

Original Research Article

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Isolation and Antibiotic Sensitivity Pattern of Extended Spectrum Beta Lactamases (ESBL) Producing *Escherichia coli* Isolated from Urinary Tract Infection

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ABSTRACT

Escherichia coli is the most common organism causing urinary tract infection (UTI). This organism has the ability to produce Extended Spectrum Beta Lactamases (ESBLs), which confer multiple drug resistance making urinary tract infection difficult to treat. So treatment of UTI requires constant updating of the antibiotic sensitivity profile. Objectives of this study were to detect prevalence of ESBL production among *E. coli* isolates causing urinary tract infection and to detect their antibiotic susceptibility pattern. A total of 400 consecutive, non-repetitive *E. coli* isolates were studied. Antimicrobial susceptibility test was performed using Kirby Bauer disk diffusion method. ESBL detection was done for all isolates according to latest CLSI criteria. Out of 400 *E. coli* isolate, 244(61%) were ESBL producers and 156(39%) were Non ESBL producers. The isolates were highly susceptible to imipenem (100%) and Piperacillin/Tazobactam (88.1%) and were least susceptible to Ampicillin (100%) and Cotrimoxazole (89.7%). This study demonstrate the importance of regular review of empirical antibiotic therapy for UTI in view of the evolving resistance of ESBL producing *E. coli* to commonly used antimicrobial agents.

Keywords

E. coli,
UTI, ESBL,
Kirby Bauer disk
diffusion method,
CLSI.

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Introduction

Extended spectrum beta lactamases (ESBLs) producing bacteria are typically resistant to penicillins, first and second generation cephalosporins as well as the third generation oxyiminocephalosporins (e.g., Ceftazidime, Ceftriaxone) and Monobactam (Aztreonam) except cephamycins and carbapenems (Rawat *et al.*, 2010).

The persistent exposure of the bacterial strains to a multitude of β -lactams has induced a dynamic and continuous production

and mutation of β -lactamases in the bacteria. ESBL enzymes are plasmid borne and they have evolved from point mutations which altered the configuration of the active site of the original and long known β -lactamases, which have been designated as TEM-1, TEM-2 and SHV-1 (Nathisuwan *et al.*, 2001). The resistance to newer β -lactams which are a result of these β -lactamases has emerged quickly. These enzymes are commonly produced by many members of Enterobacteriaceae, especially *E. coli* and *K.*

pneumoniae. First isolated in 1983 in Germany, ESBLs spread rapidly to Europe, United States and Asia and are now found all over the world (Suganya *et al.*, 2014). Since ESBL positive isolates show false susceptibility to extended spectrum cephalosporins in standard disk diffusion tests (Kumar *et al.*, 2006). It is difficult to reliably detect ESBL production by the routine disk diffusion techniques. Specific detection methods such as double disk potentiation methods recommended by CLSI (2016) have to be adopted. ESBLs are inhibited by β lactamase inhibitors like clavulanic acid, sulbactam and tazobactam and this property of specific inhibition can be utilized for the detection and confirmation of ESBLs.

It is estimated that there are about 150 million urinary tract infections per annum worldwide (Stamm *et al.*, 2001). *Escherichia coli* is the most common organism causing urinary tract infection (UTI). This organism has the ability to produce ESBLs, which confer multiple drug resistance making urinary tract infection difficult to treat (Kariuki *et al.*, 2007).

Delay or failure in identifying and reporting ESBL production contributes to their uncontrolled spread. Infections with ESBL are associated with prolonged hospital stay, increased morbidity, mortality, and health care costs. Many clinical laboratories are still not aware of the importance of screening for ESBL-producing *E. coli*.

A heightened awareness of these organisms by clinicians and enhanced testing by laboratories is the need of the hour. Knowledge of antibiotic resistance pattern will help in the appropriate and judicious antibiotic use. The main objectives of this study includes to detect prevalence of ESBL production among *E. coli* isolates causing urinary tract infection and also to detect their antibiotic susceptibility pattern.

Materials and Methods

The patients admitted and / or attending the outpatient department in Basaveshwara teaching and general hospital, Kalaburagi, Karnataka from September 2016 to January 2017 with signs and symptoms suggestive of urinary tract infection were included in the study. The study was approved by the institutional ethics committee. Informed consent was taken from all the patients. A total of 400 consecutive, non-repetitive *E. coli* isolates were studied during this period.

Isolation of pathogens

Urine specimens were inoculated onto Blood agar, MacConkey agar and CLED agar by using standard techniques. Plates were incubated at 37°C for overnight before the plates were inspected for growth. Gram's staining was performed (Cheesbrough, 1989).

