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| **Phenotypic Detection of β-Lactamase Resistance in Gram Negative Bacilli: Testing & Interpretation Guide [Microbiology Manual]** |  |
|  | **Section: MICROBIOLOGY** |  |

SCOPE: This policy applies to UPMC Hanover.

**KEYWORDS: ESBL, 4 disk**

**PURPOSE:** To detect isolates with ESBLs, it is desirable to test all Enterobacteriaceae with a combination of antibiotics that will allow detection of the ESBL resistant mechanism. The 4 drug ESBL confirmation test includes 4 antibiotic disks and will be applied to all members of Enterobacteriaceae that have a susceptibility pattern that is suspicious for the presence of an ESBL resistance gene. With this test, detection of ESBL resistance is detected/confirmed that would not have been detected using just the Vitek GN AST panels in use.

**SPECIMEN:**

When to perform a 4-Disk test: (pure isolate of the Enterobacteriaceae)

1. Any *E. coli*, *Klebsiella* or *Proteus* when phenotype does not agree with the ESBL confirmation test on Vitek. Ex., 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 confirmatory test result is available—e.g. *Proteus mirabilis.*
3. Any Enterobacteriaceae when atypical or multi-drug resistant pattern exists (e.g. Proteus mirabilis resistant Any Enterobacteriaceae when atypical or multi-drug resistant pattern exists (e.g. Proteus mirabilis resistant to multiple drugs).
4. Any Enterobacteriaceae resistant to all drugs except imipenem.

**EQUIPMENT/MATERIALS:**

2-Disk Dispenser, kept in refrigerator at 2-8ºC

Individual antibiotic cartridges in following denominations:Ceftazadime (30), Ceftazadime + clavulanate (30/10), Cefotaxime (30), Cefotaxime + clavulanate (30/10)

Sterile saline or tryptic soy broth (TSB) Sterile swabs Small Mueller-Hinton agar plates, 150 mm

0.5 McFarland barium turbidity standard/colorimeter 35ºC incubator

**QUALITY CONTROL** Controls are done each week, having been performed daily for at least 20 days at start-up.

**PROCEDURE:**

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| 1 | Allow 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 testing in sterile saline or TSB. Standardize the inoculation using the colorimeter/densichek. |
| 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 dispenses all disks and tamps them into place. |
| 5 | All of the disks must be placed on the same MH agar plate in a specified order. (See Figure 1). Assure all disks are firmly on the agar. |
| 6 | Incubate the MH agar plate overnight in a non-CO2 incubator at 35ºC. |
| 7 | After 24 hours, read and record all zones of inhibition. Interpret results based on information regarding the various resistance mechanisms under “Expected Results”. Record all disk diffusion mm zone size reading in the culture workup. (See worksheet for this.) |
| 8 | **Detection of ESBLs**(Ceftazadime 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 to be an ESBL. Only one antibiotic must be “reversed” by the clavulanate to be an ESBL. For example: CAZ/CLA = 22 mm. CAZ=11mm. 22-11= >5 = ESBL.B. If an “enhancement” or extension of the zone of inhibition is seen between any of the cepahlosporin 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.**Detection of AmpC beta lactamases**A. AmpC strains are resistant to cephamycins (i.e., Cefoxitin and Cefotetan).B. AmpC strains are susceptible to Cefepime.C. High level AmpC producers cause resistance to all 1st , 2nd and 3rd generation cephalosporins, the beta lactam- inhibitor drugs and the monobactams (i.e., Aztreonam).D. AmpC may occur with ESBL. |

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|  | **Detection of K1 beta lactamases** (Kleb oxytoca only)1. Aztreonam = R
2. Ceftazidime = S
3. Ceftriaxone= R
4. Cefotaxime= S
 |
| 9 | **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- S19.) Report cephamycins (i.e., cefoxitin & cefotetan) and beta lactam inhibitior drugs as they test (in other words, report as susceptible if they test susceptible, do not override). **If the ESBL is not confirmed**, report drugs as they test.**If the organism is shown to be an ampC or K1**, report drugs as they test, do not override or add resistance.**If ESBL is present along with ampC or K1,** apply the ESBL reporting rules & report all penicillins, cephalosporins & monobactams resistant. |
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**REFERENCES:**

Schreckenberger, P., Rekasius, V., May 2009.Phenotypic Detection of β-Lactamase Resistance in Gram-Negative Bacilli: Testing and Interpretation Guide. Loyola University Medical Center. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, Nineteenth Informational Supplement. CLSI document M100-S19.

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