

Detection of extended spectrum beta-lactamase from clinical isolates in Davangere

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ABSTRACT

Extended spectrum β -lactamase enzymes (ESBLs) are enzymes that have the ability to hydrolyze oxyiminocephalosporins and infections by isolates producing them are often difficult to treat. A study to detect the presence of these enzymes in isolates was conducted by our hospital. A total of 207 non repetitive isolates were screened for resistance to any of five screening agents. Those with suspicious profiles were checked for ESBL production by double-disk approximation or a synergy test. The isolates were also subjected to a phenotypic confirmation test as recommended by CLSI (formerly NCCLS). Various cephalosporins- β -lactamase inhibitor combinations were also tested. Of the 204 (98.5%) screen-positive isolates, only 126 (61.7%) were identified as ESBL producers. Of these, 26.1% of the isolates were positive by using the double-disk synergy test (DDST) method alone, 13.4% were positive using the method recommended by CLSI, and 60.3% of the isolates were positive by both the DDST and CLSI methods. We also report a high percentage of resistance to cefoxitin (96.8%) indicating changes in porins.

KEY WORDS: Cefoxitin resistance, double-disk synergy test, extended spectrum β -lactamase enzyme, oxyimino-cephalosporins

INTRODUCTION

Resistance to third generation oxyimino-cephalosporins is mediated by extended spectrum β -lactamase enzymes (ESBLs),^[1] which are derivatives of narrow spectrum TEM and SHV β -lactamases. ESBLs are defined as β -lactamases capable of hydrolyzing oxyimino-cephalosporins (but not cephamycin such as cefoxitin and cefotetan). They are inhibited by clavulanic acid and are placed into Bush's functional group 2be.^[2] The diversity of ESBLs results in various susceptibility profiles with different β -lactam antibiotics. Some variants (TEM-3 and -4) give high-level resistance to all second and third generation cephalosporins, while other variants (TEM-10, -12, and -26) give obvious resistance to ceftazidime but give moderate resistance to cefotaxime, ceftriaxone, and to the fourth generation cephalosporins. ESBL production is known commonly to occur in *E. coli* and *Klebsiella* but have also been found in other members of the Enterobacteriaceae family.^[3] ESBL-producing bacteria may appear falsely susceptible when tested by routine in vitro susceptibility methods and such a resistance to cephalosporins is not always obvious in disc or dilution tests.^[4] Even though certain strains may demonstrate in vitro susceptibility, there have been instances of clinical failure.^[5] Similarly, instances of ESBL-producing strains successfully treated by cephalosporins have also been documented.^[6] There has been no detailed study till date on ESBL detection from this part of the country.

MATERIALS AND METHODS

A total of 207 randomly chosen non repetitive isolates obtained from cultures of various

specimens such as urine (100), pus (52), blood (22), sputum (24), stool (1), suction tip (2), bronchial lavage (1), and vaginal swab (1) were studied for ESBL production. The samples were obtained from both outpatients and from those admitted to the hospitals attached to our medical college between January and August 2006. A total of 204 isolates that included *E. coli* (73), *K. pneumoniae* (28), *P. mirabilis* (12), *E. cloacae* (9), *K. oxytoca* (2) and one isolate each of *M. morganii* and *Providencia* sps were obtained following culture. Each strain was screened for possible ESBL production by testing against 30 μ g each of ceftazidime, cefotaxime, ceftriaxone, and aztreonam and 10 μ g of cefpodoxime. Colonies were picked up and emulsified in sterile saline, adjusted to 0.5 McFarland standard and inoculated on Mueller Hinton (MH) agar plates. The zone diameters were carefully measured by scale and an interpretation was made as per recent Clinical and Laboratory Standards Institute (CLSI) recommendations. Every isolate that showed resistance to at least one of the screening agents was tested for ESBL production by both the double-disk synergy test (DDST method) described by Jarlier, *et al.*^[7] and the phenotypic confirmation test recommended by CLSI (formerly NCCLS).^[8] Even though the present recommendations by CLSI are only for *E. coli*, *K. pneumoniae*, and *P. mirabilis*, we took the liberty of extending it to other members of the Enterobacteriaceae family.

