ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephemycins or carbapenems) by hydrolysis of these antibiotics, and are inhibited by β-lactamase inhibitors such as clavulanic acid. First described in Germany (1983) and France (1985) among *Klebsiella* spp., ESBLs exist in every region of the world and in most genera of enterobacteria. Currently, ESBLs are becoming a major threat for patients in the hospital, long-term care facilities, and community. These bacteria have not only caused outbreaks but have become endemic in many hospitals throughout the world. When a significant proportion of Gram-negative isolates in a particular unit is ESBL producers, empirical therapy may change towards use of imipenem, quinolones, or β-lactam/β-lactamase inhibitor combinations. In some centers this has been associated with emergence of imipenem resistance in *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and in ESBL-producing organisms themselves. Control of endemic ESBL producers is difficult, and may only be possible after significant nursing and medical reorganization, at substantial financial cost. Patients at high risk for developing colonization or infection with ESBL-producing organisms are often seriously ill patients with prolonged hospital stays and in whom invasive medical devices are present (urinary catheters, endotracheal tubes, central venous lines) for a prolonged duration. A myriad of other risk factors have been implicated including the presence of nasogastric tubes, gastrostomy or jejunostomy tubes and arterial lines, administration of total parenteral nutrition, recent surgery, haemodialysis, decubitus ulcers, and poor nutritional status. Heavy antibiotic use is also a risk factor for acquisition of an ESBL-producing organism. A strong relationship exists between third-generation cephalosporin use and acquisition of an ESBL-producing strain. Other antibiotic classes have been found to be associated with subsequent infections due to ESBL-producing organisms include quinolones, trimethoprim- sulphonamide, aminoglycosides and metronidazole.

**ESBL detection: Clinical and epidemiological relevance**

ESBL detection has both clinical as well as epidemiological relevance. Poor outcome occurs when patients with serious infections due to ESBL-producing organisms are treated with cephalosporins even when the cephalosporin MICs are in the intermediate or susceptible range. A final argument in favour of ongoing efforts aimed at ESBL detection is the infection control significance of detecting plasmid-mediated multidrug resistance. There are epidemiologic implications for the detection of ESBL-producing organisms in that this resistance issue may not be as apparent if organisms are simply reported as intermediate or resistant to individual cephalosporins. Outbreaks of ESBL-producing organisms can be halted using appropriate infection control interventions. Endemic transmission of ESBL producers can also be curtailed using infection control measures and antibiotic management interventions. Detection of ESBL production in organisms from samples such as urine may be important because this represents an epidemiologic marker of colonization (and therefore the potential for transfer of such organisms to other patients). Therefore, it is imperative that microbiology laboratories should detect such infections promptly and with accuracy.

In spite of Clinical and Laboratory Standard Institute’s (CLSI) recommendations that clinical microbiology laboratories perform specialized tests for detection of ESBLs, many clinical microbiology laboratories...
laboratories make no effort to detect ESBL production, or are ineffective at doing so. In a 1998 survey of 369 American clinical microbiology laboratories, only 32 per cent (117 of 369) reported performing tests to detect ESBL production by *Enterobacteriaceae*. In a study from Europe, just 36 per cent of 91 ESBL-producing klebsiellae were reported by their original clinical laboratories as cefotaxime resistant. Situation of both ESBL prevalence and their detection is definitely worse in resource-poor settings of developing nations. Factors like overcrowding, poor nutrition, and hygiene status and lack of infection control measures combined with antibiotic misuse has led to high endemic levels of these resistant bugs. In some centers from India as many as 86 per cent of klebsiellae have been found to be ESBL producers. An ESBL prevalence and their detection is definitely worse in resource-poor settings of developing nations. Factors like overcrowding, poor nutrition, and hygiene status and lack of infection control measures combined with antibiotic misuse has led to high endemic levels of these resistant bugs. In some centers from India as many as 86 per cent of klebsiellae have been found to be ESBL producers.

**CLSI recommended methods for ESBL detection**

As ESBLs are found throughout the world, numerous detection strategies have been developed. According to CLSI, screening methods for ESBL producers include disk diffusion and dilution antimicrobial susceptibility tests.

(i) Disk diffusion methods: The CLSI has proposed these for klebsiellae, *Escherichia coli*, and *Proteus mirabilis*. Laboratories using disk diffusion methods for antibiotic susceptibility testing can screen for ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production. Cefpodoxime, ceftazidime, aztreonam, cefotaxime, or ceftriaxone are used. However, the use of more than one of these agents for screening improves the sensitivity of detection. If any of the zone diameters indicates suspicion for ESBL production, phenotypic confirmatory tests should be used to ascertain the diagnosis.

