Detection of extended spectrum β-lactamase production in clinical isolates of *Klebsiella* spp.

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**Background & objectives**: Clinical laboratories need to develop quick screening methods for detection of extended spectrum β-lactamase (ESBL) producing strains, so that the appropriate medication can be started without delay. In this study, we report the screening sensitivity of four representative antimicrobial agents *i.e.*, cefpodoxime, cefotaxime, ceftazidime and aztreonam, commonly used for ESBL detection in *Klebsiella* spp.

**Methods**: A total of 100 clinical isolates of *Klebsiella* spp. from the cases of neonatal septicaemia at a tertiary care hospital from north India, were screened for ESBL production by Kirby- Bauer’s disc diffusion (cefpodoxime, cefotaxime, ceftazidime and aztreonam) and minimum inhibitory concentration (MIC) test by agar dilution methods. Confirmation was done by double disc method.

**Results**: Results showed that 58 of the 100 isolates tested were ESBL positive by confirmatory test and cefpodoxime was more efficient ESBL screening antimicrobial agent than ceftazidime, cefotaxime and aztreonam.

**Interpretation & conclusions**: Using the standard disk diffusion as screening test for identifying ESBL producers, cefpodoxime was found to be the most efficient antimicrobial agent in screening isolates as potential ESBL producers followed by ceftazidime in *Klebsiella* spp. isolates.

**Key words** Cefpodoxime - ESBL - *Klebsiella* spp. - neonatal septicaemia

The most common method of testing for extended spectrum β-lactamases (ESBLs) is screening for reduced susceptibility to cefpodoxime/cephotaxime/ceftaxime/ceftazidime followed by phenotypic confirmatory testing by demonstrating a synergistic effect between an indicator cephalosporin and a β-lactamase inhibitor *i.e.*, clavulanic acid. The sensitivity of screening for ESBLs can vary depending on the type of antimicrobial agent tested. According to Clinical Laboratory Standards Institute (CLSI) M100-S13 guidelines, use of more than one of the five indicator cephalosporins suggested for screening improves the sensitivity of ESBL detection. Some studies reported that if it should be necessary to rely on a single screening substance, cefpodoxime or ceftazidime would be the best choice for ESBL detection in *Klebsiella* spp.

In the present study, we evaluated the screening sensitivity of four representative antimicrobial agents *viz.*, cefpodoxime, cefotaxime, ceftazidime and aztreonam for the detection of ESBL in *Klebsiella* spp.
Material & Methods

A total of 100 clinical isolates of *Klebsiella* spp. isolated from 2995 blood samples from the same number of suspected cases of neonatal septicemia, referred to the Department of Microbiology, C. S. M. Medical University, Lucknow, for culture during a period of three years (January 2004 - December 2006) were included in the study. All *Klebsiella* spp. isolates were screened for ESBL production by Kirby- Bauer’s disc diffusion method\(^6\) demonstrating reduced susceptibility to cefpodoxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), and aztreonam (30 µg). Minimum inhibitory concentration (MIC) of all isolates was tested for cefpodoxime, cefotaxime and ceftazidime by agar dilution method using different drug concentrations (0.2 -128 µg/ml)\(^5\).

Cut-off zone sizes as an indicator of ESBL producer were < 27 mm for cefotaxime, < 22 mm for ceftazidime, < 27 mm for aztreonam and <17 mm for cefpodoxime. Isolates with a breakpoint of MIC > 2 µg/ml for cefotaxime, ceftazidime and aztreonam and > 8 µg/ml for cefpodoxime were designated as ESBL producers\(^5\). All the antibiotics and media were purchased from Hi-media laboratories, Mumbai (India) and *Escherichia coli* ATCC25922 was used as standard strain for quality control testing of antibiotics [Kindly provided by Christian Medical College & Hospital (CMCH), Vellore].