Additionally, we compared zone sizes to cefoperazone (75 μ g) and cefoperazone+sulbactam combination (75 μ g +15 μ g), ticarcillin (75 μ g) alone and ticarcillin+clavulanic acid

combination (75 µg+10 µg), and piperacillin (100 µg) alone and piperacillin+tazobactam combination (100 µg+10 µg) on separate plates. All isolates were also tested against cefoxitin (30 µg), cefepime (30 µg), and imipenem (10 µg). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 strains served as controls.

RESULTS AND DISCUSSION

A total of 204 (98.5%) isolates out of 207 screened were presumptively considered ESBL-positive on the basis of their resistance to the five screening agents. The predictive value was highest with cefpodoxime (79.2%) and least with aztreonam (62.3%). The role of cefpodoxime as a good screening agent for *E. coli* and *K. pneumoniae* has been described before.^[17] All ESBL producers (except one *Morganella* isolate) were uniformly resistant to all screening agents. We conclude from our study that any of the five screening discs can be used to look for potential ESBL producers. However, it was observed that not all screen positive isolates were ESBL producers. Thus, there may be other mechanisms of resistance to these agents. In a similar study by Lee, *et al.*^[9] only 7.8% *E. coli* and 30.2% *K. pneumoniae* isolates that were cefoxitin-susceptible and cefpodoxime disk screen-positive were found to be positive for ESBL indicating that cefpodoxime resistance does not necessarily signify ESBL production. Overall, ESBL was detected in 126 (61.7%) out of 204 screen-positive isolates. Of the 126 isolates, 33 (26.1%) isolates were positive by the DDST method but negative by the CLSI method. Only 17 (13.4%) out of 126 were positive by the CLSI method and negative by the DDST method. A total of 76 (60.3%) isolates were positive by both the DDST and CLSI methods. Of the total 126 ESBL-positive isolates, the DDST method detected 109 (86.5%) and the CLSI method detected 93 (73.8%) cases. ESBL production was highest in *Proteus* spp (70.5%) and almost equally in *E. coli* (62.9%) and *Klebsiella pneumoniae* (62.2%). Since the numbers of *Enterobacter* spp, *Morganella morganii*, and *Klebsiella oxytoca* tested were far too few, their results are statistically irrelevant. Detection rates across the country have been varied, the presence of ESBL have ranged from 27.2% to 63.7% in *E. coli* and 14% to 97.1% in *K. pneumoniae*. Table 1 compares the present study with other studies across India.

It is not possible for all laboratories to perform both DDST and CLSI phenotypic tests routinely. Our study indicates that the DDST method (86.5%) is better than CLSI method (73.8%) in detecting ESBL producers, but we would have missed 17 cases had we performed only DDST tests and we would have missed 33

cases had we performed only the phenotypic test recommended by CLSI. Since there is some variance between the two methods, it is difficult to detect false positives, if any. All the four discs were useful in detecting zone enhancement towards the disc of Amoxicillin-Clavulanic acid combination (amoxyclav); however, the activity in decreasing order, were to cefotaxime, ceftazidime, aztreonam, and ceftriaxone. All the four discs produced an enhanced zone of inhibition towards amoxyclav in 68.8% isolates, while enhancement by three discs were observed in 19.2% isolates, two discs in 9.1% isolates, and a single disc in 2.7% isolates. Even though ceftazidime or cefotaxime is recommended, we believe that the use of all four discs enhances the sensitivity of the DDST. ESBLs do not confer resistance to cefoxitin, however, cefoxitin resistance among ESBL-positive isolates in our study was 96.8%. Resistance to cefoxitin could be due to mutations resulting in impermeability through porins and is being described more often than before.^[10] The high degree of resistance to piperacillin (93.4%), ticarcillin (78.8%), cefepime (64.2%), and cefoperazone (92.8%) was also noted in this study. All the strains were uniformly susceptible to imipenem.

