Detection for colistin resistance for Enterobacterales and *Pseudomonas aeruginosa*

using Colistin Broth Disk Elution (CBDE)

Purpose

To determine the lowest concentration of colistin that can inhibit the growth of Enterobacterales and *P. aeruginosa*.

Materials

1. Overnight culture of bacterial isolate on non-selective media (Tryptic soy agar (TSA), Sheep blood agar (SBA))
2. Sterile cotton swab
3. 0.5 McFarland standard
4. Nephelometer
5. Cation adjusted Muller Hinton Broth (CAMHB)
6. 10-µl loop
7. Sterile forceps
8. Antimicrobial agent
    - 10-µg-colistin disks
9. Vortex mixer
10. Sterile 0.85 % saline solution
11. Autopipette (10-100 µL)
12. Filtered tip (10-100 µL)
13. 35°C ambient-air incubator
14. Quality control strains
    - *Escherichia coli* NCTC 13846 (*mcr-1*) (≤1 – >4 µg/mL, with a target of 2 µg/mL)
    - *P. aeruginosa* ATCC 27853 (1–4 µg/mL)

Procedure

1. Let the CAMHB tubes (10 mL) and colistin disks warm to room temperature.

2. Label 4 tubes of CAMHB for each isolate to be tested with 1, 2, and 4 µg/mL and control.

3. Using aseptic technique, carefully add:

    - 1 colistin disk to the tube labeled “1 µg/mL”
    - 2 colistin disks to tube labeled “2 µg/mL”
    - 4 colistin disks to the tube labeled “4 µg/mL”

4. Gently vortex the tubes with the added disk and let the colistin elute from the disks for at least 30 mins but **no longer than 60 mins at room temperature**.

5. Prepare the standardized inoculum.

6. Using 10-µl loop, pick 3–5 colonies from an 18- to 24-hrs non-selective agar plate and transfer to sterile saline (4–5 mL).

7. Adjust turbidity with saline solution to equivalent of a 0.5 McFarland turbidity standard.

8. Add 50 µL standardized inoculum to the control and 1-, 2-, and 4-µg/mL tubes to attain a final inoculum concentration of approximately 7.5×10^5 CFU/mL.

9. Using a 10-µL loop, subculture from the original inoculum tube to a blood agar plate as a purity check.

10. Cap the tubes tightly and **vortex each inoculated tube on slow speed to mix**. Slow speed is suggested to prevent colistin from sticking to the cap and glass surface above the meniscus of liquid.

11. Loosen the caps slightly before incubation.

12. Incubate the tubes and purity plate at 33 to 35°C; ambient air for 16–20 hrs.

Interpretation of results

1. Examine the purity plate to ensure inoculum was pure.

2. Examine the growth control tube, which must demonstrate obvious turbidity for the test to be valid. NOTE: Some *P. aeruginosa* isolates may grow only near the meniscus.

3. Read the MIC as the lowest concentration that completely inhibits growth of the test isolate

For Enterobacterales and *P. aeruginosa*:

≤ 2 µg/mL = intermediate

≥ 4 µg/mL = resistant

Reference

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