

Detecting Resistance to Beta Lactams in Gram-Negative Bacilli

*What you need to know about ESBL's.
A clear explanation and a complete procedure for your lab.*

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ESBL-mediated resistance to cephalosporins is not always obvious in disk or dilution tests because the MICs of cephalosporins for ESBL-producers are often low (0.5-2 µg/ml) and inhibition zones of disks are correspondingly large. Nevertheless, such ESBLs have been associated with clinical failure in patients; therefore, reliable detection is imperative.

Many laboratories detect and report ESBLs only in *E. coli* and *Klebsiella* species; however, ESBLs are known to occur in most species of Enterobacteriaceae. Furthermore, not all cephalosporin resistance in *E. coli* and *Klebsiella spp.* is due to ESBL production, and detection of isolates with other potent beta-lactamase types presents further challenges to the laboratory. This review highlights the types of beta-lactamases that occur in various members of the Enterobacteriaceae and describes a disk screening procedure for detection of beta-lactamase

mediated resistance that can be applied to all species of Enterobacteriaceae.

ESBLs.

Beta-lactamases are bacterial enzymes that inactivate beta-lactam antibiotics. Beta-lactamases that inactivate all the penicillins and cephalosporins including the extended spectrum cephalosporins are termed Extended Spectrum Beta-Lactamases, abbreviated as ESBLs. There are approximately 500 different ESBLs described all of which are mutations of the classical broad-spectrum beta lactamase enzymes that were initially named TEM and SHV (TEM-1, TEM-2, SHV-1). ESBL's are

Caution: *ESBLs can be produced by organisms other than E. coli and Klebsiella. In addition, there are potent beta-lactamases other than ESBLs.*

named TEM-3, -4 etc., SHV-2, -3 etc., CTXM-1, -2 etc., OXA-1, -2 etc. ESBLs hydrolyze penicillins, cephalosporins and the monobactam, aztreonam, conferring resistance to all of these drug classes.

ESBLs do not hydrolyze the cephamycin antibiotics (ie; cefoxitin and cefotetan), which are close relatives to the cephalosporins. ESBL's are also inhibited by beta-lactamase inhibitors such as clavulanate, sulbactam and tazobactam. ESBLs are generally inactive against the carbapenem antibiotics (Imipenem, Meropenem, Ertapenem).

The Clinical and Laboratory Standards Institute (CLSI) has published guidelines for performing an ESBL confirmatory test that involves testing cefotaxime and ceftazidime alone and in combination with clavulanate. Clavulanate inhibits the activity of the ESBL enzyme and makes the organisms appear more sensitive to drug + clavulanate combinations. This "greater sensitivity" with clavulanate can be demonstrated when a disk containing drug + clavulanate has a zone diameter that is ≥ 5 mm larger than the zone diameter of the drug tested alone, or when a zone of enhanced sensitivity is observed when a cephalosporin antibiotic is placed in close proximity to a clavulanate containing disk. This is commonly referred to as the "keyhole phenomenon." (see illustration 1B) Currently, the CLSI ESBL confirmatory test is recommended only for testing *E. coli*, *Klebsiella spp.* and *Proteus mirabilis*. There are currently, no CLSI recommendations for detection and reporting of ESBLs in other members of the Enterobacteriaceae.

AmpCs

AmpC beta-lactamases differ from ESBL's in that they are cephalosporinases and are resistant to beta-lactamase inhibitors. They hydrolyze the cephamycins, but not the 4th generation cephalosporins (eg. cefepime). AmpC is normally produced in low levels by many organisms and is not associated with resistance, but it can be produced at high levels and cause resistance. High-level production of AmpC usually causes resistance to all beta-lactams, except carbapenems and 4th generation cephalosporins.

The AmpC gene is found on the chromosome in 100% of the following organisms:

Enterobacter spp, *Hafnia alvei*, *Morganella morganii*, *Citrobacter freundii*, *Serratia marcescens*, *Providencia spp*, *Aeromonas spp*, and *Pseudomonas aeruginosa*. Chromosomal AmpC beta-

lactamases can be produced inducibly or constitutively. Inducible expression of the AmpC gene occurs when the enzyme is produced at a high level when the organism is exposed to inducing agents, such as cephamycins (ie; cefoxitin), ampicillin and carbapenems (ie; imipenem, meropenem, ertapenem). Induction is temporary and may be reversed when the antibiotic inducer is removed. In some organisms, mutations occur that cause the AmpC gene to become permanently expressed at high levels. These organisms are termed permanently de-repressed

mutants. Plasmid-mediated AmpC beta-lactamases can be found in organisms that do not carry the chromosomal AmpC. Plasmid-mediated AmpC's have been detected in organisms such as *E coli*, *Klebsiella spp*, *Proteus spp* and *Salmonella spp*. There are currently no CLSI approved methods to detect AmpC gene chromosomal or plasmid-mediated resistance.

K1 β -lactamase.

Klebsiella oxytoca produces a chromosomal beta-lactamase, the K1 enzyme. Hyperproduction of the K1 enzyme can occur by mutation. The K1 enzyme is predominantly a penicillinase that can also significantly hydrolyze aztreonam, cefuroxime and ceftriaxone and has weak activity against cefotaxime or ceftazidime. A distinctive feature of hyperproducers of K1 is greater

Dr. Schreckenberger proposes a disk method to detect the presence of an ESBL, AmpC, K1 or KPC type resistance gene in Enterobacteriaceae.

activity against ceftriaxone than cefotaxime and greater activity against aztreonam than ceftazidime. Organisms producing K1 beta lactamases may give false positive ESBL confirmatory tests with automated testing systems. There are currently no CLSI approved methods to detect K1 beta lactamases.

KPCs

Class A carbapenamases have recently been described that hydrolyze all beta lactam antibiotics including the carbapenem class of antibiotics. These have been named KPCs (Klebsiella Pneumoniae Carbapenemase) because they were first found in strains of K. pneumoniae. They are now known to occur in other members of the Enterobacteriaceae as well. KPC resistance is often not detected in automated susceptibility testing systems and leads to false susceptible reporting of imipenem. Ertapenem has been shown to be a good screening reagent for detecting KPC resistance. There are currently no CLSI approved methods to detect KPC-mediated resistance.

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 10 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 a many species of Enterobacteriaceae that would have not been detected using our automated susceptibility testing system. The following is a full description of our 10-Disk Procedure.

[Click here](#) for the procedure...

