Phenotypic detection and antibiotic susceptibility pattern of ESBL producing *Escherichia coli* from UTI patients at a tertiary care hospital in Jaipur

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**Abstract**

*UTI* caused by *Escherichia coli* (E. coli) is reportedly the most common infection. According to current knowledge, antimicrobial resistance of these gram-negative bacteria especially to *Beta* lactam antibiotics, further add on to the problem. Extended-Spectrum beta-Lactamase (ESBL) producing *E. coli* in urine cannot be detected by routine disk diffusion susceptibility test, which may lead to inappropriate use of antibiotics and thus ineffective treatment. The aim of this study was thus to determine the susceptibility pattern and phenotypic detection of ESBL producing *E. coli* from UTI patients. A total of 247 *E. coli* isolated insignificant number from UTI patients were included in the study and identified by conventional biochemical tests as per laboratory protocol. Antimicrobial susceptibility testing was performed by Kirby Bauer's disc diffusion method for ten antibiotics of different groups. Isolates showing resistance to third-generation cephalosporin were further tested for confirmation of ESBL production by Double disc approximation test, combined disc diffusion test and confirmed by ESBL E-test. During the study period 6084 urine samples from suspected UTI patients were tested. 56% (3397) were found to be caused by *E. coli*. Out of which 247 *E. coli* isolates chosen for the study. 20% were found to be ESBL producers by various phenotypic methods. The majority of the isolates were from males i.e., 53.8% (133) and females were 46.2% (114). *UTI* was observed highest amongst outpatients (61.9%) followed by various IPD wards (34.8%) and ICUs (0.3%). Among the isolates high degree of resistance was seen against Norfloxacin (81.7%), ciprofloxacin (77.7%) followed by amoxyclav (70.4%). Results indicate that *UTI* due to ESBL producing *E. coli* is common in the community. Thus, routine ESBL detection should be made compulsory not only in indoor patients but in outdoor patients also.

**Keywords:** *UTI*, *Escherichia coli*, Extended spectrum beta lactamase, 3rd Generation Cephalosporins, Antimicrobial susceptibility.
Introduction
Urinary tract infection (UTI) is one of the most common infectious diseases. Worldwide about 250 million people are diagnosed with UTI each year. Bacteria is the key causative organism of UTI and E. coli is the most prevalent-causative bacteria of UTI followed by Klebsiella, Staphylococcus, Proteus & Pseudomonas. Several studies show that the pattern of antibiotic usage affects the development and emergence of resistant microorganism. E. coli has shown increasing antimicrobial resistance to most antibiotics particularly Cephalosporins due to the production of an arsenal of enzymes known as Extended spectrum β-lactamases. ESBLs are β-lactamases which are able of hydrolyzing oxyimino- cephalosporins and are inhibited by beta-lactamase inhibitors. The incidence of ESBL producing E. coli has been increasing worldwide due to the misuse of antibiotics. Extended-spectrum beta-lactamases (ESBLs) have emerged as a challenge with a higher incidence of resistance for the treatment of UTIs. ESBLs are clinically significant as they render cephalosporins ineffective, given as first-line antibiotics which may lead to improper treatment and increased mortality.

In community and hospital-acquired infection, the aetiology of UTI and the antibiotic resistance pattern of uropathogens have been changing over the past years. And we are left with limited therapeutic options to treat urinary tract infections. Hence, the present study was conducted to identify ESBL producers by different phenotypic methods, their frequency and resistance pattern in Jaipur. For the empirical therapy and judicious management of UTI patients, the present study will prove to be very useful. Furthermore, it will also help the authorities to plan advanced antibiotic prescribing strategy.

Materials and Method
Sample collection and analysis: The study was conducted on 247 isolated uropathogenic E. coli over 1 year (October 2016 to October 2017). Urine samples from clinically suspected UTI patients were analysed for significant bacteriuria. The urine samples received were inoculated by semi-quantitative method on MacConkey agar and Blood agar media by calibrated loop technique and followed after 24 hours of aerobic incubation at 37°C. All the isolates were identified to the species level by their colony morphology, staining characters, motility and other standard biochemical tests as per laboratory SOP.

