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EXTENDED SPECTRUM BETA LACTAMASE - A RISING THREAT IN URINARY TRACT INFECTIONS

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ABSTRACT

Antibiotic resistant urinary tract infections are increasing in frequency with Extended Spectrum beta Lactamase (ESBL) producing organisms on the rise. Routine antibiotic susceptibility testing may fail to detect these strains, leading to treatment failures. This study aims at the detection and analysis of ESBL producing Gram negative bacteria in hospitalized patients with UTI.Mid-stream clean catch urine samples from 600 patients with symptoms of hospital acquired UTI were subjected to culture and antimicrobial susceptibility testing by standard methods. ESBL production was confirmed by standard CLSI guidelines. E.coli (47.1%) was the predominant pathogen. Sixty ESBL producers were found and all were sensitive only to Imipenem. Beta-lactam with beta-lactamase inhibitor combination was also found to be effective. Co-resistance to two or more other classes of drugs along with the third generation cephalosporin resistance indicates multi-drug resistance in these strains.ESBL producing Gram negative bacilli are a serious challenge because of their changing trend, multi-drug resistance and their frequent isolation in hospital acquired UTI. For the therapeutic benefit in resistant cases, additional confirmatory tests for screening ESBL will be of great use.

KEYWORDS : Extended Spectrum beta-Lactamase (ESBL), Gram negative bacteria, Multi-drug resistance, Urinary Tract Infection.

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INTRODUCTION

Urinary tract infections (UTI) are amongst the common infections acquired in hospital settings. The antimicrobial susceptibility, resistance patterns and the diverseness of the uropathogens have been changing over the past years[1]. The indiscriminate use of antibiotics, especially beta-lactams has resulted in a selective pressure, which in turn increased the frequency of multi-drug resistant hospital acquired UTI[2]. Extended Spectrum Beta-Lactamase (ESBL) producing Gram Negative bacteria especially of the Enterobacteriaecae family are being increasingly isolated in these cases. ESBL are Class A plasmid mediated enzymes that hydrolyze the oxyimino-cephalosporins and monobactams. These do not inactivate the cephamycins or Carbapenems but are inhibited by β-lactamase inhibitors[3].Organisms that produce ESBLs remain an important reason for cephalosporin inactivity leading to treatment failure which affects the infection control practices. In view of the above facts, it is important that clinical microbiology laboratories detect and report ESBL-producing organisms. Our present study was aimed at detection and characterization of the organisms causing UTI in hospitalized patients and simultaneously determine the ESBL producing isolates amongst them. During the course of the study, analysis of the therapeutic options for treatment of UTI was undertaken.

MATERIALS AND METHODS

This prospective study was conducted in Yenepoya Medical College and Hospital, a tertiary care set-up in Mangalore, from June 2013 to January 2014. A total of 600 non-repeat urine samples collected with proper techniques from hospitalized patients who developed symptoms of UTI after 48 hours of admission/ post-catheterization were subjected to semi-quantitative culture [4, 5]. Those organisms showing significant growth (>10^5 colony forming units per milliliter) were first identified with standard biochemical techniques [6] and then were subjected to susceptibility testing by Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates as per CLSI guidelines using ampicillin (10mcg), cefotaxime (30mcg), ceftazidime (30mcg), aztreonam (30mcg), piperacillin-tazobactam (100/10mcg), gentamicin (10mcg), norfloxacin (10mcg), nitrofurantoin (300mcg), co-trimoxazole (1.25/23.75mcg) (HiMedia, India) discs.[7]

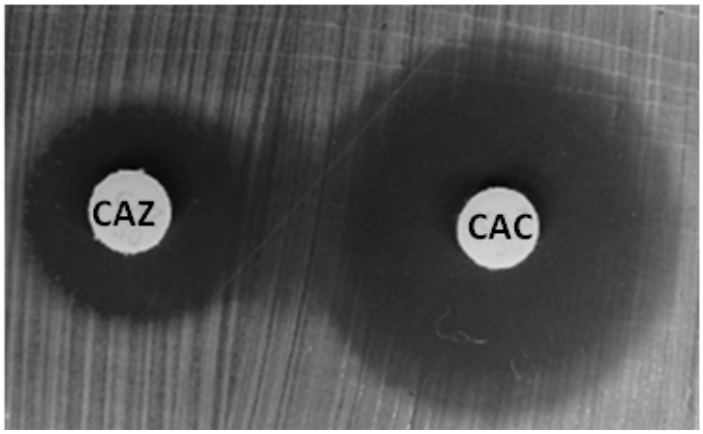
1. Screening for ESBL

The isolates of *E.coli* and *Klebsiella pneumoniae* were screened for ESBL production according to the current CLSI guidelines. [8]The zone diameters around Ceftazidime(30µg), Cefotaxime (30µg) and Aztreonam (30µg) discs were measured and recorded. Inhibition zonesof <22mm for Ceftazidime, <27mm for Cefotaxime and Aztreonam were taken to indicate ESBL production and further confirmed.

