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Research Article

Extended Spectrum B-Lactamases among Clinical Isolates of Enterobacteriaceae Ssp.: Prevalence and Susceptibility Pattern at A Tertiary Care Hospital

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Abstract: The purpose of this study was to know prevalence and susceptibility pattern of extended spectrum β-lactamases(ESBLs) at our tertiary care centre. A total of 660 non-repetitive clinical isolates of Enterobacteriaceae obtained from various clinical samples of which isolates that exhibited intermediate/ resistance to third generation cephalosporins were screened to detect ESBL producers by double disk synergy test. ESBLs were confirmed by phenotypic confirmatory test and E-test ESBL strips. Among 660 Enterobacteriaceae isolates, 125(18.9%) of which were ESBL producers. *E.coli* 53.6% was the largest group followed by *K. pneumoniae* 32.8% and all other species together comprised 13.6%. Continued monitoring of drug resistance is necessary in clinical settings for proper disease management.
Keywords: Extended spectrum β-lactamases (ESBLs), *E. coli, K. pneumoniae,* E-test.

INTRODUCTION

Among the various antibiotics, β-lactams are the most varied and widely used agents accounting for over 50% of all systemic antibiotics in use [1]. The most common cause of bacterial resistance to β-lactam antibiotics is the production of β-lactamases. β-lactamases continue to be the leading cause of resistance to β-lactam antibiotics in gram negative bacteria [2, 3]. Extended-spectrum β-lactamases (ESBLs) are mutant, plasmid-mediated B-lactamases derived from older, broad-spectrum β-lactamases and confer resistance to all extended-spectrum cephalosporins and aztreonam and inhibited by β-lactamase inhibitors (Clavulanic acid, Sulbactam and Tazobactam) but have no detectable activity against Cephamycins and Carbapenems(Impipenem, Meropenem) [4, 5]. ESBLs, although most commonly encountered in *Klebsiella spp.* and *Escherichia coli*, have also been detected in other gram-negative bacteria, including *Enterobacter, Salmonella, Citrobacter, Serratia marcescens, Proteus ssp.* and *Pseudomonas aeruginosa* [5, 6]. Extended spectrum B-lactamase producing strains of Enterobacteriaceae have emerged as a major problem in hospitalised as well as community based patients [7, 8]. These organisms are responsible for a variety of infections like urinary tract infection (UTI), septicaemia, hospital acquired pneumonia, intra-abdominal abscess, brain abscess and device related infections [9]. Major risk factors for colonization or infection with ESBL producing organisms are long term antibiotic exposure, prolonged ICU stay, nursing home residency, severe illness, residence in an institution with high rates of ceftazidime and other third generation cephalosporin use and instrumentation or catheterisation [10]. This study was undertaken to determine the prevalence and sensitivity pattern of Extended-spectrum β-lactamases in our tertiary care hospital.

MATERIALS AND METHODS

A total of 660 non-repetitive clinical isolates of Enterobacteriaceae from various clinical samples viz., blood, urine and exudates were included in this study and processed at Department of Microbiology, Pacific Medical College and Hospital, Udaipur, Rajasthan. The antibiotic sensitivity test was performed by disc diffusion technique with commercially available discs (Hi Media, Mumbai, India) on Muller Hinton agar plates. The discs used were Amikacin (30 ug), Gentamicin (10 ug), Ciprofloxacin (5 ug), Levofloxacin (5 mcg), Norfloxacin (10 ug), Nitrofurantoin (300 ug), Co-Trimoxazole (25 ug), Ceftazidime (30 ug), Cefaclor (30 ug), Cefixime (5 mcg), Ceftizoxime (30 ug), Cefotaxime (30 ug), Ceftriaxone (30 ug), Ceftazidime

/Clavulinicacid (30/10 ug), Ceftriaxone/Sulbactam (30/15 ug), Piperacillin/Tazobactam (100/10ug), Aztreonam (30 mcg), Meropenem (10 mcg), Imipenem (10 ug). Susceptibility and resistance was determined based on the interpretative criteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [11].

Screening for ESBLs by double disk synergy test

Enterobacteriaceae cultures that exhibited intermediate/resistance to third generation cephalosporins were screened to detect ESBL producers. A modified double disk synergy test first described by Jarlier et al in 1988 [12] was carried out, amoxicillin+clavulanic acid(20ug+10ug) disk was placed in the centre and the ceftazidime (30ug) and cefotaxime (30ug) disks were placed on either side at a distance of 15mm centre to centre from amoxicillin+clavulanic disk. Plates were incubated at 35^0C for 18-20 hours and the pattern of zones of inhibition was noted. Isolates that exhibited a distinct shape/size with potentiation towards amoxicillin+clavulanic disks were considered potential ESBL producers and short listed for confirmation of ESBL producers.

