

## Occurrence of ESBL and Multiple Drug Resistance in UTI caused by *Escherichia coli*

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The objective of this study was to evaluate the prevalence of multidrug resistance (MDR) and production of extended spectrum beta-lactamase (ESBL) by *Escherichia coli* (selected serotypes) isolated from patients suffering from urinary tract infection. Urine cultures were obtained from 308 patients with urinary illnesses. Isolates of *Escherichia coli* were serotyped and antibiotic susceptibility testing was carried out using disk diffusion (Kirby-Bauer). ESBL screening and phenotype confirmation were done following National Committee for Clinical Laboratory Standards (NCCLS) recommendations for *Escherichia coli*. A total of 180 serotyped isolates of *E. coli* were grown. 124 isolates (68.8%) were resistant to two or more antibiotics (MDR isolates), with a greater prevalence among serotype O75 (97%). Out of 124 urinary isolates exhibiting MDR, 54 isolates were suspected of ESBL production i.e. Ceftazidime screen positive. And out of 54 only 15 isolates were confirmed for ESBL production in DDST. A greater prevalence of uropathogenic *E. coli* serotypes with higher rates of multidrug resistance and ESBL production is concerning as well as the escalating incidence of ESBL-producing organisms has been attributed to the increased use of expanded-spectrum cephalosporins in clinical practice.

**Key words:** Multiple drug resistance, ESBL, uropathogens, *Escherichia coli*.

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Resistance to antimicrobials is thought to be a major worldwide problem. The clinical management of urinary tract infection is complicated by the increasing incidence of infections caused by strains of *E. coli* that are resistant to commonly used antimicrobial agents. While a number of drugs are available for UTI

management, increasing resistance rates among infecting organisms have limited the use of a number of antibiotics, leading to greater difficulty in treating these infections<sup>1</sup>.

The various mechanisms of drug resistance in Gram negative bacilli includes extended spectrum beta lactamase (ESBL) production also.

ESBL-producing *E. coli* can cause a wide range of infections, ranging from urinary tract infections to severe blood poisoning. Infections with ESBL-producing *E. coli* most commonly hit the elderly, people who have recently been in hospital and people who receive or have received antibiotic treatment. ESBL-producing *E. coli* are

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exceptionally uncommon in simple cystitis.

Many clinical laboratories currently test *Escherichia coli* and *Klebsiella* spp. for production of ESBLs. These enzymes are typically associated with multiple antibiotic resistances, leaving a few therapeutic options.

Since both MDR and Extended spectrum  $\beta$  – lactamases are encoded on plasmids and confer a selective advantage to strains harbouring these in a hospital setting<sup>2</sup>. It is important to know the occurrence of ESBL producing strains as well as their antibiotic susceptibilities to newer agents to guide empirical therapy for various infections. Keeping in view the high-level drug resistance in the community we conducted this study to determine the occurrence of Multi drug resistance and ESBL producing strains among the uropathogenic *Escherichia coli*.

## MATERIAL AND METHODS

A prospective study was conducted over a period one year. Urine samples were collected from patients suspected to have urinary tract infection from five different hospitals in and around Thane.

These included clean catch midstream urine samples. Urine (1 ml) was inoculated onto cysteine lactose electrolyte deficient (CLED, Hi-Media Laboratories, Mumbai, India) medium. Organisms grown in pure culture and in significant numbers ( $>10^5$  cfu/mL) were identified by standard biochemical tests and sent to Central Research Institute, Kasauli, H.P., for serotyping. Selected strains from each serogroups were taken for antibiotic susceptibility test by disc diffusion method (Kirby-Bauer)<sup>3</sup>.

Following antibiotics were used in the study

### Test for extended spectrum $\beta$ -lactamase production

Those serogroups which revealed multiple drug resistance (MDR) were taken for studying the ESBL activity.

