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EXTENDED SPECTRUM β-LACTAMASES (ESBL) IN UROPATHOGENIC *ESCHERICHIA COLI*, PREVALENCE AND SUSCEPTIBILITY PATTERN IN A SOUTH INDIAN CITY

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ABSTRACT
The treatment of *Escherichia coli (E.coli)* infections is becoming difficult because of multidrug resistance. Extended spectrum β-lactamase (ESBL) production among *E.coli* resulted in limitations of therapeutic option. In this study, 300 strains of uropathogenic *E.coli* strains were studied for ESBL production by disc approximation test and double disc synergy test. Antibiotic sensitivity pattern of ESBL producers and Non ESBL producers are performed by Kirby-Bauer's disc diffusion technique. 56% of uropathogenic *E.coli* strains were ESBL producers. High degree of antibiotic resistance to gentamycin, norfloxacin, cotrimoxazole was seen among ESBL producers. Both ESBL producers and non-ESBL producers were sensitive to imipenum (100%). ESBL producers were susceptible to amikacin (84%), nitrofurntoin (91%) respectively.
Keywords: Uropathogenic *E.coli*, Extended spectrum β-lactamase, Multiple Drug Resistance

INTRODUCTION

Uropathogenic *E.coli* is responsible for approximately 85% of urinary tract infections. Treatment of *E.coli* infections is becoming difficult because of multidrug resistance. Production of extended spectrum β-lactamase results in multidrug resistance and limitations of therapeutic options.[1-5] ESBL is a plasmid encoded β-lactamase capable of hydrolyzing extended spectrum cephalosporins i.e., first, second, third and forth generation cephalosporins, penicillins and aztreonam except cephamycins and carbapenems.[6]

The number of ESBL producing strains among *E.coli* has been steadily increasing over the past years resulting in limitation in cephalosporin therapy.[7] *E.coli* has the ability to produce ESBL in large quantities and can spread among other strains during conjugation. The aim of the study is to find out the prevalence of ESBL production among uropathogenic *E.coli* and the drug resistance pattern of ESBL producers and non-ESBL producers, which is essential to guide appropriate antibiotic treatment.

MATERIAL AND METHODS

300 non-repeating strains of uropathogenic *E.coli* included in the study. Strains were isolated from hospitalized patients of different age groups with clinical symptoms of urinary tract infections, which yielded $>10^5$ bacteria per ml of urine on semi quantitative urine culture. The *E.coli* strains were collected from three major tertiary care centre, Yenepoya Medical College hospital, Father Muller Medical College hospital and K.S. Hegde Charitable hospital which receive patients from Dakshina Kannada and Udupi district of Karnataka, Kasaragod and Kannur District of Kerala during July 2009 to June 2011. All the strains identified by IMVIC (Indole, Methyl red, Voges - Proskaur and Citrate) reactions and sugar fermentation reactions[8]. The strains were subjected to antibiotic sensitivity test and ESBL detection test.

Antibiotic Sensitivity Test

All the *E.coli* strains are subjected to Antimicrobial susceptibility test by standard Kirby-Bauer's disc diffusion test. norfloxacin(10 µg), nitrofurantoin (300µg), gentamycin (15µg), cotrimoxazole (25µg), amoxiclav (20/10µg) cefotaxime (30µg) ceftazidime(30µg) amikacin (30µg), imipenem (10µg) were the antibiotics tested. The result is interpreted according to NCCLS criteria.[9]

Test for ESBL production

Screening of ESBL production was done by standard NCCLS criteria. Two disc of ceftazidime (30µg) and cefotaxime (30µg) were used for invitro susceptibility testing by Kirby-Bauer's disc diffusion method.[8,9] Inhibition zone of <22 mm for ceftazidime and <27 mm for cefotaxime indicate a probable ESBL producing strain.