Antibiotic susceptibility testing: Susceptibility to different antibiotics was determined by Kirby Bauer’s disc diffusion method on Mueller Hilton agar media as per the standard CLSI guidelines (2016). Following antibiotic discs were used: Amikacin (30µg), Amoxyclav (30µg), Cefotaxime (30µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Cotrimoxazole (25µg), Gentamycin (10µg), Meropenem (10µg), Imipenem (10µg), Nitrofurantoin (100µg), Norfloxacin (10µg), Piperacillin/tazobactam (110µg).

Detection of ESBL production (Phenotypic)
Screening test: Isolates of E. coli found resistant to 3rd generation Cephalosporins (Cefotaxime and/or ceftazidime discs) were further tested for ESBL production by phenotypic tests.

The Combined disc diffusion test [13] While performing antibiotic testing, cefotaxime (30µg) & ceftaxime plus clavulanic acid (30/10µg) and ceftazidime (30µg) & ceftazidime plus clavulanic acid (30/10µg) were applied on Mueller-Hinton agar and incubated. If there was an increase in inhibitory zone diameter of the disc containing clavulanate and that of the antibiotic disc alone by ≥5, then the organism was considered as ESBL producer.

Double disc approximation test [14] The organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic discs of amoxicillin/clavulanic acid (20/10µg) and
cefotaxime (30μg) were placed at a distance of 15 mm apart and incubated. ESBL producing organism showed a clear extension of cefotaxime inhibition zone towards the disc containing clavulanic acid.

Phenotypic Confirmatory test
The E-test
The E test ESBL CT/CTL strips comprise of an inert, thin and non-porous plastic carrier (5 x 60 mm). One side of the strip was calibrated with MIC reading scales in μg/mL while the reverse surface carries two predefined exponential gradients. CT codes for the cefotaxime (0.25-16μg/mL) gradient and CTL for cefotaxime (0.016-1μg/mL) plus 4μg/mL clavulanic acid. The set up was made according to standard E test protocols for Gram-negative aerobes, though, an inhibition ellipse may appear at each end of the strip.

In the presence of clavulanic acid, if the MIC of CT was reduced by ≥ 3 log2 dilutions either there is a formation of the phantom zone or CT ellipse is deformed, the ESBL production was confirmed. From an overnight agar plate, some well-isolated colonies were emulsified in saline to obtain a turbidity equivalent to a 0.5 McFarland standard. To make sure a uniform distribution of the inoculum, the entire agar surface was swabbed three times by rotating the plate approximately 60° each time. To make the surface dry excessive moisture was allowed to be absorbed for about 15 minutes, before applying E test ESBL strips. E test ESBL strips were applied to the inoculated agar surface with a pair of forceps placing the MIC scale upward. The agar plates were then incubated in an inverted position at 35 ± 2 °C for 16-20 hours.

The plates were analysed after incubation. CT and CTL MIC values were recorded where the inhibition ellipses intersect the MIC strip. Growth along the entire gradient indicates that the MIC was greater than the highest value on the scale. An inhibition ellipse below the gradient indicates a MIC less than the lowest value on the scale. When the mutant colonies are present in the inhibition ellipse, MIC was read where the colonies were completely inhibited. ESBL production was determined by the ≥3 two-fold concentration decrease in the MIC of CTL in the presence of clavulanic acid[15]. Occasionally, a rounded zone (phantom zone) was seen below the CTL gradients while no ellipse was seen around the CT end. Presence of a phantom zone or ellipse deformation also indicates ESBL production due to synergy between CT and clavulanic acid diffusing across the CTL sections.

Statistical Analysis
Chi-square test was performed to check the significant difference among the different groups. A difference was considered significant if the probability that chance would explain the results was reduced to less than 0.1% (P ≤ 0.001).

