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Brief Communication

OCCURRENCE OF EXTENDED SPECTRUM ß-LACTAMASES AMONG *ENTEROBACTERIACEAE* SPP. ISOLATED AT A TERTIARY CARE INSTITUTE

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Abstract

Increasing resistance to third generation cephalosporins has become a cause for concern especially among *Enterobacteriaceae* that cause nosocomial infections. The prevalence of extended spectrum β-lactamases (ESBLs) among members of *Enterobacteriaceae* constitutes a serious threat to current β-lactam therapy leading to treatment failure and consequent escalation of costs. A detailed study was initiated to identify the occurrence of ESBLs among the *Enterobacteriaceae* isolates at a tertiary care hospital using the double disk potentiation technique. Antibiogram profiles were determined to commonly used antibiotics and confirmation of ESBLs production was carried out by the disk diffusion assay using ceftazidime and cefotaxime in the presence and absence of clavulanic acid. Our results indicate that the majority of ESBLs were expressed in *Escherichia coli*.

Key words: *Aztreonam, cefotaxime, ceftazidime, clavulanic acid, E. coli, ESBLs, K. pneumoniae*

Among the wide array of 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. Many of the second and third generation penicillins and cephalosporins were specifically designed to resist the hydrolytic action of major β-lactamases. However, new β-lactamases emerged against each of the new classes of β-lactams that were introduced and caused resistance. The latest in the arsenal of these enzymes has been the evolution of extended spectrum β-lactamases (ESBLs). These enzymes are commonly produced by many members of *Enterobacteriaceae*, especially *E. coli* and *K. pneumoniae* and efficiently hydrolyze oxyimino-cephalosporins conferring resistance to third generation cephalosporins such as cefotaxime, ceftazidime and ceftriaxone and to monobactams such as aztreonam.[2] First isolated in 1983 in Germany, ESBLs spread rapidly to Europe, US and Asia and are now found all over the world.[3] Being plasmid mediated, they are easily transmitted among members of *Enterobacteriaceae* thus facilitating the dissemination of resistance not only to β-lactams but to other commonly used antibiotics such as quinolones and aminoglycosides.

ESBLs have emerged as a major problem in hospitalized patients worldwide and have been involved in epidemic outbreaks in many institutions in Europe and USA and constitute a serious threat to the current β-lactam therapy as these enzymes cause resistance to most penicillins, cephalosporins and aztreonam.[4] Typically, nosocomial outbreaks were associated with previous antibiotic therapy, especially ceftazidime monotherapy. Hospital colonization by ESBL producing bacteria is usually a complex phenomenon involving many different mechanisms, dissemination of several epidemic strains and dissemination of plasmids and resistant genes.[5] Specific risk factors include prolonged hospital stay, severity of illness, ICU, urinary or arterial catheterization, intubation and mechanical ventilation. ESBLs commonly occur in surgical wards as well as most other areas of the hospital and frequently from patients from extended care facilities.[6]

Since ESBL positive isolates show false susceptibility to expanded spectrum cephalosporins in standard disk diffusion tests[7] it is difficult to reliably detect ESBL production by the routine disk diffusion techniques. Specific detection methods such as double disk potentiation methods recommended by NCCLS[8] have to be adopted. ESBLs are inhibited by β-lactamase inhibitors like clavulanic acid, sulbactam and tazobactam and this property of specific inhibition can be utilized for the detection and confirmation of ESBLs.

There have been few published reports regarding occurrence of ESBLs from the Indian subcontinent and most of the reports dealt with phenotypic identification of ESBLs.[9,10] This study was initiated to identify the incidence of ESBLs among *Enterobacteriaceae* isolated over a 12 month period at Nizam's Institute of Medical Sciences (NIMS), a tertiary care hospital at Hyderabad, India.

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Materials and Methods

Clinical isolates

A total of 1699 *Enterobacteriaceae* spp. culture isolates from different clinical specimens during the period of March 2000 to February 2001, were screened for potential ESBL activity. Based on routine antibiotic disk sensitivity tests, isolates that exhibited intermediate/resistance to any one of the third generation cephalosporins, ceftazidime/ cefotaxime were short listed to detect and confirm ESBL producers. Purified cultures were identified by both conventional and Enterorapid 24 test, a Micro-well ID system (Mikro Test- Lachema). *E. coli* ATCC 25922, ATCC 35218 and *K. pneumoniae* ATCC 70063 were used as controls to validate the susceptibility tests.