The combined disk method was used to confirm the presence of ESBL on all the isolated isolates of *Klebsiella* spp. by placing a disk of ceftazidime (30 µg) alone and ceftazidime (30 µg) in combination with clavulanic acid (10 µg) on a Muller-Hinton (M-H) agar plate\(^5\). The disks were placed at least 20 mm apart from each other on M-H agar plate. Any of the following two parameters was taken as indicator of ESBL production: (i) the zone diameter around ceftazidime + clavulanic acid disc is >5mm larger than that around ceftazidime disk, and (ii) the ratio of zone diameter around disks with ceftazidime + clavulanic acid and ceftazidime alone is >1.5\(^7,8\).

Of the 100 clinical isolates of *Klebsiella* spp. from neonatal intensive care unit, 58 were ESBL positive after confirmatory test. Screening test result for ESBL production revealed that four cefpodoxime sensitive, six cefotaxime sensitive, 11 ceftazidime sensitive and 9 aztreonam sensitive isolates were ESBL positive on confirmatory test. The sensitivity and specificity for cefpodoxime was highest among tested antibiotics for screening of ESBL production in *Klebsiella* spp. by disc diffusion method (Table). MIC determined by agar dilution method also showed that cefpodoxime was more accurate than other tested antibiotics for screening ESBL production as 98.2 per cent isolates met the criteria for ESBL production by cefpodoxime, while ceftazidime screened only 96.5 per cent isolates as ESBL producers (Table).

The degree of resistance against third-generation cephalosporins can be highly variable among different ESBL enzymes and the sensitivity of screening for ESBL can vary depending on the type of antimicrobial agent tested. While some ESBL enzymes confer frank resistance to extended spectrum cephalosporins, many isolates test only intermediate resistant or even susceptible to one or more of these antimicrobial agents, despite carriage of an ESBL\(^9\). This may be at least in part due to the inoculum effect. It is reported\(^10,11\) that ESBL producers appear susceptible at a standard inoculum of 10\(^5\), but have highly elevated MICs at higher inocula of 10\(^6\) or 10\(^7\). As demonstrated by animal models of endocarditis and intra-abdominal abscess, there are many types of infections in which the bacterial load could reach these levels\(^12,13\). The inoculum effect is typically seen with the third generation cephalosporins, cefotaxime, ceftriaxone and ceftazidime. A review article referring to studies reporting CLSI disk diffusion or MIC breakpoints of collections of ESBL producing organisms showed that 13 to 49 per cent of isolates were cefotaxime

### Table

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<th>Antibiotics</th>
<th>Sensitivity of DD</th>
<th>Specificity of DD</th>
<th>Sensitivity of MIC</th>
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<td>Aztreonam</td>
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DD, disk diffusion; MIC, minimum inhibitory concentration
susceptible, 11 to 52 per cent ceftazidime susceptible, 36 -79 per cent ceftriaxone susceptible and 10-67 per cent aztreonam susceptible14. Approximately 40 per cent tested isolates were susceptible to at least one oxyimino β-lactam and 20 per cent to all oxyimino β-lactams due to various degrees of hydrolysis of cephalosporins by different β-lactamase and enhanced penetration through the outer bacterial membrane of cephalosporins compared to others15.

It is mandatory that the routine clinical laboratories employ efficient ESBL detection methods, which are sensitive enough to recognize the level of resistance that would be achieved by the situation given in vivo. Although high level ESBL producers are detectable by using standard disk and MIC interpretative criteria, many other enzymes do not always increase the MIC to level high enough to be called clearly resistant by the standard interpretive criteria when tested at standard inoculum15. CLSI has proposed revised disk diffusion and MIC interpretative criteria3, which should be applied when using these techniques in a screening mode. Notably, most ESBL screening breakpoints are below the MIC upper limit for susceptibility, the only exception being cefpodoxime. For cefpodoxime, some investigators demonstrated that screening with this antimicrobial can lead to a high number of false positives if the former breakpoint of <22mm or <2μg/ml for disk diffusion and MIC testing respectively, were applied16. Therefore, the CLSI has reevaluated the susceptibility testing data and advocated a new breakpoint for cefpodoxime i.e., ≤17 mm for disk diffusion and ≤ 8 μg/ml for MIC. In this study, we found that cefpodoxime was most efficient antimicrobial agent in screening isolates as potential ESBL producers.

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References


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