Results
During 1 year of the study period, a total of 6084 urine samples were tested from UTI patients out of which 56% (3397) were caused due to Escherichia coli. Out of 247 significant E. coli isolates, it was identified that 53.8% (133) were from male patients and 46.2% (114) were from females. Most of the cases of UTI were recorded among young and middle-aged patients i.e., 21-30 years of age group included 23% (57) of the total population followed by 31-40 years age group, which recorded about 18% (45) of the total population. Results show that there was more number of males affected by UTI in certain age groups. In the patients between 41-60 years of age frequency of male patients affected by UTI was greater than female patients. Elderly patients comprised about 10% of the total number. Age and gender-wise distribution revealed that the prevalence of E. coli as uropathogen was found to be more in middle-aged and elderly males and adolescent females. And as the age increases this prevalence decreases.
A statistically significant association was found between age and sex of UTI affected patients. According to the screening test, 126 isolates were accounted resistant to Cefotaxime and 152 isolates were resistant to Ceftazidime and thus considered to be probable ESBL producer. Double disc approximation test (DDAT) and Combined disc diffusion test (CDDT) were conducted on the above positive isolates. According to CDDT results 35 isolates were ESBL producers and DDAT showed 29 isolates to be ESBL positive. For further confirmation E test was conducted on the above positive isolates. 49 isolates were confirmed to be ESBL producers.

**Antimicrobial susceptibility testing**

The susceptibility pattern of UTI patients caused by *E. coli* showed the highest resistance towards norfloxacin (82%) followed by ciprofloxacin (78%) and amoxyclav (70%). Association between ESBL production and antimicrobial resistance were found highly significant in Cefotaxime, ceftazidime and significant in Piperacillin/tazobactam and Cotimoxazole.

### Table 1: Percentage of resistant *E. coli* strains against various groups of Antibiotics

<table>
<thead>
<tr>
<th>Class of antibiotics</th>
<th>Antibiotics used</th>
<th>Resistance n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furandantins</td>
<td>Nitrofurantoin</td>
<td>42 (17)</td>
</tr>
<tr>
<td>Folate pathway Inhibitor</td>
<td>Cotrimoxazol</td>
<td>167 (67.6)</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Ciprofloxacin</td>
<td>192 (77.7)</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>200 (80.9)</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Gentamycin</td>
<td>109 (44.1)</td>
</tr>
<tr>
<td></td>
<td>Amikacin</td>
<td>70 (28.3)</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Cefotaxime</td>
<td>126 (51)</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime</td>
<td>152 (61.5)</td>
</tr>
<tr>
<td>Beta-lactam + Beta-lactam inhibitor</td>
<td>Amoxyclav</td>
<td>175 (70.8)</td>
</tr>
<tr>
<td></td>
<td>Piperacllin/Tazobactam</td>
<td>67 (27.12)</td>
</tr>
<tr>
<td></td>
<td>Cefotaxime+Clavulanate</td>
<td>93 (37.6)</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime+Clavulanate</td>
<td>115 (46.5)</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>Imipenem</td>
<td>50 (20.2)</td>
</tr>
<tr>
<td></td>
<td>Meropenem</td>
<td>58 (23.4)</td>
</tr>
</tbody>
</table>

### Table 2: Association of ESBL/Non ESBL uropathogens with antimicrobial susceptibility (No. of patients susceptible)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>ESBL</th>
<th>Non ESBL</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>40</td>
<td>136</td>
<td>0.106</td>
</tr>
<tr>
<td>Amoxyclav</td>
<td>11</td>
<td>62</td>
<td>0.297</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>4</td>
<td>117</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>1</td>
<td>94</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>7</td>
<td>48</td>
<td>0.191</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>16</td>
<td>64</td>
<td>0.0001</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>25</td>
<td>113</td>
<td>0.547</td>
</tr>
<tr>
<td>Imipenem</td>
<td>42</td>
<td>155</td>
<td>0.337</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>39</td>
<td>166</td>
<td>0.620</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>6</td>
<td>41</td>
<td>0.251</td>
</tr>
<tr>
<td>Pip/Tazobactam</td>
<td>48</td>
<td>132</td>
<td>0.0001</td>
</tr>
<tr>
<td>Meropenem</td>
<td>39</td>
<td>150</td>
<td>0.705</td>
</tr>
</tbody>
</table>
Figure 1 Resistance pattern of *E. coli* in UTI patients among various departments