**i) Screening test for ESBL detection:** by standard disc diffusion method

Screening for ESBL production was done according to criteria recommended by NCCLS<sup>4</sup>. Two discs, ceftazidime (30 mg) and cefotaxime (30 mg), were used for *in vitro* sensitivity testing

**Table 1.** Various drugs used in the study

S. No.	Antibiotics	Disc content
i)	Amikacin	30 $\mu$ g
ii)	Ampicillin	10 $\mu$ g
iii)	Cefopime	30 $\mu$ g
iv)	Ciprofloxacin	5 $\mu$ g
v)	Co—Trimoxazole (Trimethoprim/ Sulphamethoxazole)	1.25 $\mu$ g
vi)	Gentamicin	10 $\mu$ g
vii)	Kanamycin	30 $\mu$ g
viii)	Nalidixic acid	30 $\mu$ g
ix)	Norfloxacin	10 $\mu$ g
x)	Piperacillin	100 $\mu$ g
xi)	Streptomycin	10 $\mu$ g

by Kirby-Bauer disc diffusion method. Zone diameters were read using NCCLS criteria. An inhibition zone of  $\leq 22$  mm for ceftazidime and  $\leq 27$  mm for cefotaxime indicated a probable ESBL producing strain requiring phenotypic confirmatory testing.

### ii) Phenotypic confirmatory test

#### Double disk Synergy method

Disk diffusion method was used to confirm ESBL production by *E.coli* strains (5). Ceftazidime (30  $\mu$ g) vs. ceftazidime /clavulanic acid (30/10  $\mu$ g) and cefotaxime (30  $\mu$ g) vs. cefotaxime/clavulanic acid (30/10  $\mu$ g) were placed onto Mueller Hinton agar plate lawned with the test organisms and incubated overnight at 35°C. Regardless of zone diameters, a  $\geq 5$  mm increase in a zone diameter of an antimicrobial agent tested in combination with clavulanic acid vs. its zone size when tested alone, indicated ESBL production.

The source of Muller Hinton and antibiotic disks was Hi-Media, India.

## RESULTS AND DISCUSSION

Despite the widespread availability of antibiotics, UTI remains the most common bacterial infection in the human population. A high incidence of resistance to an antibiotic within a given population has long been accepted as evidence of widespread use of that antibiotic. In recent years, a significant increase in ESBL

