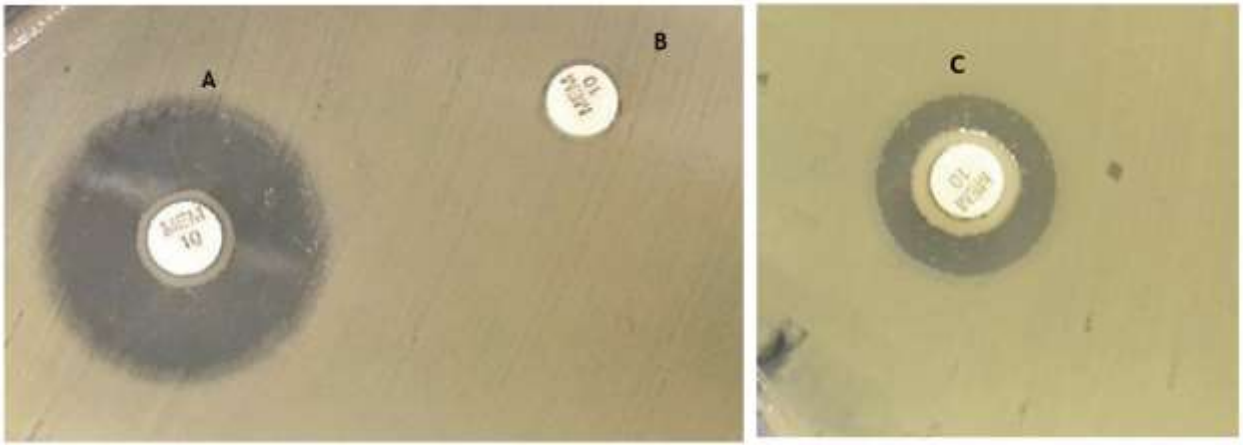

Figure 1 mCIM results for QC strains and test strain

(A) Negative Control *K. pneumoniae* ATCC BAA-1706™
(B) Positive Control *K. pneumoniae* ATCC BAA-1705™
(C) mCIM positive results

Note:

- For mCIM indeterminate results:

  – Check test isolate and *E. coli* ATCC 25922 indicator strain for purity.

  – Check meropenem disk integrity by confirming acceptable results were obtained when disks were subjected to routine disk diffusion test QC.

  – Repeat the mCIM for test isolate and QC strains.

- mCIM only: For some tests, pinpoint colonies of the indicator organism (*E. coli* ATCC 25922) may be observed within the zone of inhibition. If the colonies are present within a 6- to 18-mm zone of inhibition, the test should be considered carbapenemase positive. If colonies are present within a ≥ 19-mm zone, the test should be considered indeterminant.
- CLSI has currently standardized mCIM for Enterobacterales with a 1-µL loopful of bacteria and *P. aeruginosa* 10-µL loopful of bacteria only.

Reference

CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2022.