

## Detection of extended-spectrum $\beta$ -lactamases (ESBLs) in *E. coli* and *Klebsiella* species

Increasing resistance to third-generation cephalosporins amongst *E. coli* and *Klebsiella* spp. is predominantly due to the production of extended-spectrum  $\beta$ -lactamases (ESBLs). These plasmid-mediated enzymes mostly evolved via point mutations of the classical TEM-1 and SHV-1  $\beta$ -lactamases but other groups are increasingly prominent, notably the CTX-M types, which evolved via the escape and mutation of chromosomal  $\beta$ -lactamases from *Kluyvera* spp.

ESBL producers are associated with increased morbidity and mortality, especially amongst patients on intensive care and high-dependency units. Accurate laboratory detection is important to avoid clinical failure due to inappropriate antimicrobial therapy.

As a general rule, laboratories should test all isolates of *E. coli* or *Klebsiella* spp. from in-patients using **both** ceftazidime (the best indicator for TEM and SHV-derived ESBLs) **and** cefotaxime (the best indicator for CTX-M types). Alternatively, they can test with cefpodoxime, as a good indicator for all ESBL types. Earlier advice to screen only with ceftazidime is no longer adequate in view of the emergence of CTX-M types. Any organism showing reduced susceptibility to cefotaxime, ceftazidime or cefpodoxime should be investigated for ESBL production.

Many different techniques exist for confirming ESBL production but those utilising similar methodology to standard susceptibility tests are the most convenient for the routine diagnostic laboratory. These all depend on detecting synergy between clavulanic acid and the indicator cephalosporin(s) used in the primary screening.

Two disc detection methods have been described. The first is based on the original double disc method of Jarlier *et al*<sup>1</sup>, and examines for the expansion of the cephalosporin's inhibition zone adjacent to a disc containing co-amoxiclav 20 + 10 mg. In the second, the zone of inhibition around a 'combination' disc containing the cephalosporin combined with clavulanic acid is compared to the zone around a disc containing the cephalosporin alone. An expansion of >5 mm or 50% (according to the particular product and manufacturer's guideline) indicate ESBL production. Combination discs are available from Oxoid, Mast and Beckton Dickinson. An Etest method for ESBL detection is also available (Cambridge Diagnostic Services Ltd, Cambridge, UK). These techniques are generally reliable for detection of ESBLs in *E. coli* and *klebsiellae* but the double disc method has the disadvantage that the optimum distance between the discs varies with the strain, meaning that some tests will inevitably be done with sub-optimal spacing for ESBL detection. None of the methods is ideal for *Enterobacter*, *Citrobacter* and *Serratia* spp., which have inducible AmpC  $\beta$ -lactamases; these may be induced by clavulanate and may attack then indicator cephalosporin, masking the synergy contingent inhibition of any co-present ESBL. Etests combining cefepime and clavulanate have recently been marketed to detect ESBLs in these species, but have mostly been applied to specific epidemic strains of TEM-24-producing *E. aerogenes* that are prevalent in France and Belgium; their wider applicability awaits confirmation.

### Double disc method

Iso-Sensitest agar, or equivalent, is inoculated with the test organism to give a semi-confluent growth. Positive (*K. pneumoniae* ATCC 700603) and negative (*E. coli* ATCC 25922) control strains are inoculated onto further plates. A ceftazidime 30 mg disc and an amoxycillin/clavulanic acid 20+10 mg disc are then placed 25 - 30 mm apart, centre-to-centre. Following overnight incubation in air at 37°C, ESBL production is inferred when the zone of inhibition around the ceftazidime disc is expanded by the clavulanate - see figure 1 overleaf.



**Figure 1**  
**Detection of ESBL production using the double disc method**

The disc on the left is cefotaxime (30 mg); the disc in the centre is co-amoxiclav (20+10 mg); the disc on the right is ceftazidime (30 mg). Note the expansion of the zones around the cefotaxime and ceftazidime discs adjacent to the co-amoxiclav. The organism is *E. coli* with a TEM-24 enzyme.

Additional extended-spectrum cephalosporins may be tested concurrently eg cefotaxime (30 mg), aztreonam (30 mg) and ceftriaxone (30 mg), providing the co-amoxiclav disc is placed in the centre of the plate and the distance of 25 - 30 mm between the cephalosporin and clavulanate-containing discs is observed. The use of multiple cephalosporins may be helpful as ESBLs other than common TEM and SHV mutants begin to spread.

#### Combination disc methods

Plates of test and control organisms are inoculated as above. Pairs of discs containing an extended-spectrum cephalosporin (cefotaxime, ceftazidime or cefpodoxime) with and without clavulanic acid are placed on opposite sides of the same inoculated plate. Zones of inhibition are measured following overnight incubation in air at 37°C. According to the particular product used, the test organism is regarded as an ESBL producer if the zone of inhibition around the combination disc is at least 5 mm larger than that of the cephalosporin alone, or if the zone diameter is expanded by <sup>3</sup> 50% in the presence of the clavulanic acid. Such discs are available commercially (Oxoid Ltd., Basingstoke, UK; Mast Diagnostics, Bootle, UK and BD Biosciences, Oxford, UK) and have been found to perform well for the detection of ESBLs in *E. coli* and *Klebsiellae*<sup>2,3</sup>.

#### ESBL Etest

The detection of ESBLs by Etest is based on a similar principle to that of the combination disc method and has been shown to compare well with the disc methods<sup>2,4</sup>. Double-ended strips containing gradients of cefotaxime or ceftazidime at one end and cefotaxime or ceftazidime plus clavulanic acid at the other end are tested in parallel. The methodology should follow the manufacturer's directions (AB Biodisc, Solna, Sweden or Cambridge Diagnostics Services Ltd, Cambridge, UK). A decrease in MIC of <sup>3</sup> 3 doubling dilutions in the presence of clavulanate indicates ESBL production.

#### Reporting

**Clinical isolates of *Klebsiellae* and *E. coli* found to produce ESBLs should be assumed to be resistant to all extended-spectrum cephalosporins irrespective of the results of susceptibility testing.**

**Note:** It should be noted that not all resistance to third generation cephalosporins in *Klebsiellae* and *E. coli* is due to ESBL production - other potent  $\beta$ -lactamases such as AmpC and K1 enzymes may be responsible. Further details on these and other  $\beta$ -lactamase mediated resistance mechanisms are given elsewhere<sup>5,6,7</sup>.

#### References

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