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INTRODUCTION:
To detect isolates with AmpC, K1, and KPC enzymes, as well as those with ESBLs, it is desirable to test all Enterobacteriaceae with a combination of antibiotics that will allow detection of resistant mechanisms. In our laboratory we have expanded the ESBL confirmatory disk test to include 12 antibiotic disks and have applied the test to all members of the Enterobacteriaceae that have a susceptibility pattern that is suspicious for the presence of an ESBL, AmpC, K1 or KPC type resistance gene. Through the application of this test we have been able to successfully detect antibiotic resistance in many species of Enterobacteriaceae that would have not been detected using our automated susceptibility testing system.

WHEN TO PERFORM 12-DISK TEST:
Set up 12-disk test to confirm presence of ESBL in following instances:
1. Any E. coli or Klebsiella when phenotype does not agree with ESBL confirmation test on Vitek, MicroScan, or other automated susceptibility system (e.g. ceftazidime is I or R but ESBL confirmatory test is Neg)
2. Any Enterobacteriaceae when one of the 3rd gen. cephalosporins tests I or R and no ESBL confirmatory test result available
3. Any Enterobacteriaceae when atypical or multi-drug resistant pattern exists (e.g. P. mirabilis resistant to multiple drugs)
4. Any Enterobacteriaceae resistant to all drugs except imipenem
5. Any Enterobacteriaceae resistant to ertapenem, imipenem or meropenem

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 + clavulanate (30/10)
   - Cefotaxime (30)
   - Cefotaxime + clavulanate (30/10)
   - Cefoxitin (30)
   - Cefotetan (30)
   - Ceftriaxone (30)
   - Cefepime (30)
   - Ertapenem (10)
   - Imipenem (10)
   - Meropenem (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.
8. 

**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. Note: For MYSPACE organisms if the zone size increases 3 mm or more with clavulanic-containing disk compared to the drug alone then the isolate is considered an ESBL. See reference 14.

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

   ![Combination Disk (CLISI) Method](image)

   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

2. **Detection of AmpC beta lactamases** *(cefepime and cefoxitin disks are used to detect ampC beta lactamases)*

   a. AmpC strains are resistant to the cephemycins (ie; cefoxitin and cefotetan).
   b. AmpC strains are susceptible to cefepime.
   c. High level AmpC producers causes resistance to all 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} generation cephalosporins, the beta lactam-inhibitor drugs and the monobactams (ie; aztreonam).
Double Disk Potentiation Method - *E. coli* with plasmid mediated AmpC, ESBL Negative

No Clavulanic Effect

Cefepime (FEP) – S

Cefoxitin (FOX) – R

= AmpC

Double Disk Potentiation Method - *E. cloacae* with chromosomal AmpC, ESBL Positive

Keyhole Formation between cefepime and clavulanic containing disk

= ESBL

Cefepime (FEP) – S

Cefoxitin (FOX) – R

= AmpC
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

- Cefotaxime S: Ceftriaxone R

Cefotaxime S: Ceftriaxone R

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

4. Detection of Carbapenemase (ertapenem and imipenem disks are used to screen for carbapenemase resistance) Note: On 12 disk test meropenem can also be used to screen for carbapenemase resistance)

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

- Imipenem - S
- Ertapenem - R

Suggests possible KPC or metallo-beta-lactamase. Confirm with Hodge test and MBL Etest or send to reference lab for confirmation
5. **Metallo-Beta-Lactamases** are resistant to ALL antibiotics. May be susceptible to Aztreonam, Colistin And/or Tigecycline

Suggests possible metallo-beta-lactamase. Confirm with MBL Etest or EDTA Disks (See below) or send to reference lab for confirmation
5. Metallo-Beta-lactamase (MBL) Etest

POSITIVE FOR MBL – MIC of Imipenem (IP) is reduced by at least three doubling dilutions in presence of EDTA (IPI)

NEGATIVE FOR MBL - MIC of Imipenem (IP) is NOT reduced by at least three doubling dilutions in presence of EDTA (IPI)
6. SME – Chromosomal carbapenemase

SME are resistant to Imipenem by a chromosomal mechanism. Contact isolation is NOT necessary.

REPORTING RESULTS:
1. Record all disk diffusion mm zone size readings in the culture work up and on recording form.
2. Reporting of detected resistance mechanisms:

ESBL:
- If ESBL is detected, change/override any previous susceptibility result to resistant, for all penicillins, cephalosporins, and monobactams, regardless of how the drug tests, following CLSI interpretive guidelines for ESBL. Refer to current CLSI M100-S21 document. Report beta lactam inhibitor drugs as they test.
- If ESBL is not detected, report drugs as tested. For example if organism is shown to be an ampC or K1, report drugs as they test, do not override and make resistant
- If ESBL is present along with AmpC or K1, apply the ESBL reporting rules and report all penicillins, cephalosporins and monobactams as resistant.

AmpC:
- Chromosomal AmpC detected in MYSPACE organisms, do NOT change any antibiotics, report as they test.
- Plasmid-mediated AmpC detected in E. coli, Klebsiella species, Proteus, do NOT change any antibiotics, report as they test with comment “Plasmid-mediated...”
transmissible resistance mechanism detected (AmpC). Patient requires contact isolation.”

**K1:**
- Report all drugs as they test. Do NOT apply ESBL reporting rules.

**KPC:**
- Report confirmed positive Modified Hodge Test as: “**Carbapenem resistant Enterobacteriaceae (CRE) detected (plasmid mediated KPC type). Patient requires contact isolation.”**

**MBL**
- Report confirmed MBLs as: “**Carbapenem resistant Enterobacteriaceae (CRE) detected (plasmid mediated metallo-beta-lactamase MBL type). Patient requires contact isolation.”**

**SME**
- Report Serratia marcescens SME as: “**Imipenem resistance due to carbapenemase production (chromosomal-type). The effectiveness of other beta-lactams (that test susceptible) in treating infections due to carbapenemase-producing Serratia marcescens has not been established.”**

**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**


**Review of Phenotypic Detection Methods**


ESBL Review Articles

AmpC ß-Lactamases Review Articles

Double-Disk Potentiation Method

Hodge Test


Carbapenemase-Producing Klebsiella pneumoniae (KPC)


New Delhi Metallo beta-lactamase (NDM-1)


SME Carbapenemases

NMCA Carbapenemases

OXA-type Carbapenemases
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 cefoxitin
6 cefotetan
7 meropenem
8 ertapenem
9 imipenem
10 cefotaxime
11 ceftazidime-clavulanate
12 ceftazidime