ESBL Confirmation Test

A lawn culture of the test organism on Muller Hinton agar is prepared. A disc of amoxycillin (20µg) plus clavulanic acid (10µg) is placed on the surface, then discs of cefotaxime (30µg) and ceftazidime (30µg) were kept 20 mm apart from the amoxiclav disc (centre to centre) incubated at 37^0c overnight. The enhancement of the zone of inhibition of the cephalosporin disc towards the amoxiclav disc was considered as synergy and strain was considered as an ESBL producer. *E.coli* ATCC 25922 is used as the negative control in the procedure.[7, 9]

RESULTS

Out of 300 uropathogenic *E.coli* 168(56%) were ESBL producers. Multiple drug resistance was observed in ESBL producing strains of *E.coli*. However, all the strains of ESBL producer and non-ESBL producers were sensitive to imipenem.[10,11,12] amikacin and nitrofurantoin are the other two drugs to which more number of ESBL producers and non ESBL producers were sensitive. The sensitivity of ESBL producing and Non-ESBL strains to the other antibiotics tested is shown in Table 1.

Table 1: Antibiotic susceptibility of ESBL producer and non-ESBL producer

Antibiotics	ESBL Producer n=168	ESNL non-producer n=132
Norfloxacin	16(10%)	118(90%)
Gentamycin	45(27%)	117(89%)
Nitrofurantoin	152(91%)	122(93%)
Co-trimoxazole	50(30%)	66(50%)
Amoxyclav	105(62%)	117(89%)
Amikacin	141(84%)	117(89%)
Imipenem	168(100%)	132(100%)

DISCUSSION

Production of β-lactamase is a major means by which gram negative bacteria exhibit resistance to β-lactam antibiotics. Extended spectrum β-lactamases (ESBL) are a group of enzymes that can hydrolyse a variety of β-lactams including cephalosporins, monobactem and penicillins. The global spread of ESBL producing

E.coli into community has a potential to cause major problems in treatment in the coming years. Increased prevalence of ESBL production is detected among uropathogenic *E.coli*.

The first ESBL isolates were discovered in Europe in the mid 1980 and later in the US in late 1980[7]. Majority of ESBL producing strains are of *Klebsiella spp.* and *E.coli.* In India ESBL producing strains of Enterobacteriaceae have emerged as a challenge over last 15 years. Numerous outbreaks of infections both in community based and in hospitalized patients had reported. The emergence of ESBL production has posed a large threat to the use of many antibiotics especially the cephalosporins. The detection of ESBL has difficulties in laboratories because the resistant ESBL producing strains appear to be susceptible in vitro testing and results in treatment failure.

The wide spread use of cephalosporins and aztreonam is probable reason for emergence of mutant strains which produce ESBL. The prevalence of ESBL producing strain infections are high in tertiary care centers where the patients are referred from peripheral centers after profuse use of antibiotics. ESBL production is encoded by plasmid genes which also carry co-resistant genes for other antibiotics. Such a co resistance was observed among ESBL producing *E.coli* towards gentamycin, norfloxacin, co-trimoxazole. These finding are reported by other investigators.[13-16]

The present study shows that ESBL producing strains among *E.coli* is steadily increasing and the prevalence of ESBL strains varies in different geographical areas.[7,9,11] ESBL production among *E.coli* was 58% in Delhi (Mathur et al. 2002)[3], 41% in Coimbatore (Baby Padmini et al. 2004) [13], 32% in Bijapur, Karnataka (Basavaraj C Metri et al. 2010)[17], and 51% in Mangalore, Karnataka (Sharma et al. 2007).[9] The variation in percentage of ESBL producing strains of *E.coli* in different areas is probably due to the variation in the risk factors and the extent of antibiotic use.

The present study shows the increasing spread of ESBL producing uropathogenic *E.coli*. Constant survey of antimicrobial resistance is very important in the empiric treatment of UTI. Study also reveals the resistance pattern of uropathogenic strains of *E.coli.* Since co resistance towards commonly used non- β-lactam antibiotic like gentamycin, norfloxacin, cotrimoxazole was observed, amikacin and nitrofurantoin becomes the alternative for treating UTI with β-lactamase producing *E.coli* infections with low cost.[18]

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