

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

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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, ie.; 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:

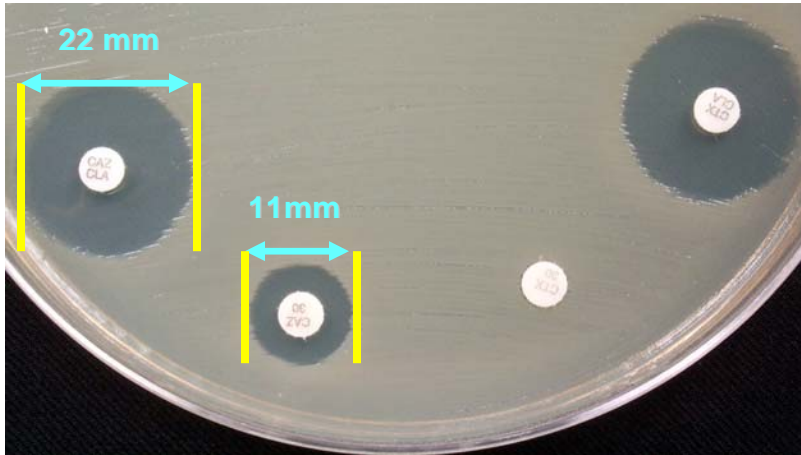
1. Allow the MH agar plate and disk dispenser to come to room temperature before use.
2. Prepare a 0.5 McFarland standard of the organism to be tested in sterile saline or TSB. Standardize the inoculum using the colorimeter.
3. Streak the bacterial suspension evenly in 3 planes onto the surface of the 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

Combination Disk (CLISI) Method – E.coli with

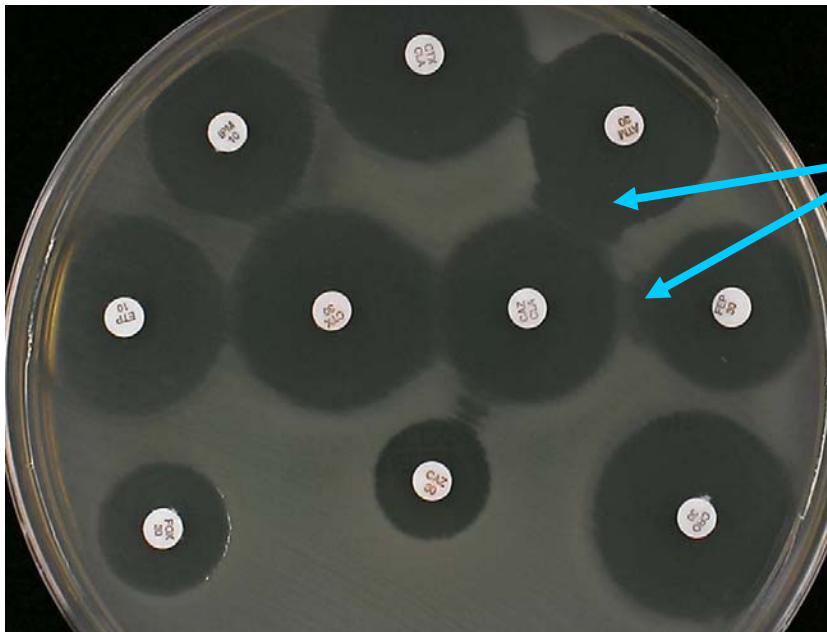


CAZ/CLA – 22 mm
CAZ – 11 mm

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

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.