Mention should be made of the use of cefpodoxime as a screening antibiotic since this antibiotic is not widely used in inpatient facilities. In 1995, Thomson and Sanders noted that cefpodoxime susceptibility by disk diffusion reliably discriminated between ESBL-producing *Klebsiella pneumoniae* and *E. coli*. The CLSI initially recommended a zone diameter of <22 mm for a 10 µg cefpodoxime disk as a suitable screening test for ESBL production. Unfortunately, the cefpodoxime screening test using a zone diameter of <22 mm lacks specificity when used to screen *E. coli* isolates for ESBL production. Therefore, the CLSI now recommends a change in the cefpodoxime screening breakpoint to <17 mm; that is, isolates with a cefpodoxime zone diameter of <17 mm should undergo phenotypic confirmatory tests for ESBL production. In the study by Jain and Mondal in this issue, the newly recommended breakpoints for cefpodoxime were used. When they compared cefpodoxime with cefotaxime, ceftazidime and aztreonam in screening method for ESBL production by disc diffusion, it was found that sensitivity and specificity for cefpodoxime was highest for ESBL-negative isolates of *Klebsiella pneumoniae*.

(ii) Screening by dilution antimicrobial susceptibility tests: The CLSI has proposed dilution methods for screening for ESBL production by klebsiellae and *E. coli*. Ceftazidime, aztreonam, cefotaxime, or ceftriaxone can be used at a screening concentration of 1 µg/ml. Growth at this screening antibiotic concentration (i.e., MIC of the cefalosporin >2 µg/ml) is suspicious of ESBL production and is an indication for the organism to be tested by a phenotypic confirmatory test. The originally proposed screening criterion for cefpodoxime was that isolates which were potential ESBL producers had cefpodoxime MIC of >2 µg/ml. However, in a study of the mechanisms of decreased susceptibility of *E. coli* to cefpodoxime, Oliver *et al.* found that none of 59 isolates with cefpodoxime MICs of 2 or 4 µg/ml produced ESBLs. The most common mechanism of reduced susceptibility to cefpodoxime was production of the TEM-1 β-lactamase associated with the loss or alteration of a major porin protein. Other isolates lacked production of TEM-1 but had porin changes, sometimes coupled with modest elevation in production of the AmpC chromosomal β-lactamase. Finally, some of the isolates produced the OXA-30 β-lactamase. There are neither reports of clinical failure nor of outbreaks of infection with ESBL-negative isolates of *E. coli* with cefpodoxime MICs >2 µg/ml. Therefore, a more clinically useful screening test for cefpodoxime is to use a cefpodoxime MIC of ≥8 µg/ml, as a trigger to perform phenotypic confirmatory tests for ESBL production. In the study by Jain and Mondal using a cut-off of 8 µg/ml for cefpodoxime, 98.2 per cent isolates met the criteria for ESBL production while ceftazidime screened only 96.5 per cent isolates as ESBL producers, thus demonstrating the superiority of this agent as a screening method above other cefalosporins.

**ESBLs and the inoculum effect**

The degree of resistance against third generation cephalosporins can vary among different ESBL enzymes. This may in part be due to the inoculum effect. *In vitro*, the MICs of cefalosporins rise as the inoculum of ESBL-producing organisms increases. For example, for a
K. pneumoniae isolates producing TEM-26, at an inoculum of $10^5 \text{ cfu/ml}$ the cefotaxime MIC was 0.25 $\mu$g/ml, rising to 64 $\mu$g/ml when the inoculum rose to $10^7 \text{ cfu/ml}$. Some, but not all, animal studies have demonstrated that the inoculum effect may be clinically relevant. In animal models of infection, failure of cefalosporin therapy has been demonstrated despite levels of antibiotics in serum far exceeding the MIC of the antibiotic when tested at the conventional inoculum of $10^5 \text{ cfu/ml}$. Intra-abdominal abscesses and pneumonia are some of the clinical settings where organisms are present in high-inoculum, physicians should avoid cephalosporins if an ESBL-producing organism is present.

**Phenotypic confirmatory tests for ESBL production**

The CLSI advocates use of cefotaxime (30 $\mu$g) or ceftazidime disks (30 $\mu$g) with or without clavulanate (10 $\mu$g) for phenotypic confirmation of the presence of ESBLs in Klebsiellae and E. coli. A difference of >5 mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disk is taken to be phenotypic confirmation of ESBL production. It should be emphasized that both cefotaxime and ceftazidime with and without clavulanate should be used. One reason for this is that the use of ceftazidime alone has resulted in the inability to detect CTX-M-producing organisms.

