



DIFFERENT PHENOTYPIC METHODS FOR DETECTION OF ESBL PRODUCTION IN BACTERIA: A REVIEW

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DOI: 10.7897/2277-4572.033139

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Received on: 15/04/14 Revised on: 12/05/14 Accepted on: 02/06/14

ABSTRACT

ESBLs are β -lactamases capable of conferring bacterial resistance to the penicillins, first, second, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) but these are inhibited by β -lactamase inhibitors such as clavulanic acid. The ESBLs are typically plasmid-mediated enzymes, mostly produced by Gram negative bacteria. Extended spectrum beta lactamase (ESBLs) enzymes were first reported in Germany in 1983 from *Klebsiella pneumoniae*. ESBL-producing Enterobacteriaceae have been responsible for numerous outbreaks of infection throughout the world and pose challenging infection control issues. Different phenotypic test for ESBL detection have been developed, which are easy to use and cost effective.

Keywords: ESBL, β lactamase, Clavulanic acid, phenotypic method, Enterobacteriaceae

INTRODUCTION

ESBL Detection Methods

Screening Methods

CLSI has developed both disk diffusion and micro dilution screening tests using selected antimicrobial agents. Each *Klebsiella pneumoniae*, *K. oxytoca*, or *Escherichia coli* isolate should be considered a potential ESBL-producer if the test results are as follows:

Disk diffusion	MICs
cefepodoxime < 22 mm	cefepodoxime > 2 μ g/ml
Ceftazidime < 22 mm	Ceftazidime > 2 μ g/ml
aztreonam < 27 mm	Aztreonam > 2 μ g/ml
cefotaxime < 27 mm	Cefotaxime > 2 μ g/ml
ceftriaxone < 25 mm	ceftriaxone > 2 μ g/ml

The sensitivity of screening for ESBLs in enteric organisms can vary depending on which antimicrobial agents are tested. The use of more than one of the five antimicrobial agents suggested for screening will improve the sensitivity of detection. Cefepodoxime and ceftazidime show the highest sensitivity for ESBL detection.^{1,2}

Confirmatory Methods

Disk diffusion method

A Mueller Hinton agar plate is taken and inoculated with the test organism. Then ceftazidime (30 μ g) and cefotaxime (30 μ g) disks are used, alone and with clavulanic acid (10 μ g). A ≥ 5 mm increase in zone diameter for either antimicrobial agent tested in combination with Clavulanic acid versus its zone when tested alone confirms an ESBL producing organism.³

Note: Other combinations like Piperacillin/tazobactam (100/10 μ g), and cefoperazone/sulbactam (75/30 μ g) can be used.⁴

Double disk diffusion synergy test

The test was performed as described by Jarlier *et al.* A sterile Mueller-Hinton agar was prepared and a 0.5 McFarland equivalent standard of the test organisms was streaked on the surface of the agar with a sterile loop and allowed for 15-20

minutes to pre-diffuse. An Augmentin which is a combination of clavulanic acid (20 μ g) and amoxicillin (10 μ g) was placed at the center of the plate and cefotaxime (30 μ g), ceftazidime (30 μ g), aztreonam (30 μ g) ciprofloxacin (30 μ g) were placed 15 mm apart center to centre on the plates with a sterile forceps. These were incubated at 35oC for 18-24 h. An enhanced zone of inhibition from 5 mm above in the presence of Augmentin is regarded as positive for phenotypic production of ESBL enzyme.^{5,6}

Three Dimensional test

Two types of inocula are prepared. A disk diffusion test inoculum (0.5 McFarland standard) and a three dimensional inoculums (109 – 1010 CFU/ml); in the direct three-dimensional test, after the surface of the susceptibility plate was inoculated similar to conventional disk diffusion method as per CLSI guidelines, the agar was stabbed vertically with a sterile no. 11 scalpel blade so that the point of the blade passed to the bottom of the agar at a predetermined point 3 mm inside the position at which the antibiotic disks were to be placed. The three dimensional inoculum was dispensed into the slit so that the slit was filled but there was no overflow onto the agar surface. Conventional disk diffusion susceptibility test results are measured according to the recommendations of CLSI. Distortion or discontinuity in the circular inhibition zone is interpreted as positive for ESBL production. The advantage of this method is simultaneous determination of antibiotic susceptibility and β lactamase detection (but not specific for ESBLs).^{7,8}

