



## Antibiotic resistance pattern in urinary isolates of *Escherichia coli* with special reference to extended spectrum $\beta$ -Lactamases production

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### Abstract

*Escherichia coli* (*E.coli*) species are able to produce extended-spectrum  $\beta$ -Lactamase (ESBLs) that cause high resistance to the beta-lactam antibiotics. Present study was undertaken to know the occurrence of ESBLs productions in *E.coli* and to determining the antibiotic susceptibility patterns in resistant organisms for suitable therapeutic approaches. Clinical specimens of urine were collected from patients attending the Medical Microbiology Laboratories at Surat. Urine culture was done by conventional microbiological techniques. Isolation and identification of *E. coli* was carried out by standard procedure. Antibiotic sensitivity was done by the Kirby Bauer method. Production of ESBLs was detected by phenotypic confirmatory tests. During the study period 1500 urine samples were processed. Totally, 125 clinical isolates of *E.coli* were isolated and identified. ESBLs production was seen in 66% of isolates. All the ESBLs producing isolates were multidrug resistant (drug resistant to  $\geq 3$  drugs). This study reveals existence of high percentage ESBLs producing *E. coli* as well as MDR *E.coli*. Hence, constant revision of antibiotic policies with infection control interventions are necessary for interruption of the transmission of these endemic isolates

Key-Words: *Escherichia coli*, Resistance, ESBLs

### Introduction

Urinary Tract Infections (UTIs) are the most prevalent infections worldwide. *Escherichia coli* (*E.coli*) is the most common cause of both community-acquired and nosocomially transmitted UTIs [1][2]. National Nosocomial Infections Surveillance data indicate that 26% of all hospital-associated UTIs are caused by *E. coli* in USA[3]. In community-acquired illnesses, between 80% and 90% of all UTIs are caused by *E. coli* [4][5][6]. *E. coli* causes about 90% of first episodes of UTI in children [7]. Antimicrobial resistance has been recognized as an emerging worldwide problem both in developed and developing countries [8]. The effect could be severe in heavily populated developing country such as India where there is no strict monitoring program regarding the use of antibiotics. In gram negative pathogens, the most important resistance problems are encountered in *Enterobacteriaceae*, with increasing trends observed for all major anti-gram – negative agents ( $\beta$ -Lactams, fluoroquinolones and amino glycosides).

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In *Enterobacteriaceae* antimicrobial resistance in *E. coli* is of particular concern because it is the most common Gram negative pathogen causing infections in humans, particularly urinary tract infections (UTIs). In addition, resistant *E. coli* strains have the ability to transfer antibiotic resistance determinants not only to other strains of *E. coli* but also to other bacteria within the gastrointestinal tract. Antimicrobial drug resistance is on the rise worldwide with regional differences in the frequency of occurrence [9]. The wide range of occurrence of antibiotic resistance suggests that, in principle, any organism could develop resistance to any antibiotics [10]. Due to the production of extended-spectrum  $\beta$ -Lactamases (ESBLs) *E. coli* exhibits increasing resistance to  $\beta$ -Lactams antibiotic. The extended spectrum  $\beta$ -Lactamase (ESBL) enzymes are plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of  $\beta$ -Lactams, including third generation cephalosporins, penicillins and aztreonam [11]. Plasmids responsible for ESBL production carry resistance to many antibiotics like aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and co-trimoxazole [12][13]. The ESBL

producing organisms are reported in increasing numbers worldwide<sup>[14][15][16][17]</sup> Clinical Laboratory Standards Institute (CLSI) recommends screening for ESBL production among *E. coli*, *K.pneumoniae* and *K.oxytica*<sup>[18]</sup>. Detection of ESBLs producing organism from samples such as urine may be important because this represents an epidemiologic marker of colonization, and therefore there is potential for transfer of such organisms to others. These infections have a significant impact on patient's mortality and additional financial burden. Establishing trends or pattern facilitates an understanding of its development, deciding empiric antibiotic therapy, identifying measures to reduce increasing resistance trends and to acquire resistance from other organisms<sup>[10]</sup>. The present study is aimed to document the existence of ESBL-producing uropathogenic *E. coli* and to know about the status of alternative antibiotics in case the organism is ESBL-producer.