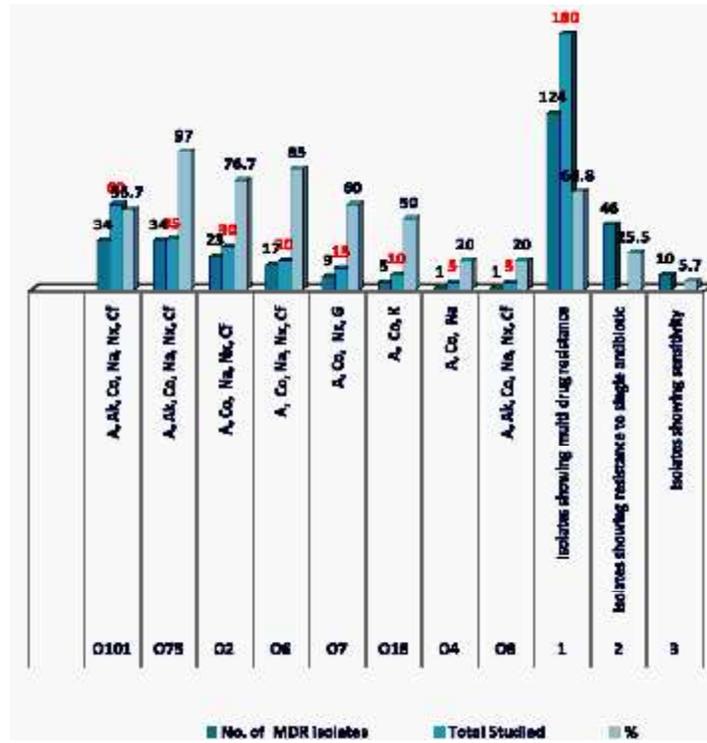


Fig. 1. Antibiotic Resistance Pattern of UPEC serogroups

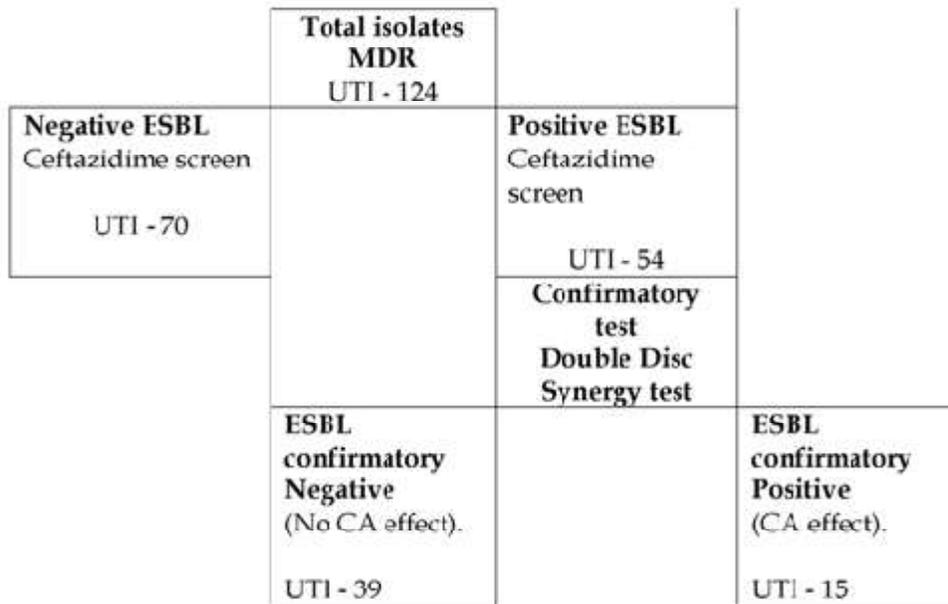


Fig. 2. The phenotypic profile of ESBL detection tests

producers was reported from USA<sup>6</sup>, Canada<sup>7</sup>, China<sup>8</sup> and Italy<sup>9</sup>.

The disc diffusion test was done for each isolates and the Mueller Hinton Agar was used as growth media with 4% sodium chloride.

In this study, the antibiotic sensitivities were determined against impregnated disks obtained from Hi media. Sensitivity and resistance pattern to various antibiotics followed in this study was based on NCCLS protocol.

With regard to the prevalence of antibiotic resistant *E.coli* from hospitalized and non-hospitalized UTI patients, 25.5% responded to one antibiotic, while 68.8% UPEC isolates showed multi drug resistance (Table 2).

Resistance to ampicillin and trimethoprim-sulfamethoxazole was observed in all the urinary serogroups. Resistance to nalidixic acid, norfloxacin and ciprofloxacin among *E.coli* serogroups ranged from 50-60 %.

The common serogroup O75 showed resistance up to 97 % or more to three or more antibiotics. All the UPEC strains revealed resistance to at least three of the antibiotics tested (Table 2 and Fig. 1).

Those serogroups which revealed multiple drug resistance (MDR) were taken for studying the ESBL activity.

#### **Extended Spectrum for Beta-lactamase activity: ESBL**

ESBL-producing *E.coli* are antibiotic-resistant strains of *E.coli*. The ESBL-producing strains manufacture an enzyme called extended-spectrum beta lactamase (ESBL). ESBL makes them resistant to cephalosporin antibiotics, as well as a number of other classes of antibiotics - making these infections much more challenging to treat.

