



Laboratory Detection of ESBLs : Issues & Problems

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ESBLS are now a problem in hospitalized patients worldwide. Most ESBLs are derivatives of classical TEM or SHV enzymes. Unlike these parent enzymes, ESBLs hydrolyze all cephalosporins, penicillins, and aztreonam except cephamycin.^{1,2} It is generally thought that patients having infections caused by an ESBL-producing organism are at an increased risk of treatment failure with an expanded-spectrum β -lactam antibiotic.³ Therefore, it is very important for clinical microbiology laboratories to detect ESBL production in gram-negative bacteria. ESBLs are most often found in *E. coli* and *K. pneumoniae*, however, they are also found in other species of Enterobacteriaceae with increasing frequency. Furthermore, ESBLs have been found in non-Enterobacteriaceae gram-negative bacteria such as *P. aeruginosa*. However, there is no ESBL reporting guideline for gram-negative bacteria, other than *E. coli* and *Klebsiella* spp.⁴

Clinically relevant ESBL-mediated resistance is not always detectable in routine susceptibility tests, as the MICs of oxyimino-cephalosporins for ESBL-producers are often low (0.5-2 $\mu\text{g/ml}$).² My presentation will focus on the issues and problems associated with the detection of ESBLs.

Several ESBL detection methods have been proposed.³ NCCLS has issued recommendations for the screening and confirmation of ESBL in isolates of *E. coli* and *Klebsiella* spp. by using disk diffusion and broth dilution methods. However, several practical problems remain in terms of following the NCCLS recommendations. One is, which agent should be used for screening test. Although cefpodoxime is the most sensitive of the NCCLS screening agents, many of the cefpodoxime screening-positive isolates are ESBL nonproducers. Therefore, NCCLS (2002) changed the breakpoints for cefpodoxime (≤ 22 mm to ≤ 17 mm, ≥ 2 $\mu\text{g/ml}$ to ≥ 8 $\mu\text{g/ml}$) to reduce the number of false-positive results.

The presence of ESBLs can be masked by the expression of chromosomal AmpC β -lactamases, which are produced by, for example, *Enterobacter*, *Serratia*, *Citrobacter* spp. Recently, plasmid-mediated AmpC β -lactamases are often found in *E. coli* and *K. pneumoniae*. ESBL-producing strains with a AmpC β -lactamase can cause a false negative in ESBL detection. Cefoxitin susceptibility testing should be useful for detecting AmpC β -lactamase production. However, a recent KONSAR survey showed about 60% of laboratories did not test cefoxitin susceptibility.⁵ Cefepime is a more reliable detection agent for ESBLs in the presence of an AmpC β -lactamase, as this drug is stable to AmpC β -lactamases, but labile to ESBLs.

It is difficult for the clinical laboratory to determine whether a screening result should be reported or not. Confirmatory testing is performed after a positive screening result. If a laboratory reports a positive ESBL screening result and the isolate subsequently proves to be ESBL negative, carbapenem could be administered unneces-

sarily. On the other hand, if the laboratory withholds a positive screening result and the isolate is subsequently confirmed as ESBL positive, appropriate therapy may have been delayed for 24 hours. ⁽⁴⁾

Double disk synergy testing using disks of amoxicillin-clavulanate and oxyimino- β -lactam antibiotics remains a reliable method for detecting ESBLs. This test is simple, easy to interpret, and cost effective, but the disk placement distance has not been standardized. Three-dimensional test is very sensitive at detecting ESBLs, but it is more labor intensive than the other methods. Etest ESBL strips are convenient and easy to use, but it is sometimes difficult to read the test.³ These three tests have common problems, that is, there is no guideline for the screening test; therefore, we should follow the NCCLS screening guidelines. As is the case for NCCLS confirmatory testing, the methods also have problems in the AmpC β -lactamase-producing strains.

The automated microbial susceptibility test system, Vitek, also has an ESBL test card that utilizes either ceftazidime or cefotaxime alone and in combination with clavulanic acid. However, the sensitivity and specificity of this system varied depending on investigators. Routinely use of this commercial system offers rapid results, but the test is costly.

Each of the ESBL detection methods has some advantages and disadvantages, and none of the detection methods is perfect for the accurate detection of ESBLs among clinical isolates of gram-negative bacteria. Moreover, as new pathogens with new resistance mechanisms are emerging, the continued education of laboratory and medical personnel is needed.

References

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