![Figure 1](image1)

Figure 2 Distribution of Uropathogenic *E. coli* with gender in different age groups

![Figure 2](image2)

Figure 3 Estimation of extended spectrum beta lactamase production by (a) Double disc approximation test, (b) Combined disc diffusion test.

![Figure 3](image3)
Figure 4 Different growth inhibition patterns by E-test MIC strips (a) A clear ESBL producer, (b) A “rounded” phantom inhibition zone below CT indicative of ESBL.

Figure 5 Antibiotic sensitivity pattern of ESBL positive uropathogenic E. coli

Discussion
UTI is a major cause of mortality these days. Effective management for the treatment of uropathogens is commonly based on the identification of the causative organisms and the selection of appropriate antibiotic treatment[16]. ESBLs are a perfect illustration of a wide variety of enzymes that inactivate β-lactam antibiotics. Urinary tract infections these days are crucial to manage due to multidrug resistance and ESBL enzymes produced by E. coli & Klebsiella spp.

In this study, we observed the antibiotic susceptibility pattern of 247 uropathogenic E. coli and further phenotypic detection of ESBL was done. Among them 133 (53.8%) were males and 114 (46.2%) were females (Fig 1). Contrary to the results given by Akram et al[17] which reported prevalence of UTI more amongst female (66.66%) in our study UTI was more prevalent in male patients (53.8%) than females (46.2%). (Fig 1) Aged males, infants and elderly shows increased incidence of UTI. Moreover, complicated UTI often strikes males having urodynamic problems like indwelling urinary catheters, neurogenic bladder, immunosuppression and prostate elongation[18]. Furthermore, hygienic factors may also contribute to more number of cases of UTI in males.

The study revealed high prevalence (n=36) of UTIs in females belonging to age group 21-30 years followed by 31-40 years (n=29) in accordance with Surinder K. et al[19] which reported 51% UTI in 21-30 years old females. These findings may correspond to other reports which showed that females are more prone to UTI during adolescence as well as adulthood.[17, 20, 21, 22] This increased risk is due to hormonally induced changes in the vaginal flora, high sexual activity, recent use of a diaphragm with spermicide and a history of recurrent UTIs.[23]

Although, in our study when compared with elderly females, elderly males (>45 years) had a higher incidence of UTI (77%) due to prostate enlargement and neurogenic bladder.[24] This
factor is also discussed by other authors whose studies showed that in males the prostate disease is responsible for the increase in the occurrence of UTI and decrease in female: male ratio in patients above 50 years.\cite{129} Surinder K et al\cite{19} and Sujatha R et al\cite{26} also reported the same factors responsible for higher UTI prevalence in aged males.

As per table no.2, we found out that most of the patients were from outpatients (OPD) i.e. 153 (61.9%) followed by IPD wards 86 (34.8%) and ICUs (0.3%). These results are in accordance with Surinder K. et al\cite{19} who reported 64.6% of patients from OPD and 35.38% from IPD. The study done by Dawoodabadi et al\cite{27} also reported more patients from OPD (68.18%) and 31.82% from inpatients department.

The antimicrobial resistance patterns in tested uropathogens of *E. coli* were found 17%, 20%, 23.4%, 27% and 28% resistant against Nitrofurantoin, Imipenem, Meropenem, Piperacillin/tazobactam and Amikacin respectively, as presented in Table 1. The present work also defined that a much higher resistance was observed in *E. coli* uropathogens isolated against commonly prescribed antibiotics, i.e., fluoroquinolones, cephalosporins, and amoxyclov. Sakina F. et al\cite{28} have also reported similar results with an increased rate of resistance in their study. This increased resistance is due to inappropriate and empiric use of antibiotics available for oral administration.