2. Confirmation of ESBL

The isolates that were screened positive for ESBL production were further evaluated by the Phenotypic confirmatory test as described by the CLSI. [9] This test was done by making a lawn culture of the isolates on Mueller Hinton agar plates and tested against combination of discs viz. Ceftazidime (30 µg) along with Ceftazidime-clavulanic acid (30/10 µg) and Cefotaxime (30 µg) along with Cefotaxime-clavulanic acid (30/10 µg). After 16-18 hours of incubation the zones sizes were measured and recorded. A ≥5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirmed ESBL production. (Fig.1.).

Figure 1
Phenotypic Disc Confirmatory test for ESBL production in Gram negative bacteria.



K. pneumoniae ATCC 700603 and *E. coli* ATCC 25922 (Himedia, India) were used as positive and negative controls respectively for ESBL production.

RESULTS AND DISCUSSION

Various organisms have been reported to be isolated from urine of patients with UTI. *E.coli*[10, 11, 12] and *Klebsiella* spp.[12] have been reported as the most common organisms causing UTI. Even in the current study, Gram negative bacilli were found to be mainly responsible for the causation of UTI in the hospitalized patients. Of the 600 urine samples 280(46.7%) yielded significant bacteriuria. *Escherichia coli* (47.3%) were the predominant pathogen followed by *Klebsiella pneumoniae*, *Acinetobacter* spp., *Pseudomonas* spp., *Proteus mirabilis*, *Enterococcus* spp. and *Candida* spp. The number and percentage of the causative organisms are as presented in the table 1.

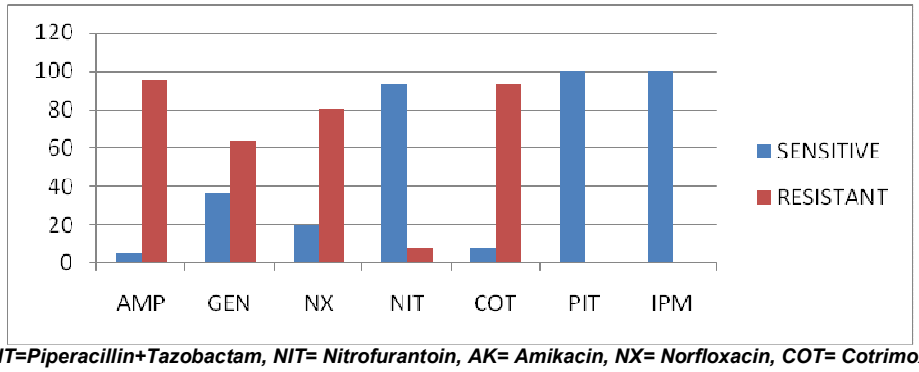
Table 1
Microbial isolates from significant culture positive urine samples and their numbers

ISOLATES	NUMBER (n=280)	PERCENTAGE (%)
Eschericia coli	132	47.1
Klebsiella pneumoniae	68	24.3
Acinetobacterspp.	16	5.7
Pseudomonas spp.	15	5.4
Proteus mirabilis	06	2.1
Enterococcus faecalis	22	7.9
***Candida* spp.**	15	5.4
Coagulase Negative Staphylococci	06	2.1

In this study, *E.coli* and *Klebsiella* caused more than half (200 out of 280) of the UTI cases. This is in concordance with the previous studies which show the same results [9, 10]. Both these organisms were tested for ESBL production as per CLSI guidelines. In the screening test, 41 out of 132 (31.1%) *E.coli* isolates and20of 68 *Klebsiella* (29.4%) isolates showed decreased sensitivity to the oxyimino-cephalosporins and aztreonam. All the 41 *E.coli* isolates and 19 of the 68 *Klebsiella* isolates were confirmed positive in the combined disc confirmatory test for ESBL production. *Klebsiella* species and *E.coli* have been reported to have higher prevalence rates of ESBL producing strains by many investigators worldwide. [12, 13, 14, 15 and 16] The production of beta-lactamase may be of chromosomal or plasmid origin [17, 18]. Acquisition of plasmids by transfer of genetic information between the Gram negative bacteria is an important factor in ESBL production. Such transferable plasmids also code for resistant determinants to other antimicrobial agents. Thus, multidrug resistance is expected to be more common in