### Material and methods

#### Collection and processing of samples

The present study was conducted by collecting the clinical samples received for the bacteriological analysis in the Microbiology laboratories at Surat. During the tenure of this study we had processed 1500 numbers of urine samples. The samples were collected by following standard procedure as per the guideline mentioned by Isenberg (1998)<sup>[19]</sup> and WHO Manuals (1980)<sup>[20]</sup>. *E.coli* from urine samples were isolated and identified using chromogenic UTI agar plate<sup>[21]</sup> and further confirmed for their biochemical characteristics using multi test media.

#### Susceptibility testing

The determination of antibiogram for the collected strain were then carried out using modified Kirby-Bauer Disc-Agar diffusion technique recommended by

Clinical Laboratory Standard Institute (CLSI)<sup>[22]</sup>. The Commercially available (Hi Media Laboratories Pvt. Limited) antibiotics disc and their concentrations ( $\mu\text{g}$ ) used in this study were as follow. The results were interpreted as per Clinical Laboratory Standard Institute (CLSI) recommendations. Reference *E. coli* (ATCC 25922) was used as control for susceptibility testing.

#### Detection of Extended Spectrum $\beta$ – Lactamases (ESBLs) production

All the isolates under study were tested for their extended spectrum  $\beta$ -Lactamase production. The initial screening and phenotypic confirmatory tests recommended by the CLSI for ESBL detection were carried to assess the prevalence of ESBLs. Combination Disk diffusion method was used to confirm ESBL production by *E. coli* strains. Plates are inoculated as per the standard disc diffusion method as recommended in CLSI guidelines. (Performance Standard For Antimicrobial Disc Susceptibility Test Approved Standard -8<sup>th</sup> edition M2A8 Volume 3 No. 1 -2003). Pairs of discs containing an extended spectrum cephalosporin with and without clavulanic acid - cefotaxime(CE) 30  $\mu\text{g}$ , cefotaxime/clavulanic acid (CEC) 30/10  $\mu\text{g}$ , ceftazidime (CA) 30  $\mu\text{g}$ , ceftazidime/clavulanic acid (CAC) 30/10  $\mu\text{g}$  were placed on opposite sides of same inoculated plate. Zone of inhibition were measured following overnight incubation aerobically at 37°C. The test organism was regarded as an ESBL producer if the zone of inhibition around the combination disc is at least 5mm larger than that of the cephalosporin alone, or if the zone diameter is expanded by 50% in the presence of the clavulanic acid regardless of zone diameters. *E. coli* ATCC 25922 was used as ESBL negative control and *E. coli* ATCC 35218 was used as ESBL positive control strain.

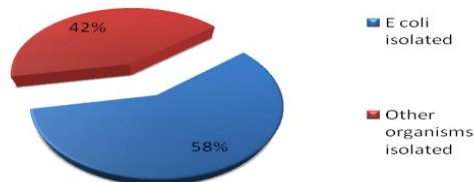
Table 1: Antibiotics used for *E.coli* isolates from urine

S/No	Name of antibiotic	Symbol	Concentration $\mu\text{g}/\text{disc}$	No.	Name of antibiotic	Symbol	Concentration $\mu\text{g}/\text{disc}$
1.	Ceftriaxone	CI	30 $\mu\text{g}$	9	Netilmicin	NT	30 $\mu\text{g}$
2.	Cephoxitin	CN	30 $\mu\text{g}$	10	Nitrofurantoin	NF	30 $\mu\text{g}$
3.	Ceftazidime	CA	30 $\mu\text{g}$	11	Norfloxacin	NX	10 $\mu\text{g}$
4.	Cephalexime	CE	30 $\mu\text{g}$	12	Ofloxacin	OF	5 $\mu\text{g}$
5.	Cefepime	CPM	30 $\mu\text{g}$	13	Co-trimoxazole	CO	1.25/23.75 $\mu\text{g}$
6.	Cefpirome	CFP	30 $\mu\text{g}$	14	Ciprofloxacin	CF	30 $\mu\text{g}$
7.	Ampicillin	A	10 $\mu\text{g}$	15	Imipenem	I	10 $\mu\text{g}$
8.	Nalidixic Acid	NA	30 $\mu\text{g}$	16	Meropenem	MR	10 $\mu\text{g}$

