Increasing incidence of ESBL type Resistance among Urinary Tract Infecting Escherichia coli in New Delhi, India

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Abstract

Reports of ESBL (extended spectrum β-lactamase) producing E. coli strain in UTI (urinary tract infections) patients is increasing. This study determines the presence of ESBL in E. coli isolates from UTI patients.

A total of 414 cultured urine samples were collected from hospital. E. coli was isolated from these cultures using conventional microbiological tools. These antibiotics of third generation cephalosporin were selected to test susceptibility of the bacterial isolates. Ceftazidime, cef tazidime plus clavulanic acid and cefotaxime, Cefotaxime plus clavulanic acid were used for Combination Disc Test (CDT). For E-test, a ceftazidime MIC greater than four-fold lower in the presence of clavulanic acid indicate ESBL production.

241 samples were positive for E. coli. Findings proved that E. coli is still the most common pathogen for UTI (58.2%).

Out of 414 UTI patients, 313 women were affected with UTI (75.6%), while male patients were only 24.4%. Also, people in the age group of 16-30 years were more prone to UTI.

Out of 241 isolates, 165 isolates were inferred to be ESBL producing E. coli and 106 were confirmed as ESBL producing E. coli.

E. coli is still the most common pathogen of UTI. The ESBL producing E. coli strains are rising in number in Delhi, India. The resulting resistance of these antibiotics might be due to continuous use of it for many years.

Key words: Extended spectrum β-lactamase, Urinary tract infections, Antibiotic resistance, E. coli and Delhi.
1. Introduction

Urinary tract infection (UTI) is generally defined as the occurrence of pathogenic microbes in the urinary tract with associated symptoms. [1] Clinically, UTIs are categorized as complicated or uncomplicated. Individuals, affected with uncomplicated UTIs are otherwise healthy and have no neurological or structural urinary tract abnormalities.[2, 3] Infections associated with factors that compromise the urinary tract or host defense, including urinary retention, urinary obstruction caused by neurological disease, immunosuppression, renal transplantation, renal failure, pregnancy and the presence of foreign objects such as calculi, indwelling catheters or other drainage equipment are defined as complicated UTIs.[4] These infections are generally categorized based on the site of infection (i) cystitis: in bladder; (ii) pyelonephritis: in kidney.[2, 5] Several risk factors i.e., a prior UTI, obesity, diabetes, vaginal infection, sexual activity, and genetic susceptibility are connected with cystitis, including female gender. [5, 6]

As much as 35%, of all the infectious disease, constitutes UTI infection alone which reveals that UTI is one of the most common infectious diseases that rank in its prevalence next to pneumonia infections. In USA, in the year 1996-97, women visit to hospitals with UTI (1.2%) was twice to that of men (0.6%),[7] suggesting that females are more prone to infection than males, and it is attributed to women’s short urethra, and certain behavioral factors such as delay in micturition, obesity, diabetes, vaginal infection, sexual activity, genetic susceptibility, use of mechanical instrumentation like catheterization, and the use of spermicides and diaphragms which promote growth of the periurethral area with coliform bacteria. [5, 6] Infection route due to perineal or periurethral bacteria among women often start with the entry of the pathogen in urethra and then move towards the bladder. [8] Symptomatic UTI is very common among those women who are sexually active. [9] According to one estimate, one in three women will have at least one UTI diagnosed requiring antibacterial treatment by the age of 24 years,[9] and 40% to 50% of women will certainly experience at least one event of UTI during their lifetime. [10, 11, 12, 13]

Significant part of the work done in clinical microbiology laboratories still hovers around UTI, and uropathogenic *Escherichia coli* (UPEC) is the most predominant causative pathogen for both
uncomplicated and complicated UTIs.[14] Prevalence of the pathogens attributed to uncomplicated UTIs are *E. coli*, followed by *K. pneumoniae, S. saprophyticus, Enterococcus faecalis, Streptococcus, P. mirabilis, P. aeruginosa, S. aureus* and *Candida spp.* [5, 6, 15, 16] For complicated UTIs, the order of prevalence for causative pathogens, following *E. coli* as most common, is *Enterococcus spp., K. pneumoniae, Candida spp., S. aureus, P. mirabilis and P. aeruginosa.* [4, 17, 18]

Urinary tract infection poses great challenge to researchers and physician, as large number of infections are reported each year, furthermore the diagnosis of UTI is not always straightforward. UTI has to be distinguished from other diseases i.e., other STIs that have similar clinical symptoms, some UTIs are present with atypical signs or asymptomatic, may require different diagnostic criteria than those used for the general UTI patient population.

Most of the UTI pathogens have acquired antibiotic resistance over a period of time because of substantial exposure excessive use of antibiotics. There are many types of resistance mechanism operating in these pathogens. One of the most prevalent mechanisms of resistance among Gram-negative bacteria is the production of β-lactamase enzyme. β-lactamases (penicillinase) are enzymes with EC 3.5.2.6, produced by some bacteria that inactivate antibiotics containing β-lactam ring such as penicillins, carbapenems (ertapenem) and cephemycins.

The emergence of ESBL type of resistance offered by *Escherichia coli* and *Klebsiella pneumonia* evidenced by increasing number of reports in the literature is a major blow to the already struggling community of physician in the hospitals who are frantically looking for alternative and novel antibacterial molecule to counter ESBL type of infections. [19] ESBLs are plasmid mediated or chromosomally mediated β-lactamases with broad activity against penicillins, cephalosporins and monobactam. They inactivate β-lactam antibiotic function by breaking amide bond of the β-lactam ring.[20] An infection with ESBL-producing pathogenic bacteria is related to a worse clinical course that entails deferred clinical and microbiological response, longer hospitalizations, higher costs, and higher death toll.[21, 22]
In view of this background, we had initiated this study to characterize the *E. coli* in UTI patients and further, to evaluate the antimicrobial activity and underlying detection of ESBL by phenotypic method in isolated strains obtained from outdoor patients of UTI in Delhi.