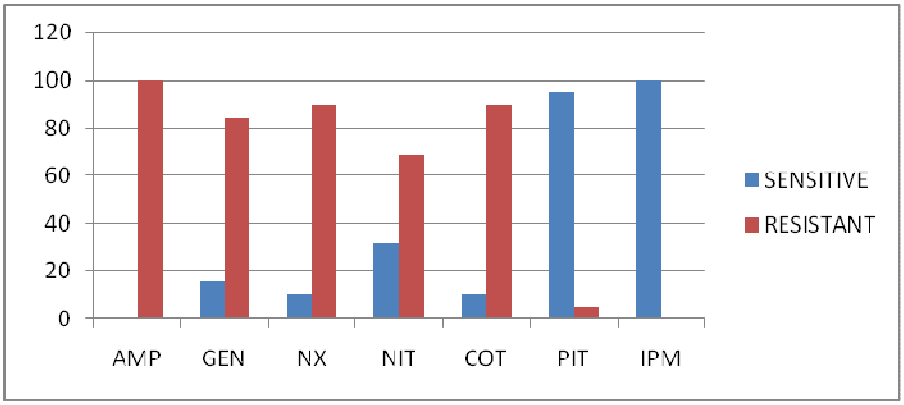
ESBL producing organisms [19]. In our study, the ESBL producing *E.coli* and *Klebsiella* isolates were all found to be sensitive to Imipenem and also maximally sensitive to β-lactam and β-lactamase inhibitor combinations. *Klebsiella* isolates were more resistant to Nitrofurantoin than *E.coli.* The susceptibility patterns are as shown below. (Graph 1 and 2).

Graph 1
Antibiotic susceptibility pattern of ESBL producing E.coli isolates



(IPM=Imipenem, PIT=Piperacillin+Tazobactam, NIT= Nitrofurantoin, AK= Amikacin, NX= Norfloxacin, COT= Cotrimoxazole)

Graph 2
Antibiotic susceptibility pattern of ESBL producing Klebsiella isolates.



(IPM=Imipenem, PIT=Piperacillin+Tazobactam, NIT= Nitrofurantoin, AK= Amikacin, NX= Norfloxacin, COT= Cotrimoxazole)

Table 2
Antibiotic resistance pattern comparing ESBL producers and ESBL non-producers

Antibiotics	ESBL producers (n=60) % resistance	ESBL non-producers(n=140) %resistance
Gentamicin	70	30.7
Norfloxacin	83.3	60
Nitrofurantoin	26.7	10
Co-trimoxazole	91.7	52.14

Resistance to the third generation cephalosporin was found to co-exist with resistance to two or more antibiotics like gentamicin, norfloxacin, co-trimoxazole indicating a multi-drug resistance pattern. It is evident from table 2 that the ESBL producers showed decreased sensitivity to gentamicin, norfloxacin, nitrofurantoin and co-trimoxazole when compared to the ESBL non-producers which is a significant observation made in this study. Similar results have been observed in other studies. [20, 21]

CONCLUSION

UTI has a diverse etiology in the hospitalized patients. *Escherichia coli* is the most common organism causing UTI in such cases. Cephalosporins are the commonly used antibiotics to treat this infection. But the causative Gram negative organisms have the capacity to produce ESBL in significant quantities conforming resistance to beta lactam antibiotics i.e. most penicillins, cephalosporins and aztreonam, thus making UTI a difficult infection to treat. The production of ESBL is difficult to detect in routine disk diffusion testing methods used in any laboratory. Hence it is necessary for specific detection methods of these inactivating enzymes irrespective of their sensitivity pattern to cephalosporins by routine methods. Over reliance on beta lactams and other higher antibiotics for the treatment of Gram negative organisms has led to a high rate of ESBL production in these clinical isolates especially in a hospital setting. Also the ESBL producing strains show a multi-drug resistant picture making these cases all the more difficult to treat. The isolates showed maximum sensitivity for Carbapenems and higher penicillin and β-lactamase inhibitor combinations making these drugs suitable for therapy. In conclusion, good infection control practice and careful inspection while prescribing β-lactam drugs in the background of the hospital flora are necessary for good antimicrobial stewardship in the hospitals.

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