Identification of isolates

Identification of all isolates was done on the basis of routine biochemical tests i.e., Gram staining, catalase test, oxidase test, motility, indole production, methyl red test, vogesproskauer test, citrate utilization test, nitrate reduction test, triple sugar iron test, urease production, sugar fermentation test and amino acid decarboxylation tests using standard techniques (Baird, 2014).

Antimicrobial susceptibility test

This was performed using Kirby Bauer disk diffusion method. Following antibiotic disks were used: Amikacin (30 μ g), Gentamicin (10 μ g), Amoxicillin/Clavulanate (20/10 μ g), Cefotaxime (30 μ g), Cefepime (30 μ g), Cefuroxime (30 μ g), Ciprofloxacin (5 μ g), Cotrimoxazole (1.25/23.75 μ g), Nalidixic acid (30 μ g), Nitrofurantoin (300 μ g), Norfloxacin (10 μ g), Piperacillin/ Tazobactam (100/10 μ g), Imipenem (10 μ g).

The disk were obtained from high media laboratories. The diameter of zone of inhibition was measured and interpreted according to CLSI guidelines (2016).

Detection of ESBL

ESBL detection was done for all isolates according to latest CLSI criteria.

Screening test

According to latest CLSI guidelines, zone diameter of *E. coli* strain for ceftazidime <22mm and for cefotaxime <21mm is presumptively taken to indicate ESBL production.

Confirmatory test

As per CLSI guidelines, ESBLs were confirmed by placing disk of cefotaxime and ceftazidime at a distance of 20mm from a disk of cefotaxime/clavulanate (30/10µg) and ceftazidime/clavulanate (30/10µg) respectively on a lawn culture of test strain (0.5 McFarland inoculum size) on Mueller-Hinton agar. After overnight incubation at 37° C, ESBL production was confirmed if there was ≥ 5mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanate versus its zone when tested alone.

Results and Discussion

Table 1 shows the number and percentage of ESBL and Non ESBL producing *E. coli* isolates. Out of 400 *E. coli* isolate, 244(61%) are ESBL producers and 156(39%) are Non ESBL producers. Graph 1 shows ESBL producers among *E. coli* isolates in UTI. Table 2 shows the number and percentage of ESBL producing *E. coli* isolates in males and females. Out of 244 ESBL producing *E. coli* isolates, 90(36.89%) were found in males and

154 (63.11%) in females. Graph 2 shows the gender distribution of ESBL producing *E. coli* isolates.

Table 3 and Graph 3 shows the antibiotic susceptibility pattern of ESBL producing *E. coli* isolates from UTI. All ESBL producers were resistant to Ampicillin. 93.8% were resistant to Cotrimoxazole, 89.7% were resistant to Nalidixic acid, 88.1% were resistant to Gentamicin, 84% were resistant to Amoxicillin/Clavulanate, 82% were resistant to Ciprofloxacin, 73.7% were resistant to Ceftazidime, 69% were resistant to Norfloxacin, 59.8% were resistant to Amikacin, 27% were resistant to Nitrofurantoin, 11.9% were resistant to Piperacillin/Tazobactam and all the ESBL producers were sensitive to Imipenem.

Urinary tract infections are the most common bacterial infection (Foxman, 2002). *Escherichia coli* is the most common organism causing urinary tract infection (UTI). Extended spectrum beta - lactamases (ESBLs) are on the rise in hospital settings across the globe (Sulochana *et al.*, 2013). The antimicrobial resistance patterns of organisms-causing UTI are changing over the years, including resistance due to ESBL producing pathogens. Correct identification of ESBL producing organisms in due time is necessary not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organism (Sasirekha, 2013). This study was a small step towards the same.

In the present study it was observed that 61% of *E. coli* isolates were ESBL producers. Studies done in other parts of the country have shown an incidence between 21% and 82% (Table 4). The wide variation in prevalence may be due to differences in the risk factors, the extent of antibiotic use, and

the selection of organisms for study. The high incidence in our center was probably due to the fact that it is a tertiary care center with high usage of antibiotics, especially 3rd generation cephalosporins.

In our study prevalence of ESBL producing *E. coli* was found to be 61%. This is in correlation with other studies such as Mahesh *et al.*, (2010) and Chaudhary *et al.*, (2013) who reported 56.2% and 54.5% ESBL production in *E. coli* isolates respectively. However our findings are in contrast with

other studies conducted by Datta *et al.*, (2014) Dugal *et al.*, (2013) DMBT Dissanayake *et al.*, (2012) and Singh *et al.*, (2016) who reported 21.4%, 24.4%, 29% and 82.6% ESBL producing *E. coli* isolates respectively.