**Broth microdilution**

Phenotypic confirmatory testing can also be performed by broth microdilution assays using ceftazidime (0.25 to 128 $\mu$g/ml), ceftazidime plus clavulanic acid (0.25/4 to 128/4 $\mu$g/ml), cefotaxime (0.25 to 64 $\mu$g/ml), and cefotaxime plus clavulanic acid (0.25/4 to 64/4 $\mu$g/ml). Again it should be emphasized that both ceftazidime and cefotaxime should be used. Broth microdilution is performed using standard methods. Phenotypic confirmation is considered as a >3-two fold serial dilution decrease in MIC of either cephalosporin in the presence of clavulanic acid compared to its MIC when tested alone.

Quality control recommendations should be followed when performing screening and phenotypic confirmatory tests. Simultaneous testing with a non-ESBL-producing organism (E. coli ATCC 25922) and an ESBL-producing organism (K. pneumoniae ATCC 700603) should also be performed.

**Implications of positive phenotypic confirmatory tests**

According to CLSI guidelines, isolates which have a positive phenotypic confirmatory test should be reported as resistant to all cephalosporins (except the cephemycins, cefoxitin, and cefotetan) and aztreonam, regardless of the MIC of that particular cephalosporin. Penicillins (for example, piperacillin or ticarcillin) are reported as resistant regardless of MIC, but beta-lactam/ beta-lactamase inhibitor combinations (e.g., piperacillin-tazobactam, amoxicillin-clavulanate, and ampicillin-sulbactam) are not affected by this rule and should be reported as obtained during routine susceptibility tests. The phenotypic confirmatory tests are highly sensitive and specific compared to genotypic confirmatory tests. However, there are a number of instances where the phenotypic confirmatory tests may be falsely positive or negative. K. pneumoniae or E. coli isolates which lack ESBLs but which hyperproduce SHV-1 may give false positive confirmatory tests. There are numerous reports in which K. pneumoniae isolates have been found to harbour plasmid-mediated AmpC-type $\beta$-lactamases. Some of these organisms have been found to harbour both AmpC-type $\beta$-lactamases and ESBLs. The co-existence of both enzyme types in the same strain results in elevated cephalosporin MICs but may result in false negative tests for the detection of ESBLs. The likely explanation is that AmpC-type $\beta$-lactamases resist inhibition by clavulanate and hence obscure the synergistic effect of clavulanate and cephalosporins against ESBLs.

**Commercially available methods for ESBL detection**

Commercially available methods include E test from AB Biodisk (Solna, Sweden), Vitek 2 (bioMerieux Vitek, Hazelton, Missouri), MicroScan panels from Dade Behring MicroScan (Sacramento, California, USA), BD Phoenix Automated Microbiology System from Becton Dickinson Biosciences (Sparks, Maryland, USA). In a recent study, in comparison to the conventional phenotypic confirmatory tests, the system with the highest sensitivity was Phoenix (99%), followed by Vitek 2 (86%) and MicroScan (84%); however, specificity was more variable, ranging from 52 (Phoenix) to 78 per cent (Vitek 2). In addition, the performance differed widely with the species investigated. In contrast, the three available E test strips and four disks combination (including ceftazidime, cefotaxime, cefpodoxime and cefpirome) showed sensitivity of 94 and 93 per cent, and specificity of 85 and 81 per cent, respectively. The double-disk test with ceftazidime, cefotaxime, cefpodoxime and cefpirome showed the highest specificity and positive predictive value among all test methods (i.e., 97 and 98%, respectively). Moreover, the semiautomated systems...
are costly and hence not affordable by many laboratories in resource-poor settings.

To conclude, ESBLs are paradigmatic as a mechanism of resistance because of the impact they have had on the therapy of infections and the insight they have offered on the relationship between structure and function, in antibiotics and in their determinants of resistance. Usually encoded on mobile genetic elements that accelerate their dissemination, the epidemiology of ESBLs illustrates the complex interactions between antibiotic use, selection and transmission of resistance, colonization and infection in different populations. The future development of novel beta-lactams resistant to hydrolysis by these versatile enzymes and the discovery of highly potent “second generation” beta-lactamase inhibitors are eagerly awaited. Considering the grave scenario of antibiotic resistance in our country, it is high time that all clinical laboratories start detecting the ESBLs routinely and accurately.

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