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Detection of Extended Spectrum Beta Lactamase Production in *Escherichia Coli* Causing Urinary Tract Infection.

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ABSTRACT

Escherichia coli is known to cause various infections, most commonly urinary tract infections. Extended Spectrum Betalactamases are one of the important causes for the emerging antibiotic resistance all over the world causing hindrance to the treating physicians. The aim of this study was to detect the production of ESBL among *Escherichia coli* isolates causing Urinary tract infection and to study their antimicrobial susceptibility pattern. A total of 100 urinary *E.coli* isolates were included in this study. Detection of ESBL was done by the combnination disc method as per CLSI guidelines. Out of the 100 *E.coli* isolates 43 isolates were found to produce ESBL. All these 43 isolates showed high degree of resistance to many drugs including cephalosporins. These isolates were highly susceptible to Amikacin (90%) followed by Nitrofurantoin (65%) and Ofloxacin (58%). Decreased susceptibility was seen towards Ciprofloxacin (32%) and Norfloxacin (30%). Current knowledge about the resistance patterns of bacterial strains in a community helps to guide appropriate usage of antibiotics. High prevalence of ESBL producing bacteria may be due to long term antibiotic exposure, prolonged ICU stay, hospital acquired strains and prolonged catheterization.

Keywords: Urinary tract infection, *E.coli*, Extended spectrum beta lactamase.

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INTRODUCTION

The most common bacterial infections in humans are the urinary tract infections (UTI), both in the community and in the hospitals. In India, the incidence of UTI is about 50,000/million persons per year [1]. Antibiotics are usually started empirically before the laboratory urine culture results are available. Regrettably, resistance to antibiotics has become a serious problem in many parts of the world [2].

E.coli is one of the important microorganisms known to cause various infections in the hospital set up. The incidence of *E.coli* is 90% in community acquired urinary tract infection. It is the common causative organism in hospital acquired urinary tract infection and complicated cases on UTI resulting from anatomical obstruction and catheterization [3, 4].

Betalactam antibiotics are one of the routinely prescribed drugs in treating various infections. The occurrence of extended spectrum betalactamase (ESBL) producing strains among clinical isolates has been progressively rising over the past few years, resulting in the restriction of therapeutic options [5]. Organisms which are responsible for Urinary Tract Infection (UTI), particularly *E.coli* and Klebsiella spp. have the capacity to produce ESBLs in huge quantities. Extended spectrum betalactamases (ESBL) hydrolyse oxyimino beta lactams like ceftriaxone, ceftazidime, cephotaxime and monobactam like aztreonam but have no effect on cefamycins (cefoxitin, cefotetam), carbapenem (imipenam, meropenam) and related compounds [6].

Resistance to cephalosporins and other antibiotics occur most commonly because these enzymes are encoded by transferable conjugative plasmids which often code resistance. The most frequent co-resistances found in ESBL producing organisms are to aminoglycosides, tetracyclines, chloramphenicol, fluoroquinolones and sulfamethoxazole-trimethroprim. Surgical care patients and patients with indwelling Foley's catheter (79%) show higher rate of multidrug resistant strains that produce ESBL [7].

The increasing reports of ESBL-producing *E.coli* from all over the world, provoked our interest to investigate and detect the rate of production of ESBL in E.coli causing urinary tract infections in our tertiary care hospital.

MATERIALS AND METHODS

This study was done in a tertiary care hospital during the period of March 2014 to June 2014. A total of 100 urinary isolates of *E.coli* were included in the study. All isolates showed a colony count of > 10^5 colony forming units per millilitre from a midstream urine sample. All *E. coli* isolates were identified in the microbiology laboratory by using standard biochemical identification methods [8].

Drug sensitivity of the isolates was done by Kirby Bauer's Method [9] using antibiotic disks. Antibiotics used were ceftazidime(30 µg), cefixime(5 µg), cefdinir(5 µg), cefuroxime(30 µg) gentamicin (10 µg), nalidixic acid(30 µg), nitrofurantoin(300 µg), cefotaxime(30 µg), aztreonam(30 µg) and amikacin(10 µg). The results were interpreted as per clinical and laboratory standards institute (CLSI) recommendations [10] .

All *E.coli* isolates which showed resistance or reduced susceptibility (intermediate as per CLSI guidelines) to 3rd generation cephalosporins were tested for ESBL production by combination disc method [11]. The detection of ESBL was perfomed according to the CLSI guidelines [12]. An overnight culture suspension of the isolate was inoculated on the surface of a Mueller hinton agar(MHA) plate using a sterile cotton swab. Cefotaxime disc (30 µg) and augmentin (20 µg amoxicillin /10 µg clavulanic acid) discs were placed on it. If the strain is an ESBL producer, then the zone of inhibition around cefotaxime disc is extended towards the augmentin disc and the distance between the two discs was roughly twice the radius of the inhibition zone produced by cefotaxime tested on its own.

Phenotypic confirmatory test was done using ceftazidime (30µg) alone and ceftazidime (30µg) + clavulanic acid (10µg). Muller Hinton agar with 4 mm depth was prepared. The test organism inoculum equivalent to 0.5 McFarland Standard was spread as lawn culture on the agar. The Ceftazidime(30 µg) and Ceftazidime-clavulanic acid(30 µg /10 µg) were placed 20 mm apart on the agar. The inoculated agar plate was incubated overnight at 37^{0}C. An increase in zone diameter of >5mm in the presence of clavulanic acid compared with ceftazidime alone was considered to be positive for the presence of ESBL production.