Gender wise distribution of ESBL revealed a female preponderance (63.11%) over males (36.89%). This may be due to the fact that UTI is more common in females, principally owing to anatomic and physical Factors. This is similar to studies done by Sasirekha *et al.*, (2013) and Rajan *et al.*, (2012).

Table.1 ESBL producers among *E. coli* isolates

Total number of <i>E.coli</i> isolates	ESBL producers		Non ESBL producers	
	Number	%	Number	%
400	244	61	156	39

Table.2 Gender distribution of ESBL positive *E. coli* isolates

Total number of ESBL isolates	ESBL producers in males		ESBL producers in females	
	Number	%	Number	%
244	90	36.89%	154	63.11%

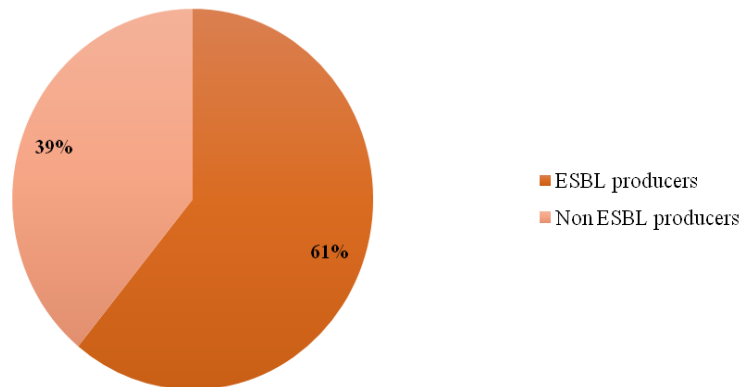
Table.3 Antibiotic susceptibility pattern of ESBL producing *E. coli* isolates from UTI

Antibiotics	Sensitive	Intermediate	Resistant
Ampicillin	0	0	244(100%)
Ciprofloxacin	44(18%)	0	200(82%)
Cotrimoxazole	11(4.5%)	4(1.7%)	229(93.8%)
Amikacin	59(24.2%)	39(16%)	146(59.8%)
Gentamicin	15(6.2%)	14(5.7%)	215(88.1%)
Amoxicillin/Clavulanate	39(16%)	0	205(84%)
Ceftazidime	55(22.6%)	9(3.7%)	180(73.7%)
Nalidixic acid	25(10.3%)	0	219(89.7%)
Nitrofurantoin	174(71.3%)	4(1.7%)	66(27%)
Norfloxacin	76(31%)	0	168(69%)
Piperacillin/Tazobactam	215(88.1%)	0	29(11.9%)
Imipenem	244(100%)	0	0

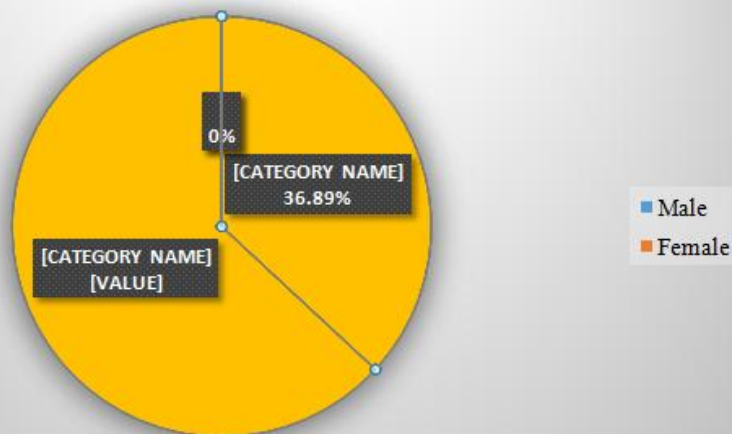
Table.4 Various studies showing the prevalence of ESBL producing *E. coli* isolated from UTI

