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ORIGINAL ARTICLE

Frequency and Antimicrobial Sensitivity Pattern Of Extended Spectrum β-Lactamases Producing *E. Coli* And *Klebsiella Pneumoniae* Isolated In A Tertiary Care Hospital

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Abstract: Infections due to extended spectrum β-lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* have become an important clinical problem. Local knowledge of antimicrobial susceptibilities of these organisms is important for implementation of effective hospital anti-infective policies. The present study was undertaken to determine the prevalence of ESBLs along with their antimicrobial sensitivity pattern in *Escherichia coli* and *klebsiella pneumoniae* isolates at a tertiary care hospital in Bangalore. A total of 243 clinical isolates comprising *E. coli* (*n*=157) and *K. pneumoniae* (*n*=86) were recovered from various clinical samples over a period of six months from June to November 2008. Antibiogram profile of these isolates was determined by Kirby-Bauer's disc diffusion method. All the isolates were screened for ESBL production as recommended by the Clinical Laboratory Standard Institute (CLSI). The present findings revealed a high occurrence of multidrug resistance ESBL producing *E. coli* (61.1%) and *K. pneumoniae* (40.6%). Imipenem was highly active against ESBL producing isolates. Results indicate that routine ESBL detection should be made imperative and empirical use of third generation cephaolsporins must be discouraged.

Keywords: Extended spectrum β-lactamases, *Escherichia coli*, *Klebsiella pneumoniae*, third generation cephalosporin.

Introduction

An extensive use of β-lactam antibiotics in hospitals and community has created major resistance problem leading to increased morbidity, mortality and health-care costs [1]. The most common cause of bacterial resistance to β-lactam antibiotics is the production of β-lactamases. The latest in the arsenal of these enzymes has been the evolution of extended spectrum β-lactamases (ESBLs). Extended spectrum β-lactamases are a large, rapidly evolving group of plasmid mediated enzymes capable of hydrolyzing and inactivating penicillins, cephalosporins and monobactams and are inhibited by β-lactamase inhibitors such as clavulanate, sulbactam and tazobactam [2, 3, 4]. Since their description in the mid-1980s, ESBLs spread rapidly to Europe, US and Asia and are now found all over the world [5]. They are also involved in nosocomial outbreaks conferring multiple drug resistant and resulting in limitation of therapeutic options [6, 7]. Specific risk factors that have led to spread of ESBL include prolonged hospitalization, severity of illinesss, intubations and mechanical ventilation, urinary or arterial catheterization and extensive use of broad spectrum antibiotics [8, 9]. Plasmid genes are easily transferred among enterobacteria, contributing to ESBL dissemination [10]. Plasmids that carry β- lactamase genes frequently harbour resistance genes to other antimicrobials [11]. Therefore, the

detection of ESBL-producing isolates is critical to assure appropriate therapy and to prevent their dissemination. This study was initiated to identify the incidence of ESBLs among *Enterobacteriaceae* isolated over a 6 month period at Sri Bhagawan Mahaveer Jain hospital at Bangalore, India. We also studied the pattern of susceptibility of the isolates to other clinically relevant antimicrobials.

Materials and Methods

Bacterial isolates: A total of 243 consecutive, non-repeat clinical isolates of *E.coli* (n=157) and *Klebsiella pneumoniae* (n=86) was collected from Sri Bhagawan Mahaveer Jain hospital, Bangalore, over a period of 6 months (June to November 2008) for the study. The isolates were obtained from different clinical specimens such as urine, sputum, wound. Both the outpatients and inpatients were included in the study. Organisms were identified to species level by conventional methods [12].

Antimicrobial susceptibility testing: The susceptibility to antibiotics was determined by Kirby Bauer method on Muller Hinton agar according to CLSI protocols [13]. The drugs tested were Ampicillin (10µg), Amoxycillin-clavulanic acid (20/10µg), Piperacillin (100µg) Piperacillin-tazobactam (100/10µg), Cephotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Cefpodoxime (10µg), Gentamicin (10µg), Amikacin (30µg) Ciprofloxacin (5µg), Tetracycline (30µg), Chloramphenicol (30µg), Trimethoprim- sulfamethoxazole 1.25/23.75µg) and Imipenem (10µg). *E. coli* ATCC 25922 were used as control strains.