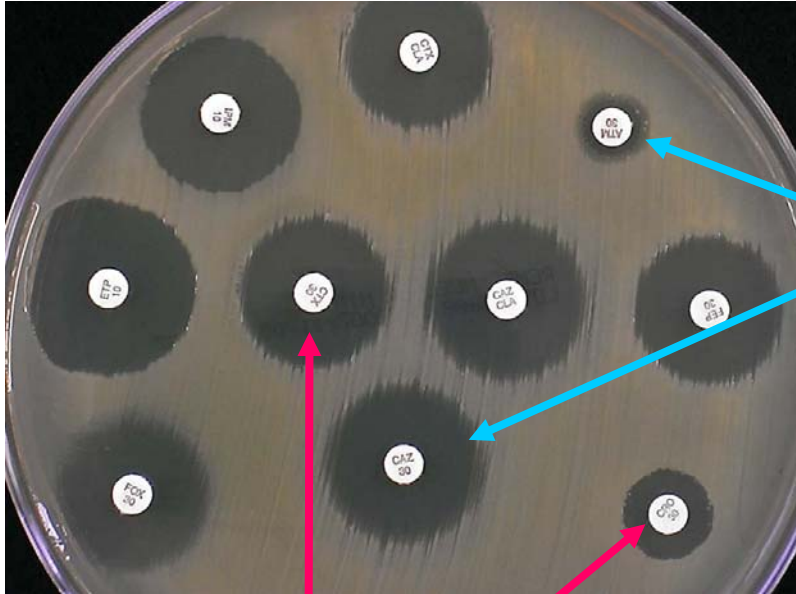
Double Disk Potentiation Method – P. mirabilis with ESBL



Keyhole Formation
Around Clavulanic
Containing Disk =
ESBL

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

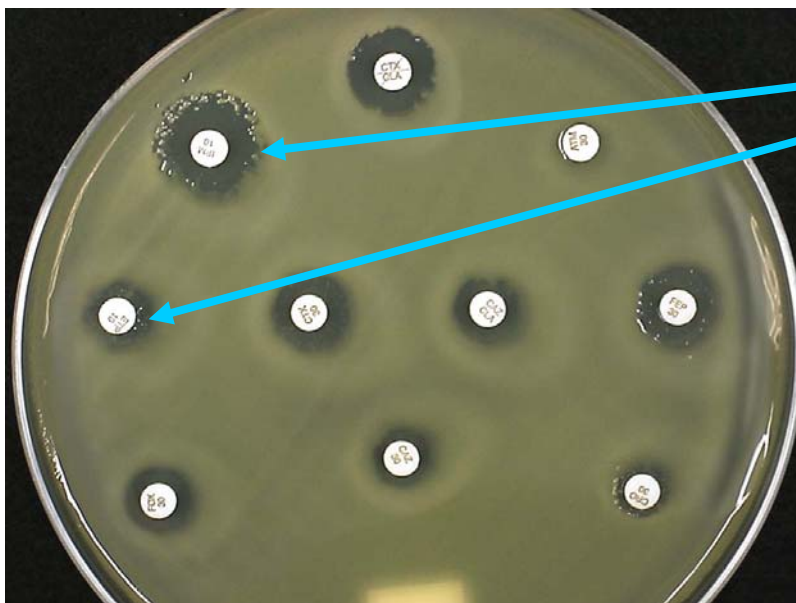
No Clavulanic Effect = Neg ESBL

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; ceftiofuran) 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:

Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing, Eighteenth Informational Supplement*. CLSI document M100-S18. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2008.

General Review Articles

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2. Livermore DM, Winstanley TB, Shannon KP. Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. *J Antimicrob Chemother*. 2001 Jul;48 Suppl 1:87-102
3. Thomson KS, Moland ES. Version 2000: the new β -lactamases of Gram-negative bacteria at the dawn of the new millennium (Review). *Microbes and Infection*, 2000;2:1225-1235

ESBL Review Articles

1. Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev*. 2001 Oct;14(4):933-51, table of contents. Review.
2. Jacoby GA, Munoz-Price LS: The new beta-lactamases. *N Engl J Med*. 2005 Jan 27;352(4):380-91. Review
3. Paterson DL, Bonomo RA: Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev*. 2005 Oct;18(4):657-86. Review.

