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ARTICLE

Detection Of Extended Spectrum Beta-Lactamase Production And Multidrug Resistance In Clinical Isolates Of *E.Coli* And *K.Pneumoniae* In Mangalore

Shiju M P *, Yashavanth R **, Narendra N ***

ABSTRACT

Purpose

The incidence of Extended Spectrum ß –Lactamase (ESBL) producing strains among clinical *Klebsiella species* and *Escherichia coli* isolates has been steadily increasing over the past years. ESBL producing organisms pose a major problem for clinical therapeutics. Identifying organisms that are ESBL producers are a major challenge for the clinical microbiology laboratory. An attempt was therefore made to study ESBL production and multidrug resistance in clinical isolates of *K.pneumoniae* and *E.coli* at a hospital in Mangalore.

Method

ESBL production and multidrug resistance was studied in a total of 228 isolates of *K.pneumoniae* and *E.coli* which were obtained from various clinical samples during one year period from January to December 2008.

Identification of the isolates was done based on cultural characteristics and reactions in standard biochemical tests. All the isolates were tested for antimicrobial susceptibility by the disk diffusion technique according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The screening for ESBL production was done by the phenotypic confirmatory test using ceftazidime discs in the presence and absence of clavulanic acid.

Result

All the isolates showed resistance or decreased susceptibility to at least one of the third generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone) which were used for the study. ESBL production was noted in 59.65% of the isolates tested. ESBL production was detected in 51.47% strains of *E.coli* and 48.53% strains of *K.pneumoniae* (70% of *E.coli* isolated from urine samples and 75% of *K.pneumoniae* isolated from exudates samples were ESBL producers). All the isolates were found to be sensitive to the antibiotic, imipenem. Sensitivity of *E.coli* to piperacillin-tazobactum (Pt) and cefaperazone-sulbactum (Cfs) was 100%, whereas *K.pneumoniae* showed 98% sensitive to Pt and 88% sensitive to Cfs.

Conclusion

The study has shown an increase in the incidence of ESBL producing *E.coli* and *K.pneumoniae* strains in Mangalore. The prevalence of ESBL and multidrug resistant strains constitute a serious threat to the current β-lactam therapy. Tests for the detection of ESBL producing bacteria should be carried out at all diagnostic centers routinely and the use of third generation cephalosporins should be restricted. This can reduce the prevalence of ESBL producing organisms.

Key Words: ESBL, multidrug resisitance, *Escherichia coli, Klebsiella pneumoniae,* double-disk synergy test.

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Introduction

The Extended Spectrum β-Lactamases (ESBL) are plasmid-mediated enzymes which are capable of hydrolyzing and inactivating a wide variety of β -lactams including third generation cephalosporins (3GC), penicillins and aztreonam [1]. Most of these plasmids not only contain DNA encoding ESBL enzymes, but also carry genes which confer resistance to several non β-lactam antibiotics. The most frequent coresistances found in ESBL producing organisms are aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and sulfamethoxazole-trimethoprim [2].

ESBL isolates were first discovered in Western Europe in the mid-1980s. ESBLs occur predominantly in *Klebsiella species* and *E.coli*. It is also been increasingly reported in other genera of the family *Enterobacteriaceae* [3], [4]. The incidence of ESBL producing strains among clinical *Klebsiella species* and *E.coli* isolates has been steadily increasing over the past years. ESBL producing strains are probably more prevalent than the currently recognized ones because they are often undetected by the routine susceptibility testing methods. Occurrence of ESBL producing members of the family *Enterobacteriaceae* have also been reported from South India [5] and Central India [6].

The present study was conducted with an objective to examine the incidence of ESBL producing strains and the multidrug resistant strains of *E.coli* and *K.pneumoniae,* which were recovered from patients attending a tertiary care hospital in Mangalore.

Materials and Methods

A total number of 228 isolates obtained from the cultures of various specimens such as urine (132), pus (70), sputum (10), bronchial lavage (12) and pleural fluid (4) were studied for ESBL production.

The samples were collected with universal safety precautions and were transported to the laboratory without delay. Samples were obtained from both outpatients and from those admitted to the hospital attached to our medical college between January and December 2008. The samples were cultured on brain heart infusion agar (BHIA) and MacConkey's agar. The specimens were processed for isolation and identification based on standard laboratory techniques [7].