Screening for ESBLs: Isolates were screened for ESBL production by using disk diffusion of cefotaxime, ceftazidime, ceftriaxone and cefpodoxime placed on inoculated plates containing Muller Hinton agar according to the CLSI recommendations [14]. Isolates showing inhibition zone size of ≤ 22mm with ceftazidime (30µg), ≤25mm with ceftriaxone (30µg), ≤ 27mm with cefotaxime (30µg) and ≤ 17 for cefpodoxime were suspected for ESBL production. *E. coli* ATCC 25922 was used as a negative control.

Confirmatory test for ESBLs: Phenotypic confirmatory test for ESBL producers was done by combined disc diffusion and MIC reduction method, for all the isolates that were screened positive for the ESBL production following CLSI guidelines.

Combined disk diffusion method: In this test a disk of ceftazidime (30µg) alone and a disk of ceftazidime in combination with clavulanic acid (30/10µg) were used. Both the disks were placed 25mm apart, centre to center, on a lawn culture of the test isolate on Muller Hinton agar plate and incubated overnight at 37° C. Difference in zone diameter with and without clavulanic acid was measured. The positive result was defined as a ≥ 5mm increase in inhibition zone diameter around combination disks with clavulanic acid versus its standard zone when tested alone [14].

MIC reduction test: The isolates positive with combination disk test were further confirmed for ESBL production by this test. MIC assay was performed on all strains that showed zone reduction for one or more of the antimicrobials used in the ESBL screening test. MIC was determined by agar dilution method as described by the National Committee for Clinical Laboratory Standard [15] against cephotaxime, ceftazidime, ceftriaxone and cefpodoxime. A break point of MIC, ≤2 µg/ml for cefpodoxime and ≤ 8 µg/ml for cephotaxime, ceftazidime and ceftriaxone were taken.

Results

A total of 243 isolates of *E. coli* (*n*=157) and *K. pneumoniae* (*n*=86) were recovered from different clinical specimens such as urine, (159 isolates; 65.4%) Sputum (61 isolates; 25.1%), Wound (23 isolates; 9.4%).Of these ESBL production was noticed in 160 (65.8%) isolates with maximal incidence in *E. coli* (73.8%; *n*=116) followed by *Klebsiella pneumoniae*, (51.1%; *n*= 44). All of them showed inhibition zone size of ≤22nm with ceftazidime during screening test. Confirmatory test for ESBL production were preformed on these 160 isolates. Out of 160 isolates, 96 *E. coli* and 35 *K. pneumoniae* isolates were found to be ESBL producers by confirmatory test with combination disks, thus, 61.1% of *E. coli* and 40.6 % of *K. pneumoniae* isolates were found to be ESBL producers. Highest number of ESBL producing *E. coli* were detected by cefpodoxime (*n*=85) followed by cephotaxime (*n*=79), ceftazidime (*n*=75) and ceftriaxone (*n*=73). For ESBL producing *K. pneumoniae* it was cefpodoxime (*n*=35) followed by ceftazidime (*n*=30), cephotaxime (*n*=22) and ceftriaxone (*n*=28). The antimicrobial resistance was significantly higher in ESBL producers than in non-ESBL producers. ESBL producers were almost always resistant to ampicillin and piperacillin. However, All the isolates were sensitive to imipenam .Cephalosporin resistance was also higher in ESBL producing *E. coli* and *K. pneumoniae* isolates when compared to ESBL non producers. Combination of β-lactam/ β-lactamase inhibitors showed greater activity in both ESBL producers and non producers. Among aminoglycosides, amikaicn showed greater activity against all the isolates irrespective of their ESBL status (Table 1).

Table 1: Comparison of antimicrobial resistance pattern ESBL producers and non ESBL producers

Antibiotics	*E. coli*		*K. pneumoniae*	
	ESBL (n=96)	non ESBL (n=61)	ESBL (n=35)	non ESBL (n=51)
Ampicillin	79	48	35	26
Piperacillin	80	50	34	17
Amoxicillin-clavulanic acid	37	18	14	11
Piperacillin-tazobactam	9	7	12	10
Cephotaxime	79	26	28	9
Ceftazidime	75	23	30	10
Ceftriaxone	73	27	22	7
Cefpodoxime	85	21	35	2
Gentamicin	41	15	17	27
Amikacin	5	0	10	4
Tetracycline	63	26	33	41
Ciprofloxacin	68	15	23	10
Chloramphenicol	79	26	29	19
Trimethoprim-sulphamethoxazole	24	15	13	15
Imipenem	0	0	0	0

Regarding the distribution of ESBL-producing isolates according to the sample source, it is found that 76% of *E. coli* isolates were obtained from urine, 18.7% from sputum and 5.2% from pus. Whereas, 60% of *K. pneumoniae* isolates were obtained from urine, 28.5% from sputum and 11.4 %5 from pus (Table 2).

