Original Article

Detection Of Extended Spectrum Of B-Lactamases In Utis Patients At Raipur Chhattisgarh

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Abstract

Enter obacteriaceae families are capable of producing the extended spectrum beta-lactamase (ESBL) enzyme, which results in high levels of resistance to all beta-lactam antibiotics. For effective antibiotic therapy, to stop the spread of resistance mechanisms, and for epidemiological reasons, their detection is crucial. 53 Gram negative isolates were detected using a standard methodology in the current investigation, which compared various phenotypic methods for ESBL detection. Antibiotic susceptibility testing (AST) was also performed on these isolates. Phenotypic confirmatory Double Disk Synergy Test (30 mm), Double Disk Synergy Test (30 mm), Modify Double Disk, and the ESBL E-Test were used to further validate the presence of ESBLs. 20 ESBLs were produced in the case of DDST (30 mm), and specificity for cefepime was 84%, rising to 86% in the case of cefepime combination. The sensitivity was high for Cefepime alone (92%), while in combination it was 87% and 92% with Ceftazidime and Cefotaxime. DDST (20mm) 15 ESBL productions was seen once more. MDDST (20mm) has a sensitivity of 94% and identified 20 isolates as ESBL. The generation of 20 ESBLs was seen in the ESBL E test, and Cefepime alone showed the highest level of sensitivity. As a result, it was discovered that cefepime was the most effective cephalosporin for ESBL detection, followed by cefotaxime, ceftazidime, and cefpodoxime.

INTRODUCTION

The ESBL are fast developing, plasmid-encoded *β*-lactamases that can hydrolyze any beta-lactamase antibiotic, including cephalosporins and azoteronam, but are blocked by clavulanic acid. They are produced by gram-negative bacteria that belong to the Enter obacteriaceae family. Older *β*-lactamases, also known as penicillinases, cause *β*-lactam antibiotics like penicillin, carbapenems, and cephamycin to lose their effectiveness against bacteria while becoming sensitive to third-generation cephalosporins. With the exception of cephamycins or carbapenems, ESBLs mediate resistance to extended spectrum cephalosporins (third generation cephalosporins, 3GCs), monobactams, and other antibiotics (Paterson *et al*., 2005). ESBLs are derived from mutations in the older *β*-lactamase TEM. CTX-M and SHV gene by one or more amino acid substitutions around the active site, in which (Paterson *et al*., 2005). The main way that beta-lactamases impede cell wall formation is by inhibiting the activity of trans peptidases through the covalent attachment to penicillin-binding protein (PBP), a peptidoglycan trans peptidase enzyme that catalyses the last steps in cell wall synthesis. The natural substrates of these enzymes are taken away by *β*-lactam antibiotics after blocking their active site. This stops the cell wall from cross-linking, and as a result, the cell eventually perishes from osmotic instability (Pitton, 1972). Beta-lactam antibiotics contain a *β*-lactam ring in their structure, which is destroyed by the *β*-lactamases enzyme when active water molecules are added. ESBLs have serine at their active site and attack the amide bond in the lactam ring of antibiotics as a result of a point mutation in previous *β*-lactamases.

MATERIALS AND METHODS

Sample collection and Processing

Over the course of 6 months, 250 clinical samples from patient units in medicine, surgery, gynaecology and obstetrics, paediatrics, and intensive care units (ICU) at various hospitals in Raipur, Chhattisgarh, were collected. These included urine samples particularly form the patients suffer from urinary tract infections. Regardless of the samples' ages and sexes, they were collected. According to accepted laboratory practices, the samples were processed, and isolates were identified (Bergey's, 1985).

Isolation and characterization

All clinical samples were initially inoculated on Mac-agar Conkey's media and incubated at 37 ± 2^0C for 24 hours in order to isolate the pathogens. Some major incubation, isolates' cultural properties were determined using several biochemical characterizations in accordance with accepted microbiological practices (Bergey's, 1985).

Antibiotic susceptibility testing

The disc diffusion technique was used to test the isolates' antibiotic susceptibility on Muller-Hinton agar (Prescott, 2003).

The suspension of inoculums was modified to meet the 0.5 Macfarland criteria for turbidity. Then, using lawn culture, this suspension was inoculated into a Muller-Hinton agar plate. Then, using sterile forceps, various antibiotic discs were positioned and gently squeezed to ensure good contact with the medium. After that, the plates were incubated for 24 hours at 37°C. As per CLSI 2008 guidance, the zone of inhibition was measured and interpreted.

Detection of ESBLs 2.4.1ESBLs Screening Test

By using the disc diffusion method or MIC estimation, screening methods can identify any third-generation cephalosporin antibiotic resistance, including that to cefotaxime, ceftazidime, ceftriaxone, cefpodoxime, or aztreonam. Using a sterile cotton swab, the test inoculum (0.5 McFarland's turbidity) was applied to the MHA. The CLSI guidelines state that isolates with inhibition zone sizes of 22 mm or less with ceftazidime (30 g), 25 mm or less with ceftriaxone (30g), 27 mm or less with cefotaxime (30g), 27 mm or less with aztreonam (30g), and 22 mm or less with cefpodoxime (10 g) were shortlisted for confirmation of ESBL production. Screen positive isolates were those that shown resistance to any of these antibiotic discs. These isolates underwent additional testing for the formation of ESBL using the Double disc synergy test (30 mm) and Double disc diffusion test (20 mm) methods (Prescott, 2003).

