**SENSITITRE Assay Protocol**

**Purpose**

Susceptibility testing of Carbapenem-resistant *Enterobacteriaceae* (CRE) to determine resistance to a selection of antibiotics.

**Materials per Assay**

* Sensititre plate
* Plate seal
* Demineralized water
* Mueller-Hinton broth
* 0.5 McFarland standard
* Disposable loops
* Multichannel pipette
* Blood agar plates
* Nephelometer
* Incubators at 35C and 37C
* Vortex mixer
* Manual viewer

**Procedure**

* **Day 1:** Growth of Bacterial Isolates
  1. Generate list of isolates to be grown.
  2. Label one blood agar plate per isolate.
  3. Remove isolates from -80C freezer.
  4. Open the vial. Using a 10uL loop, scrape the frozen TSB until some sample is in the loop.
  5. Perform a four quadrant streak for isolation on the corresponding blood agar plate.
  6. Repeat with all isolates.
  7. Incubate plates at 37C for 24 hours.
* **Day 2:** Subculture Isolates
  1. Label one blood agar plate per isolate, and designate as the second plate.
  2. For each isolate, subculture one colony for isolation on blood agar.
  3. Incubate plates at 37C for 24 hours.
* **Day 3:** Prepare Sensititre Plates
  1. Using a disposable loop, gather a small amount of bacteria from the agar plate.
  2. Emulsify in demineralized water and vortex to minimize large clumps.
  3. Calibrate the nephelometer using the 0.5 McFarland standard.
  4. Using the nephelometer, adjust the sample in demineralized water to 0.5 McFarland (1.5x108 CFU/mL) by adding more bacteria.
  5. Transfer 50uL of the suspension into a tube of 11mL Mueller-Hinton broth. Vortex well.
  6. Steps 1 through 5 must be completed within 30 minutes.
  7. Open the Sensisititre plate and check that the desiccant is orange in color.
  8. Pour the broth into a sterile trough and using a multichannel pipette, pipette 100uL into each well.
  9. Make a purity plate by pipetting 100uL of broth onto a labeled blood agar plate.
  10. Cover the plate with an adhesive seal. Press firmly to ensure proper sealing.
  11. Incubate plates at 35C for 16-20 hours. Do not stack more than three high.
* **Day 4:** Reading the Plates
  1. Do not remove the seal from the plates. Check purity plate to confirm no contaminants in culture.
  2. Place the plate on the manual viewer. Growth will appear as turbidity or a “button” of cells deposited at the bottom of the well.
  3. Record growth in the table below. Record the MIC as the lowest concentration of cells that completely inhibits growth.

**Growth Table:** check wells containing growth.

Isolate #: CRKP\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Technician Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A |  |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  |  |  |  |  |  |  |  |  |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |  |  |  |  |  |
| F |  |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H | POS |  |  |  | POS |  |  |  |  |  |  | POS |

**MIC dilutions for the various agents are noted below:** circle MIC based on growth above.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | MERO 0.25 | MEV 0.25/8 | P/T4 1/4 | CZA 0.25/4 | IMI 0.25 | IMK 0.25/4 | C/T 0.25/4 | TGC 0.25 | COL 0.25 | AMI 0.5 | PLZ 0.5 | FOS+ 1.0 |
| B | MERO 0.5 | MEV 0.5/8 | P/T4 2/4 | CZA 0.5/4 | IMI 0.5 | IMK 0.5/4 | C/T 0.5/4 | TGC 0.5 | COL 0.5 | AMI 1.0 | PLZ 1.0 | FOS+ 2.0 |
| C | MERO 1.0 | MEV 1/8 | P/T4 4/4 | CZA 1/4 | IMI 1.0 | IMK 1/4 | C/T 1/4 | TGC 1.0 | COL 1.0 | AMI 2.0 | PLZ 2.0 | FOS+ 4.0 |
| D | MERO 2.0 | MEV 2/8 | P/T4 8/4 | CZA 2/4 | IMI 2.0 | IMK 2/4 | C/T 2/4 | TGC 2.0 | COL 2.0 | AMI 4.0 | PLZ 4.0 | FOS+ 8.0 |
| E | MERO 4.0 | MEV 4/8 | P/T4 16/4 | CZA 4/4 | IMI 4.0 | IMK 4/4 | C/T 4/4 | TGC 4.0 | COL 4.0 | AMI 8.0 | PLZ 8.0 | FOS+ 16.0 |
| F | MERO 8.0 | MEV 8/8 | P/T4 32/4 | CZA 8/4 | IMI 8.0 | IMK 8/4 | C/T 8/4 | TGC 8.0 | COL 8.0 | AMI 16.0 | PLZ 16.0 | FOS+ 32.0 |
| G | MERO 16.0 | MEV 16/8 | P/T4 64/4 | CZA 16/4 | IMI 16.0 | IMK 16/4 | C/T 16/4 | TGC 16.0 | COL 16.0 | AMI 32.0 | PLZ 32.0 | FOS+ 64.0 |
| H | POS | MEV 32/8 | P/T4 128/4 | CZA 32/4 | POS | IMK 32/4 | C/T 32/4 | TGC 32.0 | COL 32.0 | AMI 64.0 | PLZ 64.0 | POS |

MERO Meropenem

MEV Meropenem/Vaberbactam

P/T4 Piperacillin/tazobactam constant 4

CZA Ceftazidime/avibactam

IMI Imipenem

IMK Imipenem/MK-7655

C/T Ceftolozane/lazobactam 4

TGC Tigecycline

COL Colislin

AMI Amikacin

PLZ Plazomicin

FOS+ Fosfomcin + glucose-6-phosphate