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Detection of Extended Spectrum Beta Lactamase Among Enterobacteriaceae from Various Clinical Samples in a Tertiary Care Hospital

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ESBL producing gram-negative organisms have posed a significant threat to hospitalized patients due to their hydrolyzing activity against extended spectrum cephalosporins often employed in the treatment of hospital-acquired infections. They are increasing rapidly and becoming a major problem in the area of infectious diseases. A delay in appropriate therapy can cause severe complication. The study was conducted on 75 isolates of enterobacteriaceae from various clinical samples._ESBL detection was done according to CLSI 2014 guidelines by Screening test (Disc diffusion method) and Confirmatory test (Combined Disc diffusion method). The most common isolate was *E.coli* (65.33%) followed by *Klebsiella* spp (26.66%) and proteus spp (8%). 45(60%) isolates were screening test positive and 30 (40%) were confirmatory test positive in ESBL detection tests according to CLSI guidelines. Maximum ESBL production was shown by Klebsiella spp (50%) followed by *E.coli* (38.77%). The prevalence of ESBL was found to be high in our hospital which cannot be ignored. We recommend disc diffusion test using multiple antibiotics in all microbiology units as a routine screening test and if possible the confirmatory test. The detection of ESBL producing strains will also help to establish and implement a strict infection control policy to stop the spread of ESBL producing organisms and in turn will reduce the morbidity and mortality of the patients.

Key words: ESBL, Enterobacteriaceae, CLSI.

Extended spectrum beta-lactamase (ESBL) producing organisms are those that hydrolyze the monobactams and oxyimino beta-lactams , but have no effect on the carbapenems and cephamycins.[1]They are inhibited by clavulanic acid and are placed into Bush's functional group 2be.[2] These enzymes are the result of mutations of Temorina (TEM-1and TEM-2) and Sulphhydryl variable (SHV-1) enzymes.[3] The total number of ESBLs characterized now exeeds 200.[4] The incidence of ESBL in major hospitals of India has been reported as high as 60% to 80%.[5] Being plasmid mediated, they are easily transmitted among the members of enterobacteriaceae, thus facilitating the dissemination of resistance not only to beta-lactams but also to other commonly used antibiotics such as quinolones and aminoglycosides.[6] ESBL are frequently encountered among clinical enterobacteriaceae, predominantly *Klebsiella pneumonia* and to a lesser extent *E. coli* and other species.[7] ESBL producing gram-negative organisms have posed a significant threat to hospitalized patients due to their hydrolyzing activity against extended spectrum cephalosporins often employed in the treatment of hospital-acquired infections.[8] They are increasing rapidly and becoming a major problem in the area of infectious diseases. Problems associated with ESBL producing isolates are

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difficult to be detected or treated, thereby causing increased mortality of patients. A delay in appropriate therapy can cause severe complication. The serious increase in the prevalence of ESBL's worldwide creates a need for effective and easy to perform screening methods for detection.[1]

MATERIALS AND METHODS

Study design-Observational cross sectional prevalence type of study. The guidelines of institutional Ethical committee were followed during the study.

Inclusion criteria

1) Specimens obtained from both genders and all ages.
2) Specimens collected from in patients and out patients.

Exclusion criteria

1) Isolates from in-situ drains or drainage bottles.
2) Isolates from stool.

The study was conducted on isolates of enterobacteriaceae from various clinical samples.

The isolates were identified based on colony morphology on blood agar, MacConkey agar and by standard biochemical tests.[9]

The antibiotic discs were obtained from HI MEDIA LTD, Mumbai.

ESBL Detection (according to CLSI 2014 guidelines).[10]

Screening test (Disc diffusion method)

Cefpodoxime(10µg), Ceftazidime(30µg), Aztreonam(30µg), Cefotaxime(30µg) and Ceftriaxone(30µg) were used. Zone diameters were measured in mm. Zones above as indicated in CLSI guidelines were taken as screen test positive for ESBL production.

Confirmatory test (Combined Disc diffusion method)

1) Ceftazidime(30µg) and Ceftazidime-clavulanic acid(30µg /10µg) and
2) Cefotaxime(30µg) and Cefotaxime-clavulanic acid(30µg /10 µg) were used.

A 5mm increase in zone diameter for either of these tested in combination with clavulanic acid VS its zone when tested alone was a confirmed ESBL.

RESULTS

As shown in table-1, a total of 75 enterobacteriaceae were obtained from various clinical specimens. Out of 75 clinical isolates, 41 were from urine, 14 were from sputum, 12 were from pus/wound swab, 4 were from blood, 4 were from body fluids.