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RESULTS

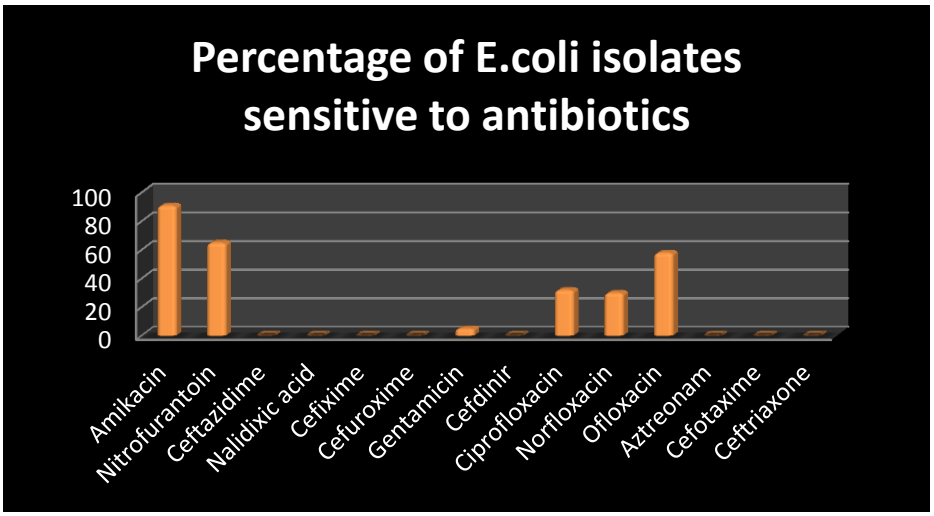
Out of the 100 *E.coli* isolates, production of ESBL was found in 43 isolates by combination disc method. Of the 43 isolates studied, antibiogram revealed that Nitrofurantoin and Ofloxacin constitute the reasonable option for treatment of UTI, as 28(65%) and 25(58%) isolates were sensitive to these antibiotics. Amikacin was found to be a good option for being drug of choice to the isolated ESBLs as 39(90%) out of the 43 isolates were sensitive to this antibiotic. All of the 43 isolates were resistant to cephalosporins. Ciprofloxacin and Norfloxacin showed least susceptibility with 32% and 30%.

Table 1: Prevalence of ESBL in *E. coli* among UTI by Combination disc method.

No.of *E.coli* isolates	No.of ESBL producing *E.coli* isolates
100	43(43%)

Table 2: Percentage of Antibiotic Susceptibility for ESBL producing *E.coli* (n=43)

Antibiotics	No. of *E.coli* isolates Sensitive	Percentage %
Amikacin (30µg)	39	91
Nitrofurantoin (300µg)	28	65
Ceftazidime (30µg)	0	0
Nalidixic acid (30µg)	0	0
Cefixime (5µg)	0	0
Cefuroxime (30µg)	0	0
Gentamicin (30µg)	2	5
Cefdinir (5µg)	0	0
Ciprofloxacin(5µg)	14	32
Norfloxacin (10µg)	13	30
Ofloxacin (5µg)	25	58
Aztreonam (30µg)	0	0
Cefotaxime (30µg)	0	0
Ceftriaxone (30µg)	0	0



DISCUSSION

Various organisms have been reported to be isolated from patients with UTI. *E.coli* has been reported as one of the most common organisms causing UTI [13,14]. The prevalence of ESBL production among the clinical isolates varies greatly globally and rapidly varying over time. In this study occurrence of ESBL among *E.coli* from urinary isolates was 43%. This rate is much higher than the reported results from Canada (*E.coli* 2.7%) and USA (*E. coli* 2.2%) [15]. In another India study, Mathur *et al*[11] observed much higher rates of ESBL production(58%).

In our study the resistance rate to Amikacin was too low (9%), thus this agent can be used as a good choice for the treatment of UTI empirically in our population. Nitrofurantoin is considered as one of the oldest urinary anti-infective drugs in use, unexpectedly; in our study, resistance to this drug was minimal (35%). The lower rate of resistance may be due to the fact that Nitrofurantoin has multiple mechanisms of action, requiring organisms to develop more than a single mutation in order to develop resistance [16].

In many countries including India, cephalosporins are one of the most favourite antibiotic agents for the practical treatment of UTI and there is much evidence signifying a relationship between prescribing them as routine and development of antimicrobial resistance [17].

Production of ESBL coexists with resistance to many other antibiotics. ESBLs are plasmid mediated, which also carry genes of resistance for other antibiotics. Multi-drug resistance was relatively high in ESBL producers (90.5%), whereas it was only 68.9% in non-producers [18]. Originally ESBL producers were restricted to hospital –acquired infections only, but now they have also been isolated from outpatient departments. Major outbreaks involving ESBL producing strains have been reported from all over the world[5]. Monitoring of ESBL production and antimicrobial susceptibility testing are essential to avoid treatment failure in patients with UTI.

CONCLUSION

The usual antimicrobial susceptibility tests done in the clinical laboratories fail to detect the production of ESBL strains and can sometimes incorrectly report such isolates as sensitive to the broad-spectrum cephalosporins such as Cefotaxime, Ceftazidime and Ceftriaxone [19]. With the increase spread of ESBL producers in hospitals all over the world, it is necessary to know the occurrence of ESBL producer strains in a particular hospital so as to devise a policy of experiential therapy [19]. A knowledge about the resistance patterns of bacterial strains in a community helps to lead appropriate and judicious antimicrobial use. In this study, high prevalence rate of ESBL producing organism may be due to long term antibiotic use, prolonged stay in hospital, ICU environment, prolonged catheterization and previous bacterial infection. It is cautious in these situations to use non beta lactam drugs primarily or use Beta lactam drugs in combination with a beta-lactamase inhibitor. The control measures include judicious use of antibiotics and implementation of appropriate infection control programme to prevent spread of these strains.

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