Antibiotics

The following antibiotic sensitivity disks used for primary screening were purchased from Hi-Media India: cefotaxime 30 μg, ceftazidime 30 μg, amoxicillin+ clavulanic acid (20 μg+10 μg). Antibiotic powders were kindly provided by the following companies: ceftazidime and lithium clavulanate- SmithKlineBeecham UK, ceftriaxone sodium, cefotaxime, ampicillin, amoxicillin- Ranbaxy Laboratories India, cefaperazone, cefixime, cefadroxil, cephalexin- Orchid Chemicals India, ciprofloxacin- Dr. Reddy's Laboratories India and gentamicin and chloramphenicol was procured from Sigma.

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 (disk approximation test) first described by Jarlier[11] was carried out, amoxicillin+clavulanic acid (20 μg+10 μg) disk was placed in the centre and the ceftazidime (30 μg) and cefotaxime (30 μg) disks were placed on either side at a distance of 15 mm centre to centre from the amoxicillin+clavulanic disk. Plates were incubated at 35°C 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 assay

ESBL production was confirmed among potential ESBL producing isolates by phenotypic tests. Sensitivity disks containing third generation cephalosporins with and without clavulanic acid were prepared as follows: ceftazidime 30 μg(Ca), ceftazidime+clavulanic acid 10 μg (Ca +), cefotaxime 30 μg (Ce), cefotaxime+clavulanic acid 10 μg (Ce+); and aztreonam 30 μg (AZT), aztreonam+clavulanic acid 10 μg (AZT+). Disk diffusion assay was carried out as per guidelines of NCCLS[8] and differences in zone diameters between disks with and without clavulanic acid were recorded.

Susceptibility profile (MIC) against select ESBL isolates

Minimum inhibitory concentration (MIC) tests were carried out by broth micro dilution[12] to determine the susceptibility profile of all *K. pneumoniae* (n=47) and select *E. coli* (n=43) against different classes of antibiotics.

Results

One thousand and six thousand and ninety-nine *Enterobacteriaceae* spp. were recovered from different clinical specimens like blood, urine and exudates submitted for routine microbiological analysis from both in and out patients of the hospital during the period March 2000 to February 2001. Data was analyzed by Whonet software to sort the identity and source of 1699 *Enterobacteriaceae* isolates. Three hundred and thirty-six out of 1699 (19.8%) *Enterobacteriaceae* isolates were identified as potential ESBL producers by the double disk potentiation test. The figure demonstrates the response seen by the position of the disks. The table describes the different species that were identified as ESBL producers among the clinical isolates. The identity of the ESBL positive isolates was as follows: *E. coli* with 63.7% (214/336) was the largest group followed by *K. pneumoniae* 14% (47/336), *Citrobacter* spp. 11.3% (38/336) and all the other species together comprised 11% while 14% (47/336) were isolated from blood, 39% (131/336) were from urine and 47% (158/336) were from exudates.

Phenotypic confirmation of ESBLs was carried out by disk diffusion assay as per the recommendations of NCCLS.[8] The zone of inhibition of the antibiotic alone was compared with the zone of inhibition in combination with clavulanic acid. According to NCCLS recommendations a difference of ≥5 mm increase in zone diameter for either agent tested in combination with clavulanic acid versus its zone diameter when tested alone confirms the presence of ESBLs. *K. pneumoniae* (n=47) and *E. coli* (n=43) exhibited a clear difference of ≥8 mm in zone diameter to ceftazidime/cefotaxime and aztreonam in combination with clavulanic acid.

Table: Identification of ESBL positive isolates (n=336)

Organism	No. of isolates	No. ESBL isolates (%)	% of ESBL isolates
E. coli	863	214 (24.8)	63.7
K. pneumoniae	464	47 (10.1)	14
E. cloacae	10	10 (100)	2.9
Citrobacter spp.	195	38 (19.5)	11.3
M. morganii	4	3 (75)	0.9
S. marcescens	2	2 (100)	0.6
Providentia spp.	3	3 (100)	0.9
Proteus spp.	97	14 (14.4)	4.2
Salmonella spp.	36	5 (13.9)	1.5
Others	25	0	0
Total	**1699**	**336**	**19.8**

