PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

ESBL Screening & Confirmation Procedure

Department of Microbiology

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1.0 Clinical Significance

Extended-spectrum β-lactamases (ESBL's) are enzymes produced by gram-negative bacilli that have the ability to inactivate β-lactam antibiotics containing an oxyimino group (i.e., third generation cephalosporins and aztreonam). They are referred to as extended spectrum β-lactamase enzymes because they are able to hydrolyze a broader spectrum of β-lactam antibiotics than the simple parent β-lactamases from which they are derived. They are most commonly found in *Klebsiella pneumoniae*, but can occur in other members of *Enterobacteriaceae* including *Escherichia coli*. Strains of *Klebsiella* species and *E. coli* that produce ESBL's may be clinically resistant to therapy with penicillins, cephalosporins or aztreonam, despite apparent *in vitro* susceptibility to these agents.

2.0 Principle

Currently there are only CLSI guidelines for testing strains of *E. coli, Klebsiella* spp. and *Proteus mirabilis* for the production of ESBL's. Some of these strains will show zones of inhibition below the normal susceptible population but above the standard breakpoints for certain extended-spectrum cephalosporins or aztreonam. Such strains should be screened for potential ESBL production by using ESBL screening breakpoints listed in Table 2A of the CLSI M100 document

before reporting results for penicillins, extended-spectrum cephalosporins, or aztreonam. Other strains may test intermediate or resistant by these standard breakpoints to one or more of these agents. In all strains with ESBL's, the zone diameters for one or more of the extended-spectrum cephalosporins or aztreonam should increase in the presence of clavulanic acid as determined in phenotypic confirmatory testing. For all confirmed ESBL-producing strains, the test interpretation should be reported as resistant for all penicillins, cephalosporins, and aztreonam.

The BD Phoenix ESBL test is also based on the principle of a differential response between the inhibitory effect of selected second- or third-generation cephalosporins used alone and in the presence of the beta-lactamase inhibitor, clavulanic acid, for the strains *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca*. While the Phoenix system is highly sensitive for detecting ESBL-producing isolates, it lacks specificity. Therefore, all isolates that are ESBL-positive on the Phoenix instrument are confirmed offline.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained perform and interpret automated and manual susceptibility testing. Testing includes but is not limited to: instrument maintenance, performance checks, basic troubleshooting, Quality Control testing, and record keeping.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

This procedure may expose you to:

- Multi-drug-resistant organisms

To perform this procedure, you must use:

- Laboratory Coat – must be worn when handling cultures.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

5.0 Specimen Requirements

Inoculum should be prepared from overnight growth of four or five isolated colonies of similar colony morphology.

6.0 Materials

6.1 Equipment and Testing System

- BD Phoenix™ , BD Phoenix™ AP, and associated consumables
- Epicenter™ software

6.2 General

- Sterile swabs
- BBL™ Prompt™ Inoculation System

6.3 Media

- Mueller Hinton Agar
- TSA II with 5% blood agar

6.4 Disks

- Ceftriaxone, 30 µg
- Ceftazidime, 30 µg
- Ceftazidime-clavulanic acid, 30/10 µg

- Cefotaxime, 30 µg
- Cefotaxime-clavulanic acid, 30/10 µg

7.0 Procedure

7.1 Automated Screening Using the Phoenix Instrument

Refer to the Phoenix Test Procedure for instructions for performing isolate testing on the Phoenix system. Isolates that test positive for ESBLs on the Phoenix system must be confirmed offline. Epicenter rules will suppress any susceptible penicillin and cephalosporin results until the confirmatory test results are available and the user manually enters the results in Epicenter (refer to the Epicenter AST Management Procedure).

7.2 Manual Screening by Disk Diffusion

1. Inoculate and incubate isolates of *E. coli, Klebsiella* spp. and *P. mirabilis* on the Mueller Hinton plate overnight at 35 ± 2°C, as outlined in the Kirby Bauer susceptibility testing procedure.
2. On the following day, examine the zone size for the ceftazidime 30 µg disk. This zone size may be used to initially screen for possible ESBL production.
3. If the ceftazidime zone measures ≤ 22 mm, confirmatory phenotypic testing should be performed to rule out ESBL.
4. For *E. coli* and *Klebsiella* species, examine the ceftriaxone 30 µg disk. If the ceftriaxone disk is ≤ 25-mm, confirmatory phenotypic testing should be performed to rule out ESBL.

7.3 Confirmatory Testing

If the zone size of the ceftazidime disk is not greater than 22 mm, a second Mueller Hinton plate should be inoculated with the isolate in order to test ceftazidime and cefotaxime alone, and each in combination with clavulanic acid. Confirmatory testing requires the use of both of these agents alone and in combination with clavulanic acid. The inoculum, incubation conditions, and incubation period are the same as the standard disk diffusion recommendations outlined in the Kirby Bauer susceptibility testing procedure.

8.0 Interpretation & Reporting of Results

After overnight incubation, the zone sizes should be measured. A ≥ 5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirms ESBL (e.g., ceftazidime zone = 16; ceftazidime-clavulanic acid zone = 21).

If the organism confirms as an ESBL-producer, attach the following comment using the code: **ESBLP** "Confirmatory testing shows that this organism produces extended spectrum beta-lactamases, and it should be considered clinically resistant to therapy with penicillins, cephalosporins, and aztreonam."

9.0 Quality Control & Quality Assurance

Performance of the ceftazidime 30 µg is checked with weekly QC using *E. coli* ATCC 25922. Quality control for the phenotypic confirmatory test should be performed weekly using *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603. The acceptable zone reactions are as follows:

- ***E. coli* ATCC 25922**:

  ≤ 2-mm increase in zone diameter for an antimicrobial agent tested alone versus its zone when tested in combination with clavulanic acid.

- ***Klebsiella pneumoniae* ATCC 700603**:

  ≥ 5-mm increase in ceftazidime-clavulanic acid zone diameter;

  ≥ 3-mm increase in cefotaxime-clavulanic acid zone diameter.

10.0 Limitations

1. Other members of *Enterobacteriaceae* produce ESBLs. However, practical clinical laboratory methods for screening and detection of these isolates have not been determined yet.
2. Some organisms with ESBLs may contain other beta-lactamases or other resistance mechanisms (e.g., porin alterations) that can mask ESBL production in the phenotypic confirmatory test, resulting in a false-negative result.
3. Phenotypic confirmation cannot detect all types of ESBL's.

11.0 References

1. Bradford, P. 2001. Extended-Spectrum β-Lactamases in the 21st Century: Characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews*. Vol. 14. No. 4. Oct. 2001. p. 933-951.
2. CLSI M100-S23. January 2013, Performance Standards for Antimicrobial Disk Susceptibility Testing; Twenty-Third Informational Supplement.
3. Patterson and Yu. 1999. Editorial Response: Extended-Spectrum β-Lactamases: A call for improved detection and control. *Clinical Infectious Diseases*. 29:1419-22.

12.0 Document Control History

Reviewed by Microbiology Director, Dr. Ann Robinson: 05/07/2004, 05/01/2014

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Updates and Revisions: 05/01/2014 Updated to include screening with the Phoenix system, added information for *P. mirabilis* testing, eliminated ESBLN reporting comment, changed QC frequency from with each isolate to weekly.