Confirmatory tests for ESBLs

Double Disk Synergy Test (30mm)

The isolated organisms that had tested positive for the production of ESBL were put to the DDST test. The MHA was covered with inoculums suspension that had been adjusted to Macfarland's normal turbidity of 0.5. The disc diffusion method incorporates the double-disk synergy method at 30 mm (DDS30) as an add-on to the common susceptibility test (Bradford PA) Placed on the Mueller-Hinton (MH) agar plate at a distance of 30 mm, centre to centre, from either a piperacillin/tazobactum (P/T; 20 and 10 g) disc, the CTX (30 g), CAZ (30 g), CTR (30 g), and CPD (10 g) discs (Hi-Media) were incubated for 18 to 48 hours at 35 0C. A uniquely shaped zone known as a "champagne-cork," "keyhole," "ellipsis," or "phantom image" was created when the inhibition zone around any of the four antibiotic discs was enhanced on the side of the tazo-containing disc, indicating the presence of ESBL.

Double Disk Synergy Test (20mm):-

On MH agar, an amoxicillin-clavulanate disc was manually positioned at a distance of 20 mm from centre to centre of the CTX, CAZ, CTR, and CPD discs. Double disc synergy test (30mm) interpretation criteria were comparable to those previously stated (Winokur *et al*., 2001; Paterson *et al*., 2005; Nakhaei Moghaddam *et al*., 2012). Any of these antibiotic discs that increased the zone of inhibition against piperacillin or tazobactum was regarded as an ESBL producer.

Modified Double Synergy Test:-

The MDDST, which employs the third generation cephalosporins cefotaxime, ceftriaxone, cefpodoxime, and fourth generation cefepime as well as an amoxicillin-clavulanate disc, was used to test a total of microorganism isolates for the development of ESBLs. For 24 hours, the plates were incubated at 37°. AmpC beta-lactamase is unaffected by fourth generation cephalosporin (Jarlier *et al*., 1988).

ESBL ETest:-

Ceftazidime and ceftazidime with clavulanic acid are the two gradients present in the E test ESBL strip (AB Biodisk, Solna, Sweden) (Pangon *et al*., 1989). The intersection of the inhibition ellipse and the edge of the E test strip is known as the MIC. When the ratio of ceftazidime to ceftazidime-clavulanic acid is 8 or above, there is an ESBL present. According to reports, the method's sensitivity as a phenotypic confirmatory test for ESBLs is from 87% to 100%, and its specificity is 95% to 100% (Pangon *et al.,* 1989; Bush *et al.,* 1989). The capacity to detect ESBL types, such as CTX- M-type enzymes, which preferentially hydrolyze cefotaxime is enhanced by the availability of cefotaxime and ceftazidime strips. (Bradford, 2001).

RESULTS

115 (46%) strains of the 250 different clinical samples from the tertiary care hospital included in this investigation exhibit considerable increase. The isolates were characterized using the gramme stain and a number of biochemical tests. *Escherichia coli* (n = 54), *Klebsiella pneumoniae* (n=32), *Pseudomonas aeruginosa* (n=21) and a typical *E. coli* (n=8) were identified as the causative agents. 21 isolates produced ESBLs, it was discovered with cefepime had the highest sensitivity and specificity of all four *β*-lactams in the Double Disk Synergy Test 30 mm, 81% and 95%, respectively. When used in conjunction with cefepime, the sensitivity was further increased to 84%, but the specificity remained the same. This approach also had the highest number of false negatives (Table 2). Again, the sensitivity was high for Cefepime alone in the Double Disk Synergy Test 20 mm (87%), but it was 87% and 89% with Ceftazidime and Cefotaxime in combination. In compared to the Double Disk Synergy Test 30 mm, the false negative rate is lower. The sensitivity of the modified double disc synergy test (20mm) was 92%, and it was able to detect one more *E. coli* isolate that was missed by the other approach. (Table 3). The single drug with the highest sensitivity in the ESBL E test was cefepime.