Table 2: Distribution of ESBL positive isolates in different clinical samples.

Clinical samples	*E. coli* ESBL positive isolates	*K. pneumoniae* ESBL positive isolates	Total ESBL positive isolates
Urine	73	21	94
Sputum	18	10	28
Pus	5	4	9
Total	96	35	131

The sensitivity for antimicrobials tested by agar dilution method for ESBL positive isolates is shown in Table 3.

Table 3: MIC $_{50}$ and MIC $_{90}$ of *E. coli* and *K. pneumoniae* against 3rd generation Cephalosporins

Antibiotics	*E.coli*				*K.pneumoniae*			
	MIC_{50} (≤µg /ml)		MIC_{90} (≤µg/ml)		MIC_{50} (≤µg/ml)		MIC_{90} (≤µg/ml)	
	ESBL	non ESBL	ESBL	non ESBL	ESBL	non ESBL	ESBL	non ESBL
Cephotaxime	1024	4	2048	8	512	2	2048	8
Ceftazidime	64	2	512	2	128	2	256	2
Ceftriaxone	2048	4	2048	16	512	4	2048	8
Cefpodoxime	2048	32	4096	256	2048	32	4096	64

Discussion

Antibiotic resistance surveillance has a central role among all strategies to manage the problem of antibiotic resistance. Since their first description in the mid 1970s, ESBLs have been isolated worldwide and form a major contributor of drug resistance in many of *Enterobacteriaceae*. ESBLs are now a problem in hospitalized patients throughout the world. The prevalence of ESBLs among clinical isolates vary greatly world wide and in geographic areas and are rapidly changing overtime [16]. Of the 243 strains included in our study 53.9% showed ESBL production, with the highest incidence in *E. coli* (61.1%) followed by *K. pneumoniae* (40.6%). The incidence of ESBL in major hospitals of India has been reported to be as high as 6%-87% [17-21]. Our results are in concordance with other studies [17]. However, lower percentage were reported from Chennai (20%) and Hyderabad (19.8%) [22-23]. One reason for such variability may be the very low number of samples studied.

In our study analysis of the 243 ESBL isolates revealed that ESBLs were predominantly present among *E.coli* (61.1%) compared to *K. pneumoniae* (40.6%). Similar findings' showing a high prevalence of ESBLs among *E. coli* [22, 24] was reported. The high incidence of ESBLs among *E. coli* may be peculiar to the Indian subcontinent. Cefpodoxime showed the highest sensitivity in detecting ESBL Producing *E. coli* and *K. pneumoniae* as reported earlier. Organisms that express an ESBL are frequently resistant to other antimicrobial agents, as many of these additional resistant genes are encoded on the ESBL associated plasmid [25-26]. In our study high level of resistance was observed against tetracycline. However, good activity was showed by β-lactam/ β-lactamase inhibitor combination. Among the non β-lactam antibiotics, amikacin showed higher sensitivity against these ESBL producers. Similar results were reported for the patients with serious infections with ESBL producers [27]. The occurrence of ESBL producers in urinary isolates in our study was found to be 71.7% which is higher than other studies [28]. In the present study, ESBL producing isolates were isolated from inpatients units as well as from clinical samples from patients attending outpatient. As indicated in many previous studies all ESBL producers were found to be susceptible to imipenem and amikacin. However, amikacin and carbapenems are usually used only as the reserve drugs. A similar study conducted by Hanstia *et al*[18] and Abigail *et al* [29] showed 100% susceptibility to amikacin and imipenem. The marked increase in β-lactamase production, including the high level constitutive ESBL producers have left us with few alternatives in combating serious infection. In conclusion, this study emphasizes the need for continued surveillance of ESBL producing bacteria as high prevalence of antibiotic resistance in ESBL positive *E. coli* and *K. pneumoniae* was observed. Phenotypic confirmatory test using combination disk is simple and cost effective for the detection of ESBL producers as it has 100% concordance with MIC reduction test. The control measure include judicious use of antibiotics, strict hygiene protocols and implementation of appropriate infection control measures in the hospital, especially while treating high risk patients.

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