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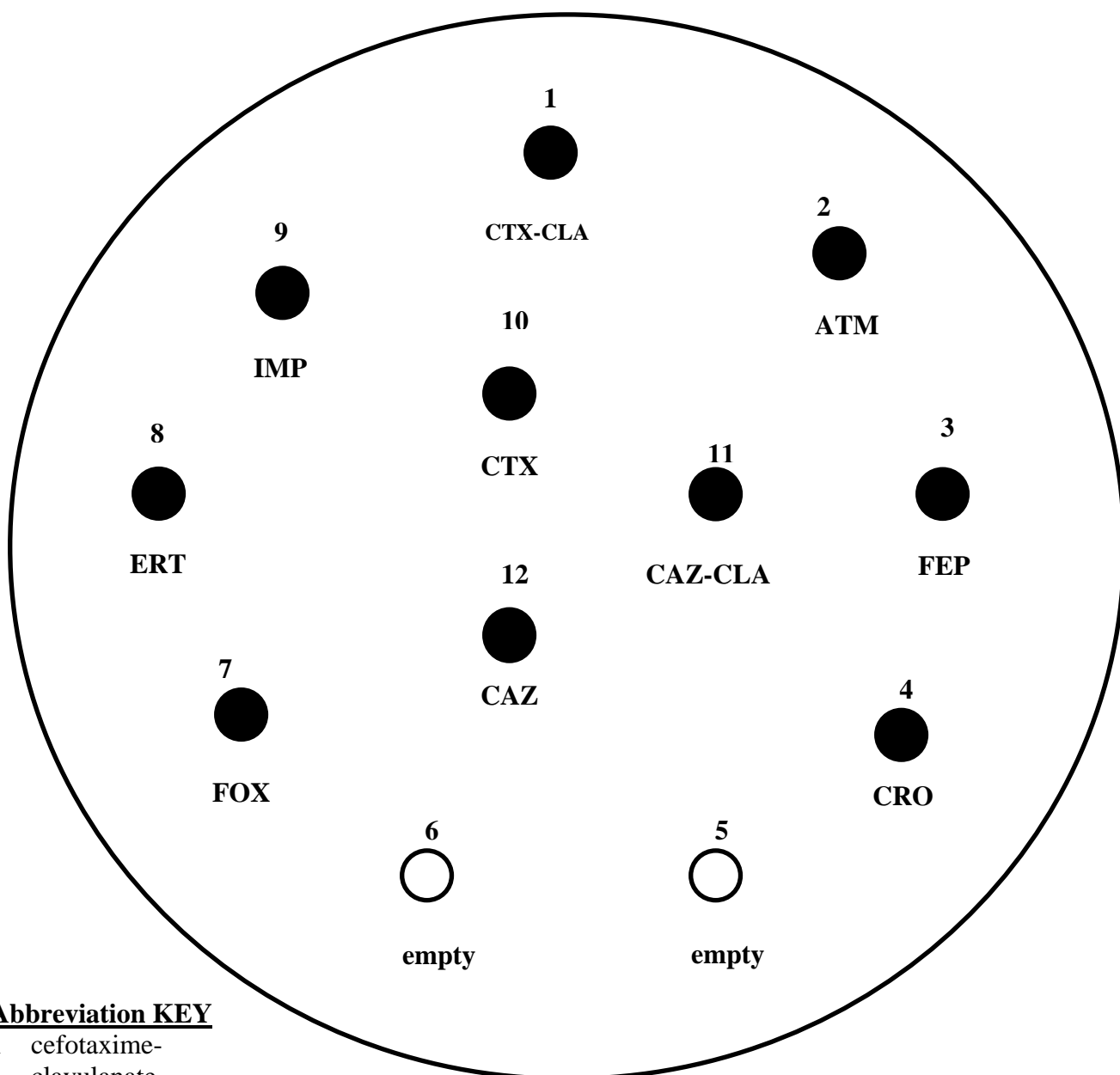
Double-Disk Potentiation Method

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Hodge Test

1. Anderson KF, Lonsway DR, Rasheed JK, Biddle J, Jensen B, McDougal LK, Carey RB, Thompson A, Stocker S, Limbago B, Patel JB. Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in Enterobacteriaceae. *J Clin Microbiol.* 2007 Aug;45(8):2723-5.
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3. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol.* 2003 Oct;41(10):4623-9

Fig 1. Template for Disk Potentiation Method for Detecting ESBL and am_pC beta-lactamases



Abbreviation KEY

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