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Frequency and Antimicrobial Sensitivity Pattern of Extended Spectrum β-Lactamases Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated from urine at a Tertiary Care Hospital

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Abstract

Background: Infections due to extended spectrum β-lactamases (ESBL) producing Escherichia coli and Klebsiella pneumoniae have become an important clinical problem. These organisms are important regarding the infection control by the physicians. **Objective:** The present study was undertaken to determine the prevalence of ESBLs along with their antimicrobial sensitivity pattern in *Escherichia coli* and *Klebsiella pneumoniae*. **Methodology:** This cross sectional study was conducted in the Department of Microbiology at Sir Salimullah Medical College, Dhaka. Urine samples were collected from patients who were clinically suspected to have UTI. After incubation, plates were checked for presence of suspected pathogens. Organisms were identified to species level by conventional methods. All isolated *E. coli* and *K. pneumoniae* were included in the study. The susceptibility to antibiotics was determined by Kirby Bauer method on Muller Hinton agar. 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. Phenotypic confirmatory test for ESBL producers was done by combined disc diffusion for all the isolates that were screened positive for the ESBL production following CLSI guidelines. Combined disk diffusion method was also done in this study. **Result:** A total of 220 non repeated urine samples were cultured of which 132(60%) cases had shown the bacterial growth. Among the 132 samples Escherichia coli had found in 103(78.0%) cases and Klebsiella spp. was found in 14(10.6%) cases. Out of 103 E coli 23(22.3%) cases was found as ESBL strain. On the other hand within 14 Klebsiella species, the ESBL strain was found in 5(35.7%) cases. Both E coli and Klebsiella species were 100% sensitive to imipenem. However, cephamycin was sensitive in 93.7% and 100% in E coli and Klebsiella species respectively. **Conclusion:** Results indicate that routine ESBL detection should be made imperative and empirical use of third generation cephalosporins must be discouraged.

Key words: Extended spectrum β-lactamases, *Escherichia coli, Klebsiella pneumoniae,* 3rd generation cephalosporin

Introduction

The most common cause of bacterial resistance to β-lactam antibiotics is the production of β-lactamases[1]. The latest in the arsenal of these enzymes has been the evolution of extended spectrum β-lactamases (ESBLs). ESBLs are defined as β-lactamases capable of hydrolyzing oxyimino-cephalosporins and are inhibited by β-lactamase inhibitors[2]. 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[3].

The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past years resulting in limitation of therapeutic options[4]. Microorganisms responsible for urinary tract infection (UTI) such as *E. coli* and *Klebsiella spp.* have the ability to produce ESBLs in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making

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urinary tract infection difficult to treat[5].

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[6,7,8]. Since their description in the mid-1980s, ESBLs spread rapidly to Europe, US and Asia and are now found all over the world[9]. They are also involved in nosocomial outbreaks conferring multiple drug resistant and resulting in limitation of therapeutic options[10,11]. Specific risk factors that have led to spread of ESBL include prolonged hospitalization, severity of illness, intubations and mechanical ventilation, urinary or arterial catheterization and extensive use of broad spectrum antibiotics[12,13]. Plasmid genes are easily transferred among enterobacteria, contributing to ESBL dissemination[14]. Plasmids that carry β-lactamase genes frequently harbour resistance genes to other antimicrobials[15]. 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 ESBL producers in urinary isolates of *E. coli* and *K. pneumoniae* and also to see the pattern of susceptibility of the isolates to other clinically relevant antimicrobials. The aim of the present study was to identify the freaqency of ESBL producing *E. coli* & *Klebsiella spp*. with their artibiogram.

Methodology

This study was conducted from January to December 2006 in the Department of Microbiology at Sir Salimullah Medical College, Dhaka. A total of 220 urine samples were collected from patients who were clinically suspected to have UTI. Both the outpatients and inpatients were included in the study. All urine samples were inoculated in Blood agar and MacConkey agar media. All the plates were incubated at 37°C aerobically for 24 hours. After incubation, plates were checked for presence of suspected pathogens. Organisms were identified to species level by conventional methods[16]. All isolated *E. coli* and K. pneumoniae were included in the study. Clinico-demographic data of the study patients was noted.