Table-2 shows, the most common isolate was *E.coli* (65.33%) followed by *Klebsiella* spp (26.66%) and proteus spp (8%). 45(60%) isolates were screening test positive and 30 (40%) were confirmatory test positive in ESBL detection tests according to CLSI guidelines. All isolates that were negative by the screening test were also found to be non-ESBL producers by the confirmatory test. Maximum ESBL production was shown by Klebsiella spp (50%) followed by *E.coli* (38.77%).

DISCUSSION

Extended spectrum beta lactams are commonly included in the empirical antibiotic

Table 1. Specimen wise distribution of organisms

Clinical sample	*E.coli*	Klebsiella spp	Proteus spp
Urine	30	5	3
Sputum	8	9	1
Pus/wound swab	7	5	2
Blood	2	0	0
Body fluids	2	1	0
Total	49	20	6

Table 2. ESBL screening and confirmed enterobacteriaceae isolates

Organisms	Number of isolates	ESBL screening test positive	ESBL confirmed
E.coli species	49(65.33%)	28 (57.14%)	19(38.77%)
Klebsiella species	20(26.66%)	14 (70%)	10(50%)
Proteus species	6(8%)	3 (50%)	1 (16.66%)
TOTAL	75(100%)	45 (60%)	30 (40%)

regimens for treatment of gram negative sepsis. The increasing use of broad spectrum cephalosporins has become one of the major factors responsible for the high rate of selection of extended spectrum beta lactamase producing microorganisms.[11]

Similar to present study, in a study done by Christine et al, they showed that *Klebsiella pneumoniae* is a potential ESBL producer by comparing Ceftazidime to Ceftazidime plus Clavulanic acid and Cefotaxime to Cefotaxime plus Clavulanic acid.[12] ESBL producing *K.pneumoniae* is an important clinical pathogen responsible for life threatening infections.[13] ESBLs were predominantly present among *E. coli* (50%) compared to *Klebsiella* spp. (37.5%) in Wadekar MD study.[14] Nachimuthu Ramesh *et al.* have reported a high prevalence of ESBLs among *E. coli*.[15] In a study done by Amita jain et al, 78% of isolates that were positive in the screening test were also positive by the confirmatory testing.[16] In a study done by MS Kumar et al, ESBLs have been predominantly reported among *K.pneumoniae*. 85-95% of the *K.pneumoniae* and *E.coli* were resistant to cefotaxime and 37-53% were resistant to ceftazidime[17]. In a study done by Patwardhan Neeta Satish et al, major ESBL producer was *Klebsiellae* (21.48%) followed by *E.coli* (7%). ESBL screening positive was 67% and confirmed positive was 28%[18]. In a study done by Chelliah et al, ESBL was detected in 70.9% of *Klebsiella pneumonia* isolates and 57.14% of *E.coli* isolates[19]. In another study done by Kenneth S et al, routine disk diffusion tests yielded moderately susceptible or resistant results in 43% to 68% of tests in which one of the four drugs ceftriaxone, ceftazidime, cefotaxime or aztreonam was tested against ESBL-producing organisms. If both cefotaxime and ceftazidime were tested, the index of suspicion increased to only 82%.[20] In study done by Fatima H et al, sensitivity of double disc diffusion test for detecting ESBLs using discs containing ceftazidime was 86% and when using cefotaxime, the sensitivity was 65.5%. The sensitivity was increased up to 93% if the results obtained using both agents are taken into consideration.[21]

In another stuy done by Ejaz et al, comparison of DDST and CLSI confirmatory test showed that the DDST detected 145 (67.8%) isolates while 213 (99.5%) ESBL *K.pneumoniae* were characterized by CLSI confirmatory test, a*nd concluded that* the use of CLSI confirmatory test is very efficient in the early detection of ESBL *K. pneumoniae* especially when the facilities for molecular characterization are not available.[13]

The results of screening tests in predicting ESBL production, it is important to remember that negative results are a better guide than positive results. Following all positive results might lead to unnecessary avoidance of conventional beta lactams in a good number of cases.

Correct identification of ESBL positive Enterobacteriaceae in due time is mandatory not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organisms.[22]

CONCLUSION

To sum up prevalence of ESBL was found to be high in our hospital which cannot be ignored. *Klebsiella* species producing ESBL were predominant. ESBL producing *K. pneumoniae* is a serious concern for the hospitals. ESBL producers can be detected by disc diffusion test and phenotypic confirmatory test. We recommend disc diffusion test using multiple antibiotics in all microbiology units as a routine screening test and if possible the confirmatory test. The CLSI confirmatory test can be used reliably especially when the facilities for molecular charaterization are not available. The detection of ESBL producing strains will also help to establish and implement a strict infection control policy to stop the spread of ESBL producing organisms and in turn will reduce the morbidity and mortality of the patients.

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