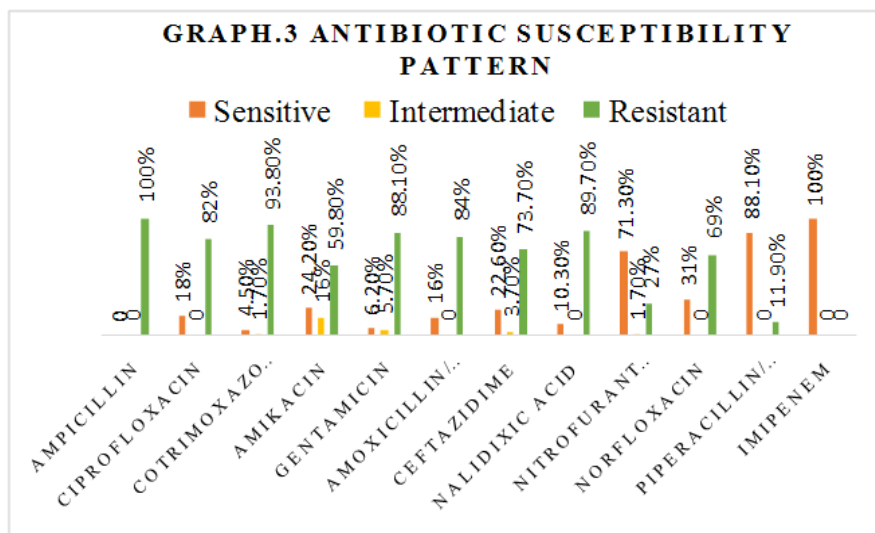
S. No.	Studies	Year	Prevalence
1	Datta P <i>et al.</i> , ¹²	2014	21.4%
2	Dugal S <i>et al.</i> , ¹³	2013	24.4%
3	DMBT Dissanayake <i>et al.</i> , ¹⁴	2012	29%
4	Chaudhary NK <i>et al.</i> , ¹⁵	2013	54.5%
5	Mahesh E <i>et al.</i> , ¹⁶	2010	56.2%
6	Singh N <i>et al.</i> , ¹⁷	2016	82.6%
7	Present Study	2017	61%

Graph 1: ESBL producers among clinical isolates



Graph 2: Gender Distribution





In the present study, we used phenotypic confirmation test (PCT) for detection of ESBL producer. PCT is technically much simpler and inexpensive compared to Double Disk Synergy Test (DDST). The interpretation is straight forward. Assuming that a laboratory is currently testing the sensitivity for ceftazidime and cefotaxime with the disk diffusion tests, only two disks are required to be added to the sensitivity plate to perform a PCT. This would screen all gram negative organisms in the laboratory for ESBL production (Selvakumar *et al.*, 2007).

Ampicillin resistance among ESBL producing *E. coli* was found to be 100% which is similar to the finding of Behroozi *et al.*, (2010) (100%). Ciprofloxacin resistance was found to be 82% which is in correlation to the finding of Shafaq *et al.*, (2011) (85%). Cotrimoxazole resistance was found to be 93.8% which is higher than the findings of Chaudhary *et al.*, (2013) (78.8%) and Behroozi *et al.*, (2010) (80%). Amikacin resistance was 59.8% which is in correlation with the finding of Behroozi *et al.*, (54%). Gentamicin resistance was found to be 88% which is higher than the findings of Behroozi *et al.*, (50%), Chaudhary *et al.*, (50.9%) and Shafaq *et al.*, (60%). Amoxicillin/Clavulanate

resistance was found to be 84% which is in correlation with Shafaq *et al.*, (2011) (85%) and Dutta *et al.*, (2014) (88.5%). Ceftazidime resistance was found to be 73.7% which is in between Daryl *et al.*, (2012) (69%) and Behroozi *et al.*, (2010) (85%). Nalidixic acid resistance was found to be 89.7% which is similar to Behroozi *et al.*, (85%). Nitrofurantoin resistance was 27% which is in between Behroozi *et al.*, (20%) and Chaudhary *et al.*, (2013) (38.8%). Piperacillin/ Tazobactam resistance was found to be 11.9% which is similar to the findings of Daryl *et al.*, (2014) (16%). Imipenem sensitivity was found to be 100% which is similar to the findings of Daryl *et al.*, (2014) (100%) and Shafaq *et al.*, (2011) (100%).

The present study demonstrates that some ESBL producing isolates show false susceptibility to third generation cephalosporin in in-vitro testing. Therefore, we recommend that detection of ESBL should be undertaken before starting UTI treatment.

In conclusion, the present study found 61% ESBL producing *E. coli* isolate in UTI. Most of the ESBL producing *E. coli* isolates were multidrug resistant making available

therapeutic choices limited. Our study also demonstrates the importance of regular review of empirical antibiotic therapy for UTI in view of the evolving resistance of ESBL producing *E. coli* to commonly used agents.

Clinicians must depend on more laboratory guidance, while laboratories must provide resistance pattern data for optimal patient management more accurately. Additionally, robust antimicrobial stewardship and strengthened infection control measures are required to prevent the spread and reduce the emergence of antibiotic resistance.

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