Phenotypic confirmatory test by disk diffusion method:

ESBL detection was performed as recommended by NCCLS confirmatory procedure using cefotaxime(30ug) and ceftazidime(30ug) discs alone and in combination with clavulanic acid. A >5mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL producing organism.

E-Test

The E-test was performed on Mueller Hinton agar accordance with the manufacturer's instructions. Briefly, after overnight growth, the colonies were suspended in saline to turbidity equal to that of a 0.5 McFarland turbidity standard. The suspension was used to inoculate Mueller Hinton agar plates by swabbing them with a cotton swab. After drying for 15 min, the Etest strips were placed on the plates and the plates were incubated for 18-24hrs at 35^0C. The MIC was interpreted as the point of intersection of the inhibition ellipse with the E-test strip edge. The E-test ESBL strip (Hi-media) carries two gradients, on the one end, ceftazidime and on the opposite end ceftazidime plus clavulanic acid. Ratio of ceftazidime MIC and ceftazidime clavulanic acid MIC equal to or greater than 8 indicates the presence of ESBL.

RESULTS

Six hundred and sixty Enterobacteriaceae species were recovered from different clinical specimens like blood, urine and exudates submitted for routine microbiological analysis. Antibiotic susceptibility test results by disk diffusion method revealed very high susceptibility to Imipenem (100%), Meropenem (100%) and Piperacillin-tazobactam (100%) followed by Amikacin (86%). Resistance to Cefotaxime, Ceftazidime, Gentamicin, Ciprofloxacin, Levofloxacin and Co-trimoxazole was found to be 100%, 100%, 89%, 84%, 76% and 83.2% respectively. One hundred and twenty five out of 660(18.93%) Enterobacteriaceae isolates were identified as potential ESBL producers by the double disk synergy test and confirmed by E-test ESBL strips. The Table 1 describes the different species that were identified as ESBL producers among clinical isolates. The identity of the ESBL positive isolates was as follows: *E.coli* with 53.6%(67/125) was the largest group followed by *K. pneumonia* 32.8%(41/125) and all other species together comprised 13.6% while 53.6%(67/125) were isolated from urine, 16.8%(21/125) were from blood and 29.6%(37/125) were from exudates.

Table 1: Identification of ESBL positive isolates (n=125)

Organism	No. of Isolates	No. of ESBL isolates (%)	% of ESBL isolates
E. coli	363	67(18.4)	n/67(53.6)
K. pneumonia	251	41(16.3)	n/41(32.8)
E. cloacae	15	12(80)	n/12(9.6)
Citrobacter spp.	18	3(16.6)	n/3(2.4)
Proteus	13	2(15.3)	n/2(1.6)
Total	660	125	660/125(18.9)

ESBL - Extended spectrum β-lactamase, n=125.

DISCUSSION & CONCLUSION

ESBLs are now a problem in hospitalized patients throughout the world. The prevalence of ESBLs among clinical isolates varies greatly worldwide and in geographic areas and are rapidly changing over time [13]. In the present study, out of 660 Enterobacteriaceae isolates, of which 18.93% were ESBL producers. Antibiotic susceptibility test results of the above isolates illustrated an alarming trend of associated resistance to gentamicin (89%), co-trimoxazole (83.2%), ciprofloxacin (84%) and Levofloxacin (76%). Such resistance has been reported in recent surveys from Canada, Italy, Spain, Greece and UK [14].

ESBLs have been predominantly reported among *K. pneumoniae* both in Europe and USA [15]. However in our study number of isolates were less in that also ESBLs were predominantly present among *E.coli*

(53.6%) compared to *K. pneumonia* (32.8%) and other Enterobacteriaceae spp. Our findings are correlates to that of MS Kumar *et al*. [16] and Ananthakrishnan *et al*. [17] who reported a high prevalence of ESBLs among *E.coli*.

ESBLs becoming an increasing problem in hospital and community setting, screening for the presence of these resistant pathogens would ultimately become a necessity, especially in high risk units where infections due to resistant organisms is much higher. Knowledge of resistance pattern of bacterial strains in a geographical area will help to guide the appropriate and judicious antibiotic use.

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