DISCUSSION

ESBL production were detected by two-step method as recommended by the CLSI, this study demonstrates the presence of ESBL-mediated resistance in gram-negative bacilli causing infections in various wards and ICU of a tertiary hospital

in Raipur. Even while only a few studies have examined the incidence of ESBL producers in Indian hospitals, it's possible that these bacteria have emerged in a number of hospitals across the nation. The significant rate of resistance found among the isolates in the current investigation, which suggests a multidrug resistance pattern, is quite concerning. Co-transmission of ESBL and resistance to other antimicrobials within the same conjugative plasmids is one potential method for co-resistance, while the exact mechanisms are unknown. ESBL can be detected in clinical samples with a high degree of sensitivity using the combination disc of cefotaxim and ceftazidime, according to prior research (Wiegand et al., 2007). In order to determine whether the recommended disc separation of 30mm was ineffective, Thompson and Sanders, 1992 performed the experiment at 20mm. In this study, it was found that a cefotaxim and ceftazidime combination had a sensitivity of 81% when employed in a double disc synergy test of 30 mm, but when cefepime was added, the sensitivity increased to 84%. The sensitivity of cefotaxim and ceftazidime combined, however, was considerably higher than the 20 mm test in the case of the double disc synergy test; it was 86%, and it increased even further to 89% when cefepime was employed in combination. Additionally, Cefepime combination was found to boost ESBL detection sensitivity as reported by (Linscott and Brown, 2005). The modified twin disc synergy test was reported by Jarlier *et al.* (1988) and Thompson and Sanders (1992) to boost the sensitivity of ESBL detection. They discovered that the method had a sensitivity of 92% when piperacillin-tazobactam was positioned in the middle and the other discs were positioned 20mm apart. They chose to use piperacillin-tazobactam instead of ampicillin-clavulinic acid because it has been shown that clavulinic acid may cause high levels of AmpC production in an organism that also produces ESBL and AmpC, and it may inhibit rather than protect the antibacterial activity of beta lactam, masking the synergy brought on by ESBL inhibition. The first line approach for ESBL detection in the case of an E-test is Cefoitaxime and Ceftazidime, as per manufacturer guidance. Cefepime was the most efficient cephalosporin in this trial, followed by cefoitaxim, ceftazidime, and cefpodoxime. Modified double disc synergy testing can further increase this sensitivity (20mm), similar findings was observed by Sturenberg *et al*., 2004; Garrec *et al*., 2011 ; However Cormician *et al*.,1996 showed maximum detection of ESBL by Ceftazidime.

CONCLUSION

It was noted that the prevalence of ESBL was observed in *E. coli* followed by *K. pneumonia*. The most effective cephalosporin was found to be cefepime, followed by cefoitaxim, ceftazidime, and cefpodoxime. With the use of the modified double disc synergy test (MDDST), this sensitivity can be further increased.

Table1: Distribution of ESBL isolates among the phenotypes methods

Method		No. of ESBL positive Isolates	No. of ESBL Negative Isolates	Total
DDST (30 mm)	Positive	17	2	19
	Negative	3	36	39
DDST (20 mm)	Positive	15	2	17
	Negative	1	36	37
MDDST	Positive	17	1	18
	Negative	2	36	38
ESBL E-test	Positive	19	2	21
	Negative	2	36	39

Table 2: Statistical analysis of the various parameters of phenotypic methods

Antibiotics & Combinations	All Isolates (53)			
	Sensitivity (%)	Specificity(%)	PPV(%)	NPV(%)
Double Disk Synergy Test (30 mm)				
Ceftazidime	79	100	100	92
Cefotaxime	80	100	100	92
Cefpodoxime	74	100	100	90
Cefepime	84	100	100	93
Ceftazidime + Cefotaxime	84	100	100	93
Ceftazidime + Cefepime	86	100	100	92
Cefotaxime + Cefepime	86	100	100	94
Double Disk Synergy Test (20 mm)				
Ceftazidime	82	100	100	93
Cefotaxime	84	100	100	94
Cefpodoxime	77	100	100	91
Cefepime	87	100	100	95
Ceftazidime + Cefotaxime	85	100	100	94
Ceftazidime + Cefepime	89	100	100	95
Cefotaxime + Cefepime	92	100	100	96
Modified Double Disk Synergy Test (20 mm)				
Cefpodoxime, Ceftazidime, Cefotaxime & Cefepime with piperacillin/tazobactam at centre	94	100	100	97
ESBL Etest				
Ceftazidime	84	100	100	93
Cefotaxime	86	100	100	95
Cefepime	92	99	99	96

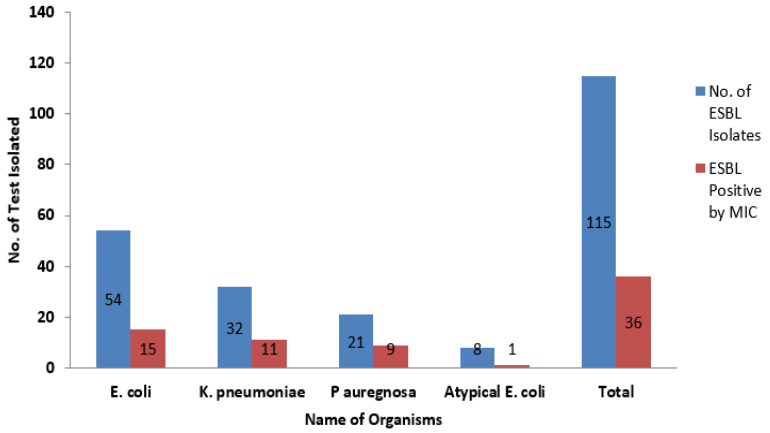

Fig. 1. Graph showing distribution of *ESBL* among tested organism

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