### Results and Discussion



A total 1500 Urine samples from both outdoor and indoor patients were analyzed. Out of these samples, a total of 215 urine samples yielded significant growth. Amongst these isolates *E.coli* was most common isolates (n=125) (58%)



**Fig. 1: Prevalence of *E.coli* in Uropathogen.**

In other two studies carried out on Uropathogens by Azra S.H. *et al*, New Delhi 2007<sup>[23]</sup> and Ritu Agrawal *et al*, Rohtak 2009<sup>[24]</sup> the prevalence of *E. coli* was reported respectively 50.7 % & 50 % among gram negative isolates, a higher incidence, i.e. 58% *E.coli* was observed in our study. Out of 125 *E.coli* isolates 75 isolates were from outdoor and 50 isolates were from indoor patients.

In our study range of patients age was between 1-80 years. Among them significant numbers of *E.coli* were isolated from age group of 15 - 50 years and above. In the study carried out by the Annabelle *et al* : 1999<sup>[25]</sup>; the most common organism causing UTI in women between 18 – 45 year of age, adults with complicated UTI and adults with a symptomatic bacteriuria was *E. coli*, which is in accordance with our findings. The more isolates were recovered from females (n=76) as compared to males (n=49). It is stated that UTI is predominantly a disease of the females due to a short urethra and proximity to anal opening. In our study too there was a female preponderance for this infection. Present findings were also in agreement with the findings of the Olafsson M *et al*, 2000<sup>[26]</sup>; and Gupta *et al*, 1999<sup>[27]</sup>; they found *E. coli* as the most common isolates in females in their study on uropathogens.

In our study 66 % of the isolates were found to be ESBL producers when tested with Cephalexime / Clavulanic Acid (CE / CEC) combination and 63 % with Ceftazidime / Clavulanic Acid (CA / CAC) combination. Tankhiwala *et al*, (2004)<sup>[28]</sup> reported 48.3% and Ritu Aggarwal, Uma Chaudhry and Rama Sikka, Rohtak: 2009<sup>[29]</sup> reported 40 % ESBL producers *E. coli* isolated from the urine, which were lower than our study findings

The production of  $\beta$ -Lactamase may be of chromosomal or plasmid origin. Plasmid mediated production is often acquired by transfer of genetic information from one organism to another. Such transferable plasmid also codes for resistant

determinants to other antimicrobial agents. Hence multidrug resistance is expected to be more common in ESBL producing organisms. In the present study, ESBL producing isolates were found to be resistant to three or more drugs whereas multidrug resistance in non ESBL producers was also seen but the difference was statistically significant. Our study showed that ESBL production was high among uropathogens. This study also shows that *E.coli* recovered from clinical specimens of Urine in this region produce ESBLs in much high number. Thus, are resistant to penicillins and cephalosporins, which are important drugs for the treatment of UTIs. Such isolates are also resistant to fluoroquinolones, aminoglycosides and cotrimoxazole. In this study, 96.8% sensitivity to imipenem and 88.8% sensitivity to Meropenem was observed thus Carbapenems are the drugs of choice against such infections caused by *E.coli*. Hence, routine ESBL testing for uropathogens along with conventional antibiogram would be useful for all cases of UTI.

Our study confirms the global trend towards increased resistance to  $\beta$ -lactam antibiotics. Moreover, the prevalence and antibiotic susceptibility pattern of ESBLs producers differs geographically. The higher multiple drug resistance in this region is a cause for concern. Due to the increasing resistance of *E.coli* to common medications it is recommended that any changes in the resistance pattern of pathogen is monitored in larger scale. Further molecular studies should be conducted to know the basis of this MDR. Strict antibiotic usage policy should be adopted for reducing resistance.