Antibiotic susceptibility of the isolates was determined against 14 antibacterial agents by the Kirby Bauer disk diffusion method. They include ceftriaxone (Ci-30µg), cefotaxime (Ce-30µg), ceftazidime (Ca-30µg), gentamicin (G-10µg), tobramycin (Tb-10µg), amikacin (Ak-30µg), netilmicin (Nt-30µg), nalidixic acid (Na-30µg), ciprofloxacin (Cf-5µg), imipenem (I-10µg), cefaperazone-sulbactum (Cfs-75/15µg), co-trimoxazole (Co-25µg), piperacillin-tazobactum (Pt-100µg) and chloramphenicol (C-30µg); (Hi-Media, Mumbai). The results were recorded and interpreted as per CLSI recommendations [8].

The isolate that showed resistance to at least one of the third generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone) was tested for ESBL production by both the double-disk synergy test (DDST method) [9] and the phenotypic confirmation test which were recommended by CLSI.[8] The *E.coli* ATCC 25922 and *K.pneumoniae* ATCC 700603 strains were used as controls.

Results

Among the 228 isolates, 64(56.14%) were *E.coli* and 50(43.86%) were *K.pneumoniae*. Of these, 228 strains tested, 136(59.65%) were found to be ESBL producers, of which, 70(51.47%) were *E.coli* and 66(48.53%) were *K.pneumoniae.* 70% of *E.coli* isolated from urine samples and

75% of *K.pneumoniae* isolated from other samples were detected as ESBL producers. Among these, 96% of the isolates showed resistance to at least one of the three third generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone), 89% of the isolates showed resistance to all the three 3GC and their resistance was found to co-exist with the resistance to other antibiotics. All the isolates were found to be sensitive to the antibiotic, imipenem. The sensitivity of *E.coli* to Pt and Cfs was found to be 100%, whereas *K.pneumoniae* showed 98% sensitivity to Pt and 88% sensitivity to Cfs. The resistance pattern of each isolate to various antibiotics used in our study was different
[Table/Fig 1].

[Table/Fig 1] Antibiotic susceptibility pattern of ESBL positive isolates

Sl .no	Antibiotics	Percentage of resistance	
		E.coli	K.pneumoniae
1.	Imipenem	0	0
2.	Cefaperazone-sulbactum	0	12
3.	Piperacillin-tazobactum	0	2
4.	Amikacin	14	52
5.	Chloramphenicol	17	70
6.	Co-trimoxazole	51	94
7.	Netilmicin	80	91
8.	Nalidixic acid	82	91
9.	Gentamicin	89	91
10.	Tobramycin	94	88
11.	Ciprofloxacin	96	76

Discussion

ESBL producing organisms pose a major problem in clinical therapeutics. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past few years, resulting in limitations of therapeutic options [10]. Previous studies from India have reported the presence of ESBL producers to be 6.6 to 68 % [11]. The frequency of ESBL producers (59.65%) in our study is comparable to previous Indian studies [12],[13],[14].Among this, 51.47% were *E.coli* and 48.53% were *K.pneumoniae*. It is an established fact that ESBL producers show cross resistance to other antibiotics also, thus limiting therapeutic choices. We have noted this in our study as well. The sensitivity to carbapenem (imipenem) was 100%, ESBL positive *E.coli* strains were 100% sensitve to cefoperozone sulbactum and piperacillin-tazobactum. ESBL positive *K.pneumoniae* strains showed 88% and 98% sensitivity to cefaperazone sulbactum and piperacillin-tazobactum, respectively. The sensitivity to gentamicin, ciprofloxacin, co-trimoxazole, nalidixic acid and netilmicin was poor and not suitable for empirical selection. Major outbreaks involving ESBL strains have been reported from all over the world, thus making them emerging pathogens [15]. A number of nosocomial outbreaks caused by ESBL producing organisms have been reported in the U.S. [16].

The routine susceptibility test done by clinical laboratories can fail to detect ESBL positive strains and can sometimes erroneously detect isolates to be sensitive to any of the broad spectrum cephalosporins like cefotaxime, ceftazidime, ceftriaxone [17]. With the spread of ESBL positive strains in hospitals, there is a need to formulate a policy of empirical therapy in a high risk unit where infection due to resistant organisms is much higher [17]. As indicated in many previous studies, the 100% carbapenem sensitivity in our study advocates the usage of the carbapenem antibiotic as the therapeutic alternative in the wake of the increasing resistance rates observed with conventional β-lactam and non-β-lactam antibiotics [18]. Equally important is the information on an isolate from a patient to avoid the misuse of extended spectrum cephalosporins, which still remain as an important component of antimicrobial therapy in high risk wards [18].

Knowledge of the resistance patterns of bacterial strains in a geographical area will help to guide appropriate and judicious antibiotic use [18]. There is a possibility that restricted use can lead to the withdrawal of selective pressure and resistant bacteria will no longer have the advantage of survival in such settings.

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