Procedure for antimicrobial susceptibility testing of *Enterobacteriaceae* by disc diffusion

Confirm identification of isolate.
Sub-culture on non-selective media

Prepare inoculum

Prepare a **0.5 McFarland** suspension of the bacteria to be tested in sterile saline

Compare prepared suspension with that of the **0.5 McFarland** standard (control) and adjust turbidity as needed with sterile saline or pure culture until correct density is achieved.

Perform **quality control** of medium as appropriate

Perform **quality control** of antimicrobial discs as appropriate.

| CLSI | EUCAST |
|-----------------------------|-------------|
| All Enterobacteriaceae | |
| Escherichia coli ATCC®25922 | |
| Escherichia coli ATCC®35218 | |
| Pseudomonas | Klebsiella |
| aeruginosa | pneumoniae |
| ATCC®27853 | ATCC®700603 |
| | |

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Culture Collection

Inoculate **Mueller Hinton Agar (MHA)** by streaking the plate with a swab multiple times in different directions to ensure even confluent growth. Allow to dry (Maximum 10 minutes).

Place discs on plate with sterile forceps/tweezers/disc dispenser. Do not move the disks once they have touched the agar surface.

Allow disks and media to reach **ambient temperature** before inoculating plates and placing
discs

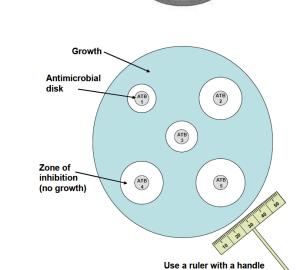
agar surface.

Incubate 35°C±2°C; Ambient air; 16-18 hours

Read zone edges at the point showing no growth against a dark background illuminated with reflected light. Measure zone diameter (mm) using ruler/calipers.

Interpret according to the <u>latest</u> Clinical and Laboratory Standards Institute (CLSI) / European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

Record and report findings.



if calipers are unavailable

Read **quality control** strain zones of inhibition first. If within limits, read test strain. If quality control fails trouble shoot whole process.

- 1. Clinical and Laboratory Standards Institute (2017); Performance Standards for Antimicrobial Susceptibility Testing; Twenty-fifth Information supplement. CLSI document M100-S27
- 2. European Committee on Antimicrobial Susceptibility Testing; Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, 2017
- 3. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Importance in the developing World; WHO Manual
- 4. Laboratory Methods for the Diagnosis of Epidemic Dysentery and Cholera; WHO Manual (1999)