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Table 2: Results of the ESBL detection in Urine isolates

Combination	Symbol	Positive	Negative	Positive (%)	Negative (%)
Cephotoxime / Clavulanic Acid	CE / CEC	82	43	66	34
Ceftazidime / Clavulanic Acid	CA / CAC	79	46	63	37

Table 3: Results of antimicrobial agents between ESBLs producing and non-ESBLs producing *E.coli* isolates

Antibiotic	ESBLs Positive (n=82)			ESBLs Negative (n=43)		
	R	MS	S	R	MS	S
Ceftriaxone	76	0	6	24	4	15
Cephoxitin	56	11	15	17	15	11
Ceftazidime	76	3	3	22	13	8
Cephotoxime	74	8	0	22	13	8
Cefepime	74	8	0	25	6	12
Cefpirome	82	0	0	40	1	2
Ciprofloxacin	72	7	3	34	5	4
Nalidixic Acid	79	3	0	36	1	6
Netilmicin	21	5	56	10	3	30
Nitrofurantoin	32	21	29	23	6	14
Norfloxacin	75	4	3	32	4	7
Ofloxacin	72	5	5	33	1	9
Co-trimoxazole	28	2	52	12	3	28
Ampicillin	80	1	1	41	1	1
Imipenem	0	1	81	3	0	40
Meropenem	5	1	76	5	3	35

S=Sensitive, MS=Moderately Sensitive, R=Resistant

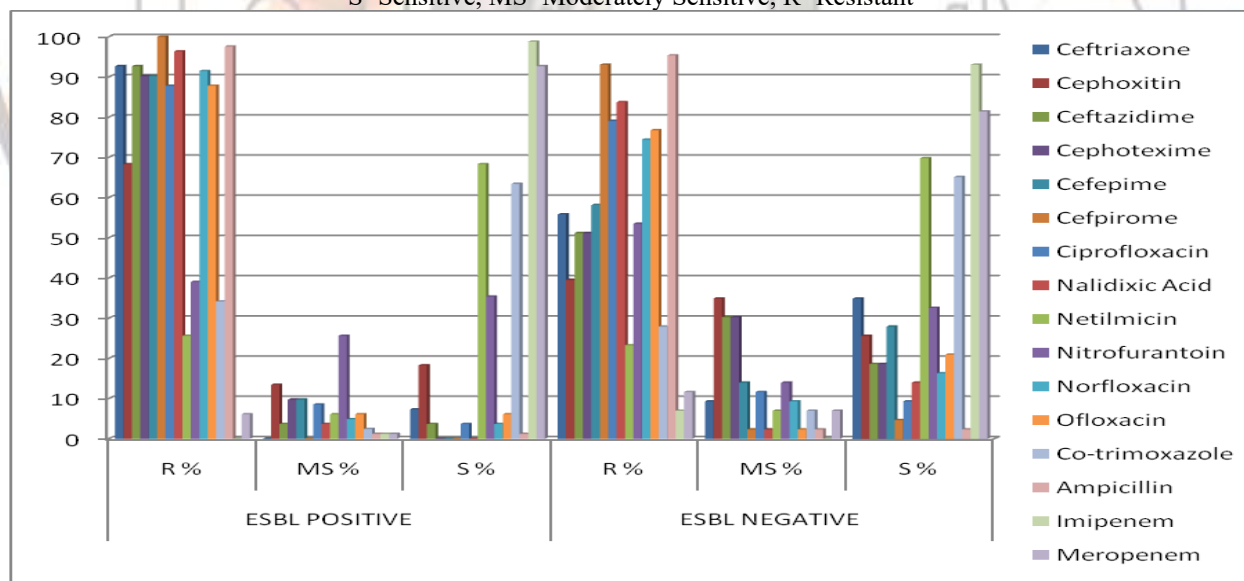


Fig. 2: Antibiotic wise resistance pattern in ESBLs producing isolate and non-ESBLs producing isolates