Modified three dimensional test

Direct method

A lawn culture of the test organism is made on a well dried MHA plate. A disc of Cefipime (30 μ g) is placed onto the MHA plate. A circular well is made (4 mm diameter) 2 mm from the antibiotic disk. Wells is then filled with 30 μ l of the three dimensional test inoculum (McFarland no 5 standard). Plates were incubated at 37°C for 18-24 h. Interpretation must be made by observing the shape of the inhibition zone.^{8,12}

Indirect method

It is same as DMTDT except, MHA plates were seeded with the inoculum of a standard sensitive strain (*E. coli* ATCC 25922) adjusted to 0.5 McFarland standard instead of test strain. Heart shaped distortion of zone of inhibition around the β -lactam disc was indicative of an ESBL production. Modified three dimensional test is used for detection of AmpC β -lactamase in ESBL positive isolates with reduced susceptibility to cefoxitin (≤ 18 mm).⁹

Interpretation

When the inhibition zone around the β lactam antibiotic is distorted in such a way that; the growth of the test organism appears within the zone behind the well and fully reaching the well so that; a heart shaped distortion of the inhibition zone around the Cefepime is seen. This is due to enzymatic inactivation of the test antibiotic by the β lactamase diffusing through the agar in the vicinity of the three dimensional test inoculums, resulting in gradually decreasing diameter of the inhibition zone towards the well.

Inhibitor potentiated disc diffusion test

This test is based on a modification of the standard disc-diffusion method as described by the CLSI. Mueller–Hinton (MH) agar supplemented with 4 mg/L of clavulanate was prepared the day before testing with the addition of clavulanate after cooling to 50°C in a water bath. The inoculum was prepared by the direct colony suspension method to match the turbidity of a 0.5 McFarland standard. Antibiotic discs containing ceftazidime (30 g), cefotaxime (30 g), ceftriaxone (30 g) and aztreonam (30 g) were placed on 85 mm clavulanate- containing and clavulanate-free MH agar plates using a BBL Sensi-Disc dispenser. Up to three antibiotic discs were tested per agar plate. After overnight incubation at 37°C, the diameters of the inhibition zones around the antibiotic discs were measured. Augmentation zone widths were obtained by subtracting inhibition zone diameters produced by the lactam discs on clavulanate-free medium with those in clavulanate-containing medium. An augmentation zone width of 10 mm was considered positive for ESBL production. Each strain was tested on three separate occasions.¹⁰

ESBL E test Strip

This combines both the principles of dilution and diffusion techniques. E strip is a thin nonporous plastic strip 5 mm wide and 60 mm long. It carries two shorter gradients aligned in opposing directions on a single strip. One end generates a

stable concentration gradient of one of the Oxyiminocephalosporins (e.g. Ceftazidime), while other end generates a gradient of Cephalosporin + Clavulanic acid (4 μ g/ml). When the E strip is applied over a lawn culture Inhibition ellipse may be seen on the both ends of the strip. MIC is interpreted as the point of intersection of the inhibition ellipse with E test strip edge. This method is more sensitive and easy to use.¹¹

CONCLUSION

Diagnostic laboratories demand cheaper and easy to use tests for detection of ESBL. These are more cost-effective and less labour intensive than genotypic methods of ESBL detection.

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Source of support: Nil, Conflict of interest: None Declared

<p>QUICK RESPONSE CODE</p> 	ISSN (Online) : 2277 –4572
	<p>Website</p> <p>http://www.jpsionline.com</p>

How to cite this article:

Insan Nitin Goel, Sharma Sachin, Chaudhary Pravin Kumar, Payal Nikhil, Yadav Suneet. Different phenotypic methods for detection of ESBL production in bacteria: A review. J Pharm Sci Innov. 2014;3(3):197-198 <http://dx.doi.org/10.7897/2277-4572.033139>