A Ten Disk Procedure for the Detection of Antibiotic Resistance in Enterobacteriacae

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SPECIMEN:
The specimen consists of a pure isolate of the Enterobacteriaceae, which has a susceptibility result that is consistent with an ESBL or an AMPC pattern, i.e.: ceftazidime is I/R, ceftriaxone is I/R, aztreonam is I/R and requires confirmation by a disk method.

MATERIALS:
1. Antibiotic disks are placed in 12 cartridge dispenser, kept in fridge (2-8C), until use:
   - Aztreonam (30)
   - Ceftazidime (30)
   - Ceftazidime + clavulante (30/10)
   - Cefotaxime (30)
   - Cefotaxime + clavulante (30/10)
   - Cefoxitin (30)
   - Ceftriaxone (30)
   - Cefepime (30)
   - Ertapenem (10)
   - Imipenem(10)
2. Mueller Hinton (MH) agar plate, 150 mm, kept in fridge (2-8C), until use
3. sterile saline or tryptic soy broth (TSB)
4. sterile swabs
5. 0.5 McFarland barium turbidity standard / photometer (colorimeter)

METHOD:
1. Allow the MH agar plate and disk dispenser to come to room temperature before use.
2. Prepare a 0.5 McFarland standard of the organism to be tested in sterile saline or TSB. Standardize the inoculum using the colorimeter.
3. Streak the bacterial suspension evenly in 3 planes onto the surface of the MH agar plate, using a cotton swab. Rim the edge of the plate.
4. Place the disk dispenser over the MH agar plate and depress the knob. This will allow the antibiotic disks to dispense and automatically “tamp” the disk into place.
5. All of the disks must be placed on the same MH agar plate in a specified order (See Figure 1)
6. Incubate the MH agar plate overnight in a non-CO₂ incubator at 35C.
7. The following day read and record all zones of inhibition.
RESULTS:

1. **Detection of ESBLs (ceftazidime and cefotaxime disks with and without clavulanic acid are used to detect ESBLs)**
   
   A. If the zone size increases 5 mm or more when clavulanate is added compared to the drug alone the isolate is considered an ESBL. Only one antibiotic must be "reversed" by the clavulanate to be an ESBL. For example: CAZ/CLA – 22 mm

   \[
   \text{CAZ/CLA} \rightarrow 22 \text{ mm}
   \]

   \[
   \text{CAZ} \rightarrow 11 \text{ mm}
   \]

   \[
   22 - 11 = > 5 \text{ mm} = \text{ESBL}
   \]

   **Combination Disk (CLISI) Method – E.coli with**

   ![](image1.png)

   B. If an “enhancement” or extension of the zone of inhibition is seen between any of the cephalosporin antibiotics and the clavulanate containing disks, the presence of an ESBL can be predicted. This phenomenon is often referred to as the “KEYHOLE” effect, or “CLAVULANIC” effect and is indicative of ESBL production.

   **Double Disk Potentiation Method – P. mirabilis with ESBL**

   ![](image2.png)

   Keyhole Formation Around Clavulanic Containing Disk = ESBL
2. **Detection of AmpC beta lactamases** *(cefepime and cefoxitin disks are used to detect ampC beta lactamases)*

   a. AmpC strains are resistant to the cephamycins (ie; cefoxitin and cefotetan).
   b. AmpC strains are susceptible to cefepime.
   c. High level AmpC producers causes resistance to all 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> generation cephalosporins, the beta lactam-inhibitor drugs and the monobactams (ie; aztreonam).

*Double Disk Potentiation Method - E. coli with plasmid mediated AmpC, ESBL Negative*

![Image](image_url)

- No Clavulanic Effect
- Cefepime (FEP) – S
- Cefoxitin (FOX) – R
3. **Detection of K1 beta lactamases** (aztreonam, ceftazidime, cefotaxime and ceftriaxone disks are used to detect K1 beta lactamases)

Double Disk Potentiation Method – *K. oxytoca* with chromosomal K1 beta lactamase, ESBL Negative, AmpC Negative

- Cefoxitin S = Neg AmpC
- No Clavulanic Effect = Neg ESBL
- Aztreonam - R
- Ceftazidime - S
- Cefotaxime S: Ceftriaxone R

4. **Detection of Carbapenemase** (ertapenem and imipenem disks are used to screen for carbapenemase resistance)

Double Disk Potentiation Method – *K. pneumoniae* with KPC beta lactamase

- Imipenem - S
- Ertapenem - R
- Suggests possible KPC which should be confirmed with Hodge test or sent to reference lab for confirmation
5. Record all disk diffusion mm zone size readings in the culture work up.

6. If ESBL is confirmed, change/override any previous susceptibility result to resistant, if the antibiotic is a penicillin, cephalosporin, or monobactam regardless of how the drug tests, following CLSI interpretive guidelines for ESBL. Refer to CLSI document M100-S18. Report cephamycins (ie; cefoxitin) and beta lactam inhibitor drugs as they test (in other words report as susceptible if they test susceptible, do not override).

7. If ESBL is not confirmed then report drugs as they test. For example if organism is shown to be an ampC or K1, report drugs as they test, do not override and make resistant.

8. If ESBL is present along with ampC or K1 then apply the ESBL reporting rules and report all penicillins, cephalosporins and monobactams as resistant.

9. If KPC is confirmed then report all beta lactam drugs as resistance regardless of how they test.

QUALITY CONTROL:

Disk diffusion testing is performed weekly with ATCC# 700603 Klebsiella pneumoniae and E. coli ATCC 25922 following CLSI guidelines. If correct quality control results are not obtained, the test is invalid and patient results cannot be reported.

REFERENCE:


General Review Articles

ESBL Review Articles

Double-Disk Potentiation Method

Hodge Test
Fig 1. Template for Disk Potentiation Method for Detecting ESBL and ampC beta-lactamases

Abbreviation KEY
1  cefotaxime-clavulanate
2  aztreonam
3  cefepime
4  ceftriaxone
5  empty
6  empty
7  cefoxitin
8  ertapenem
9  imipenem
10  cefotaxime
11  ceftazidime-clavulanate
12  ceftazidime