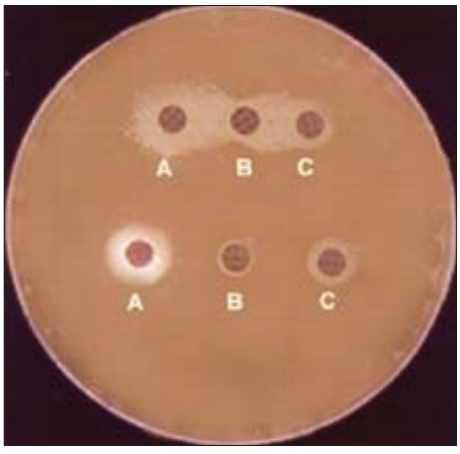

Figure: Identification of potential ESBL producers. A: Ceftazidime (30 μg), B: Amoxicillin clavulanic acid (20 + 10 μg), C: Cefotaxime (30 μg). Top row: Discs placed 15 mm centre to centre, Bottom row: Discs placed 20 mm centre to centre

Minimum Inhibitory Concentration (MIC) of select ESBL isolates

Forty-seven isolates of *K. pneumoniae* were resistant to cefazolin, cefoperazone and ampicillin (MIC ≥256 μg/mL) and 92% of the isolates exhibited MIC of ≥256 μg/mL against cefadroxil. Eighty-five percent of *K. pneumoniae* exhibited an MIC of ≥256 μg/mL to cefotaxime compared to 53% towards ceftazidime and 87% ceftriaxone. MIC of 83% of isolates was ≥256 μg /mL for gentamicin. Ciprofloxacin exhibited sensitivity wherein the MIC of 21% of isolates was 0.125 μg/mL, 10% showed MIC of 2 μg/mL and the MIC of remaining isolates was in the range of 16-256 μg /mL.

The sensitivity profile of *E. coli* (n=43) was almost similar to *K. pneumoniae*, all the isolates were resistant to ampicillin, cefazolin, cefoperazone and cefadroxil. More *E. coli* (95%) isolates were resistant to cefotaxime compared to 37% to ceftazidime (MIC ≥256 μg/mL). Twenty-eight percent of the isolates were sensitive to gentamicin (MIC 0.125 μg/mL) while MIC of the rest of isolates was in the range of 64-256 μg/mL. For ciprofloxacin MIC ranged from 64-256 μg/mL.

Discussion

There have been sporadic reports of ESBLs from major hospitals in India and some of them have recorded the incidence to be as high as 60-68%[13,14] but the sample numbers have been low. Our study indicates that 19.8% *Enterobacteriaceae* spp. (336/1699) isolated over a period of one year, were ESBLs producers. The unusually high incidence of ESBLs should be a cause of concern to the regulators of the hospital antibiotic policy. Over reliance on third generation cephalosporins to treat gram negative infections is one of the prime factors responsible for increased resistance to this class of antibiotics.

ESBLs have been predominantly reported among *K. pneumoniae* both in Europe and USA.[2] However, in our study analysis of the 336 confirmed ESBL isolates revealed that ESBLs were predominantly present among *E. coli* (63.7%) compared to *K. pneumoniae* (14%) and other *Enterobacteriaceae* spp. Our findings are similar to that of Ananthakrishnan *et al*[10] who reported a high prevalence of ESBLs among *E. coli*. The high incidence of ESBLs among *E. coli* may be peculiar to the Indian subcontinent.

While double disk potentiation test was a simple and convenient method to detect ESBLs, a phenotypic confirmatory test as recommended by NCCLS[8] is mandated to confirm the presence of ESBLs. *Enterobacteriaceae* spp. that exhibit resistance to any one of the third generation cephalosporins must be reported as resistant to all third generation cephalosporins. ESBLs are plasmid mediated and multidrug resistance is a characteristic feature of strains producing ESBLs. Our study confirms this observation, as ESBL isolates of *K. pneumoniae* and *E. coli* were resistant to different classes of antibiotics. Gentamicin and tobramycin typically demonstrate poor *in vitro* activity against ESBL producing organisms.[15] Such resistant isolates pose serious problems to the physicians as therapeutic options are limited. Carbapenams and cephamycins are uniformly active against ESBL positive isolates. Monitoring and judicious usage of extended spectrum cephalosporins, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy would go a long way in addressing some of the problems associated with ESBLs.

An interesting observation was the increased resistance of *K. pneumoniae* and *E. coli* isolates to cefotaxime as compared to ceftazidime, 85-95% of the *K. pneumoniae* and *E. coli* were resistant to cefotaxime (MIC ≥256 μg/mL) compared to 37-53% that were resistant to ceftazidime. We presume that the major ESBL enzyme being expressed in our isolates is a cefotaximase that preferentially hydrolyses cefotaxime. Further studies are warranted to establish the presence of cefotaximases.

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