Antimicrobial susceptibility testing: The susceptibility to antibiotics was determined by Kirby Bauer method on Muller Hinton agar media according to CLSI[17] protocols. 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 (1.25/23.7µg) and Imipenem (10µg). *E. coli* ATCC 25922 was 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 media according to the CLSI[17] recommendations. Isolates showing inhibition zone size of 22mm with ceftazidime (30µg), 25mm with ceftriaxone (30µg), 27mm with cefotaxime (30µg) and 17 mm 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 for all the isolates that were screened positive for the ESBL production following CLSI[17] 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 25 mm apart, centre to centre, on a lawn culture of the test isolate on Muller Hinton agar plate and was incubated overnight at 37° C. Difference in zone diameter with and without clavulanic acid was measured. The positive result was defined as 5 mm increase in inhibition zone diameter around combination disks with clavulanic acid versus its standard zone when tested alone[17].

Results

Out of the 220 consecutive, non-repeat urine samples processed, 132(60.0%) samples yielded various bacterial isolates. Among them, *E. coli* were the highest number of isolated from the specimen which was 103(78.0%) and the next to this is the *K. Pneumoniae* which was isolated in 14(10.6%) cases. ESBL production was observed in 22.3% of *E. coli* (23/103) and 35.7% of *K. pneumoniae* (5/14).

Table 1 : Age distribution among the study population (n=28)

Age group	Frequency	Percentage
<20	3	10.7
20-40	19	67.8
40-60	5	17.9
>60	1	3.6
Total	**28**	**100**

*Mean age ± SD=35 ± 24.7

All of them showed inhibition zone size of 22mm with ceftazidime during screening test. Confirmatory test for ESBL production were performed by CLSI[17] confirmatory test on these 28 isolates of *E. coli* and *Klebsiella pneumoniae.* These ESBL positive isolates were obtained from 12 male and 16 female patients with a male female ratio of 1:1.3. They were distributed in the age group of 1 month to 77 years and the mean age of the study population was 35±24.7 years. The antimicrobial susceptibility results of

ESBL producers were also done and showed that susceptibility of ESBL producers to imipenem, nitrofurantoin and amikacin were found to be 100%, 89% and 86% respectively.

Table 2 : Distribution of sex among the study population (n=28)

Sex group	Frequency	Percentage
Male	12	42.9
Female	16	57.1
Total	**28**	**100**

*Male : Female= 1:1.3

The antimicrobial resistance was significantly higher in ESBL producers than in non-ESBL producers. ESBL producers were almost always resistant to ampicillin and piperacillin. Both *E coli* and *Klebsiella* species were 100% sensitive to imipenem. However, cephamycin was sensitive in 93.7% and 100% in *E coli* and *Klebsiella* species respectively.

Table 3 : Distribution ESBL producing E coli and Klebsiella species (n=117)

Bacteria Isolated	ESBL positive	ESBL negative	Total
E coli	23(82.1%)	80(89.9%)	103(88.0%)
Klebsiella species	5(17.9%)	9(10.1%)	14(12.0%)
Total	**28(100%)**	**89 (100%)**	**117(100%)**

* Pearson Chi-Square test was corrected by Fisher's Exact Test
*p value= 0.318

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.

Discussion

Antibiotic resistance monitoring has a central role among all strategies to manage the problem of antibiotic policy. 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[11]. The prevalence of ESBLs among clinical isolates vary greatly world wide and in geographic areas and are rapidly changing overtime[18]. Of the 132 strains included in this study 23.5% showed ESBL production, with the highest incidence in *E. coli* (82.1%) followed by *K. pneumoniae* (17.9%). The ESBL production is alarming. The incidence of ESBL in major hospitals of India has been reported to be as high as 58%[19]. The range of ESBL isolation rate has been varied from 6 to 39% in different studies[20,21,22,23]. However, very similar percentage was reported from Chennai[24] (20%) and Hyderabad[25] (19.8%). One reason for such variability may be the very low number of samples studied.

Interestingly among all ESBL isolates, it is predominantly present among *E. coli* (82.1%) compared to *K. pneumoniae* (17.9%). Similar finding showing a high prevalence of ESBLs among *E. coli*[25,26] 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[27]. In this 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[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 et al[20] 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.

Conclusion

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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