Our study indicated that the combinations of inhibitors (clavulanic acid, sulbactam, and tazobactam) with cephalosporins are not equally effective. The combination of ceftazidime and clavulanic acid was ineffective against any isolate, however 24% of the isolates were susceptible to a combination of ticarcillin and clavulanic acid. Similarly, 75% of the isolates were inhibited by cefoperazone in the presence of sulbactam and 76% of the isolates were inhibited by piperacillin in the presence of tazobactam. Resistance was highest to ceftazidime/clavulanic acid (83.7%) followed by ticarcillin/clavulanic acid (24.3%), and cefoperazone/sulbactam (9.7%). Resistance was least to the combination of piperacillin and tazobactam (5.8%). In a similar study by Baby Padmini, *et al.*,^[16] isolates had displayed 100% susceptibility to piperacillin/tazobactam. We conclude that piperacillin/tazobactam is the best combination against ESBL producers, even though piperacillin/tazobactam is the most active penicillin/β-lactamase inhibitor combination against *Klebsiella* isolates with extended-spectrum β-lactamases; some variations have also been documented.^[18] Besides, the combination of piperacillin and tazobactam is known to suffer from the "inoculum effect".^[19]

We evaluated the use of other β-lactam/β-lactamase inhibitor combinations in addition to the phenotypic method recommended by CLSI. An increase in zone size by ≥5 mm to a combination of piperacillin and tazobactam was observed in 99.1% of ESBL producers and 82.5% of ESBL non producers. Similarly, zone enhancement to a combination of cefoperazone and sulbactam was observed in 96% of ESBL producers and 86.2% of ESBL non producers. Enhancement to a combination of ticarcillin and clavulanic acid was observed in 72.3% of ESBL producers and 57.1% of ESBL non producers. High percentages of zone enhancement were seen even against non ESBL producers; leaving us to wonder if they were due to the presence of other β-lactamases or false positives due to yet unknown reasons. Only additional studies involving enzyme characterization and

Table 1: Comparative studies

Authors	Year published	<i>E. coli</i> (%)	<i>K. pneumoniae</i> (%)
Jain, <i>et al.</i> ^[13]	2003	63.6	86.6
Baby Padmini, <i>et al.</i> ^[11]	2004	41	40
Ananthan, <i>et al.</i> ^[20]	2005	27.2	23.6
Kumar, <i>et al.</i> ^[12]	2006	63.7	14
Present study	2007	62.9	62.2
Prabha Lal, <i>et al.</i> ^[14]	2007	-	97.1
Varsha Gupta, <i>et al.</i> ^[15]	2007	63.8	76.2

detection at the genetic level may provide clues. Clavulanic acid failed to protect hydrolysis of ceftazidime in any isolate. Even though an increase in zone diameter around ticarcillin-clavulanic acid by >5 mm was seen in 33% of the isolates, only 24% of the isolates had been rendered susceptible to ticarcillin by the addition of clavulanic acid. Similarly, an increase in zone diameter around cefoperazone-sulbactam was observed in 82% of the isolates, but only 75% were rendered susceptible to cefoperazone by sulbactam. Likewise, of the 86% of isolates that exhibited increased zone around piperacillin-tazobactam, only 76% were actually susceptible to piperacillin due to the action of tazobactam. We observed that even though the enhancement of zone diameter by ≥ 5 mm was seen against many isolates, β -lactamase inhibitors did not necessarily provide sufficient protection. Three ESBL non producing *E. coli* isolates had a zone diameter around ceftazidime/clavulanic acid disc less than that of ceftazidime alone. Among the ESBL positive isolates, three isolates each of *K. pneumoniae* and *E. coli*, two isolates of *E. cloacae*, and one isolate of *K. oxytoca* had zone diameters around ceftazidime-clavulanic acid less than that of ceftazidime alone. Similarly, one isolate of *Morganella* displayed decreased zone size around the disc containing cefoperazone-sulbactam and piperacillin-tazobactam than discs containing cefoperazone and piperacillin alone. We presume that this could be due to low levels of induction of some β -lactamase that had hydrolyzed the cephalosporin used in combination. From the findings of this study, we conclude the following: a) ESBL production among isolates other than *E. coli* and *K. pneumoniae* must also be looked for, b) negative test results may be due to the presence of other beta-lactamases and must be investigated, c) the use of multiple screening agents increases the sensitivity of DDST, d) ESBL producers need not always be susceptible to ceftazidime since porin mutations prevent drug entry, and e) imipenem and piperacillin/tazobactam are the most effective drugs against ESBL producers.

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