In our study comparison methods used for the detection of ESBL production showed DDAT to be less sensitive than CDDT. According to CDDT 16% isolates were ESBL positive and DDAT reported 14% to be positive. Similar results were reported by M.S Kumar et al\cite{29} i.e. 19.8% with a same inter-disc distance of 15mm. Prabha R. et al\cite{30} also showed that CDDT is more sensitive than DDAT for detection of ESBLs. E-test results were confirmatory and were comparable to DDAT and CDDT.

The productivity of ESBL and antimicrobial resistance against three antibiotics, i.e., Cefotaxime, Ceftazidime and Piperacillin/Tazobactam were found significant (Table 2). Hence, these findings are limiting the use of Cephalosporins as the choice in the treatment of ESBL-positive UTIs. Our results also recommend an increasing resistance towards Piperacillin/tazobactam. β-Lactam/ β-lactamase inhibitor combinations typically act against organisms having a single ESBL. In fact, it has been noted already that, many organisms now produce multiple ESBLs, which may decrease the effectiveness of β-lactam/ β-lactamase inhibitor combinations.\cite{31} In vitro resistance of ESBL-producing isolates to such combinations is increasing.\cite{7} Our study finds Nitrofurantoin, Meropenem, and Co-trimoxazole and Gentamicin as a good choice to treat UTIs with ESBL positive *E. coli*.

**Conclusions**

This study concluded that complicated UTI in males is increasing with urodynamic problems like neurogenic bladder, indwelling urinary catheters, immunosuppression and prostate abnormalities. UTI in elderly males is growing alarmingly.

Furthermore, a high rate of resistance has been developed in uropathogens with empiric antibiotic treatment, including Cotrimoxazole, Fluoroquinolones, Amoxyclov and broad-spectrum Cephalosporins. This suggests that the use of these antibiotics is not as good as empiric treatment for community-onset UTIs. Therefore, proper screening is needed for ESBLs detection in laboratories. The study also recommends the use of Nitrofurantoin, Meropenem, Gentamycin and Co-trimoxazole to treat UTIs with ESBL producing *E. coli*. In the antibiogram, a significant difference was noticed between ESBL producers and non-producers. Resistance to non-β-lactam antibiotics like quinolones and also to aminoglycosides was shown by many ESBL producers while Co-trimoxazole, Meropenem and Nitrofurantoin were found highly effective in them. Among these three drugs, consistent
susceptibility was observed towards Nitrofurantoin only. Even though Piperacillin-tazobactam were found to be potent towards ESBLs, it is not advisable to use them routinely as empirical therapy because of co-production of Amp C which makes these combinations clinically unproductive.

Thus it is of utmost importance that antibiogram report must mention clearly whether the isolate is a doubtful or a confirmed ESBL producer. The report should also state that irrespective of their in vitro susceptibility, production of ESBL can render β-lactam antibiotics therapeutically ineffective.

Efficient infection control measures like barrier precautions and hand washing should be made mandatory to lessen the pervasiveness of antimicrobial-resistant pathogens, including ESBL producing *E. coli*. Efforts to curtail the trend of using empirical antibiotic therapy, promotion of the judicious use of extended-spectrum cephalosporins along with periodic surveillance of antibiotic resistance patterns are among the key actions which are necessary to fix the difficulties associated with these pathogens.

**Acknowledgement**

This work was carried out in the Department of Microbiology, SMS Medical College, Jaipur, India. The authors would like to thank, the staff and the HOD of the Microbiology department for providing the necessary support and a research oriented environment.

**References**


10. Collee PE, Enright DP, inventors; Baker Hughes Inc, assignee. Method and


27. Davoodabadi A, Farahani A, Ranjbaran M. Antibiotic resistance in Escherichia coli