This *E.coli* strain produces Extended-Spectrum Beta Lactamase (ESBL), an enzyme

**Table 2.** Multiple Drug Resistance (MDR) Patterns of urinary *E.coli* serogroups isolated

S. No.	Serogroup	Resistance pattern to commonly used antibiotics	No. of MDR isolates	Total Studied	%
1	O101	A, Ak, Co, Na, Nx, Cf	34	60	56.7
2	O75	A, Ak, Co, Na, Nx, Cf	34	35	97.0
3	O2	A, Co, Na, Nx, Cf	23	30	76.7
4	O6	A, Co, Na, Nx, Cf	17	20	85.0
5	O7	A, Co, Nx, G	09	15	60.0
6	O18	A, Co, K	05	10	50.0
7	O4	A, Co, Na	01	05	20.0
8	O8	A, Ak, Co, Na, Nx, Cf	01	05	20.0
		Isolates showing multi drug resistance	124	180	68.8
		Isolates showing resistance to single antibiotic	46		25.5
		Isolates showing sensitivity	10		05.7

which makes it such that infections become resistant to several antibiotic drugs. Patients develop urinary tract infections, which can develop into dangerous septicemia (blood poisoning).

There are not enough data on the prevalence of ESBL producers in urinary tract infection.

Hence, the present study was undertaken to find out prevalence of ESBL producers in urinary isolates of *E.coli*.

The phenotypic profile for ESBL detection test result is expressed in Fig. 2. Out of 124 urinary isolates exhibiting MDR, 54 isolates were suspected of ESBL production i.e. Ceftazidime screen positive. And out of 54 only 15 isolates were confirmed for ESBL production in DDST.

The percentage of ESBL producing strains in *E.coli* from UTI was found to be 12.0% (15/124).

This was significantly lower than the data available from other hospitals.

The overall prevalence of ESBL producers was found to vary greatly when the confirmatory tests were performed.

The multidrug resistance was significantly higher among ESBL producers. ESBL producers were almost always resistant to Ampicillin.

A high rate of ESBL production by *E. coli* may be due to the selective pressure imposed by extensive use of antimicrobials. The indiscriminate use of cephalosporins is responsible for the high rate of selection of ESBL producing microorganisms.

### REFERENCES

1. Murray, B.E. and Moellering, P.C. Patterns and mechanisms of antibiotic resistance. *Med. Clin. North. Am.*, 1987; **62** : 899 - 923.
2. Black, J. A., Moland, E. S. and Thomson, K.S. AmpC disk test for detection of plasmid-mediated AmpC- b lactamases in Enterobacteriaceae lacking chromosomal AmpC- b lactamases. *J Clin Microbiol.*, 2005; **43** : 3110-3.
3. Bauer, A. W., Kirby, W. M. and Sherris, J.C. Jurck M Antibiotic susceptibility testing by a standard single disc method. *Am J Clin. Path.*, 1996; **451**: 493-496.
4. National committee for clinical laboratory standards. Performance standards for antimicrobial disc susceptibility tests. Approved standard M2-A7. National Committee for Clinical Laboratory Standards: Wayne, PA; 2000.
5. Bradford, P.A. Extended spectrum b-lactamases in 21 st century: Characterisation, epidemiology and detection of this important resistance threat. *Clin Microbiol Rev.*, 2001; 933-51.
6. Saurina, G., Quale, G. M., Manikal, V. M., Oydna, E. and Landman, D. Antimicrobial resistance in enterobacteriaceae in Brooklyn NY: Epidemiology and relation to antibiotic usage patterns. *J Antimicrob Chemother.*, 2000; **45**: 895 - 8.
7. Cordero, L., Rau, R., Taylor, D. and Avers, L. W. Enteric Gram negative bacilli blood stream infections: 17 years experience in a neonatal intensive care unit. *Am J Infect Control.*, 2004; **32**: 189 - 95.
8. Xiong, Z., Zzhu, D., Zhang, Y. and Wang, F. Extended spectrum beta-lactamase in Klebsiella pneumoniae and Escherichia coli isolates. *Zhongua Yi Xue Za Zhi.*, 2002; **82**: 1476 - 9.
9. Luzzaro, F., Mezzatesta, M., Mugnaioli, C., Perilli, M., Stefani, S. and Amicosante, G., Trends in production of Extended Spectrum B-Lactamases among Enterobacteria of medical interest: Report of the second Italian nationwide survey. *J Clin Microbiol.*, 2006; **44**: 1659-64.