2. MATERIALS AND METHODS

2.1 Study design

In all 414 Gram negative cultured isolates from urine samples taken from indoor and outdoor patients visiting Al-Shifa Multispecialty Hospital located in south east Delhi, were collected over a period spanning 14 month i.e. from May 2013 to July 2014. All the isolates were subjected to identification of *E. coli* and these were analyzed for resistance to β-lactam antibiotics.

Only one colony per plate was collected to avoid duplicates, unless isolates showed different resistance profiles.

2.2 Isolation and identification of Pathogens

Gram-negative cultured colony from urine samples were collected in sterile vial containing EC broth then inoculated in plates containing MacConkey agar and EMB agar consecutively, by standard culture techniques. A calibrated loop (4mm diameter) delivering 0.01ml of EC broth containing culture sample was used to inoculate in MacConkey agar. The plates were incubated at 35⁰C to 37⁰C for 24 hrs before inspecting for growth of organisms.

Selected pink colonies were cultured on EMB agar. Isolates were further identified by a panel of biochemical test (IMViC test). Final identification was done by 16s rRNA gene analysis.

2.3 Biochemical test

Apart from colony morphology the routine biochemical tests (IMViC test) were performed to confirm the ability to produce Indole, mixed acid fermentation of glucose, the presence of acetylmethylcarbinol (acetoin) and citrate utilization.
2.4 Molecular identification:

Genomic DNA was prepared by using methods of Birnboim and Doly and PCR amplification of 16S rRNA gene was carried out using the following primers 5′-ACT CCT ACG GGA GGC AGC-3′ and 5′-CCG TCA ATT CAT TTG AGT TT-3′. PCR conditions for this gene comprised a thermal temperature of 94 ºC for 5 min, followed by 35 cycles of 94 ºC for 1 min, annealing at 70 ºC for 30 sec. and extension at 72 ºC for 1 min, followed by final extension for 10 min at 72 ºC.

2.5 Antibiotic susceptibility testing

Antimicrobial susceptibility was performed by disk diffusion method according to Clinical Laboratory Standard Institute (CLSI). The antibiotics used were as follows: ampicillin (10µg), nalidixic acid (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), streptomycine (10 µg), and imipenem (10 µg).

2.6 Phenotypic Test for ESBL production

a. ESBL Disc Screening

The organism was spread on Muller-Hinton agar (HiMedia). Disk-diffusion method for ESBL screening was performed using cefotaxime, ceftriaxone and ceftazidime according to CLSI guidelines (table 1).

b. Combination Disc Test (CDT)

While conducting antibiotic susceptibility test disc of ceftazidime (30µg), ceftazidime plus clavulanic acid (30/10 µg) and cefotaxime (30µg), Cefotaxime plus clavulanic acid (30/10 µg) were placed on Muller-Hinton agar (HiMedia) and incubated at 37 ºC. Organism was considered as ESBL producer if there was a ≥5mm increase in zone diameter between these two combinations. E. coli (ATCC 25922) and Klebsiella pneumonia (ATCC 700603) were used as negative and positive control reference strains, respectively.

c. E. test for phenotypic ESBL identification
Test was performed on Muller-Hinton agar in accordance with the manufacturer’s instruction. Overnight culture of isolates in LB broth was swabbed on Muller-Hinton agar plates (HiMedia). After drying for 15 min, the E-test strips were placed on the plates and plates were incubated for 18 h at 37°C. The MIC was interpreted as the point of intersection of the inhibition ellipse with the E-test strip edge.

3. Results:

Out of 414 cultured urine samples processed, 241 cultures were determined to be positive for *E. coli*. The remaining 173 cultures showed a growth of organisms other than *E. coli*. *E. coli* was found to be the most common pathogen for UTI (58.2%).

It was observed that women are more prone to UTI (n= 313 (75.6%)) as compared to men (n=101 (24.4%)). Further it was also observed that the age group of 16-30 years are more affected with UTI (Table 2).

All three tests differing in their stringency were employed to identify ESBL producer. Disc screening method delineated 165 *E. coli* samples as ESBL producers. A more discriminating screening test i.e. combination disc test (CDT) and E-test reduced this number to 106. And rest of the numbering 135 (56%) strains were sampled from patient harboring non-ESBL producing *E. coli* strains. Out of these 106 ESBL producing strains, 75 (70.8%) were obtained from females and 31 (29.2%) were collected from males.

Furthermore, it was observed that in the age group of 0-15 years, 10% children had UTI with ESBL producing *E. coli* (Table-3). Antibiogram was prepared by Kirby & bauer’s method for all the 165 isolates identified as *E. coli* and 7 antibiotics were used against the isolates that showed multi drug resistance. The results clearly indicate that ESBL producers were non-responsive to Ampicillin (98%), Nalidixic acid (94%), Cefotaxime (78%), Ceftriaxone (75%), Ceftazidime (73%), and showing sensitivity to Streptomycin (13%), Imipenem (5%) (Fig:1). Therefore, it is concluded that all the isolated ESBL
producing *E. coli* show their resistance to third generation cephalosporins like cefapodoxime, ceftazidime and cefatoxime and sensitive to carbapenems like Imipenem.

4. Discussion

Gram negative enterics constitute a serious health crisis in urinary tract infection among humans in many parts of the world.[23] Uropathogenic *Escherichia coli* (UPEC) remain the most frequent cause of UTI.[14] *E. coli* are becoming increasingly resistant to many of available antibiotic in the pharmaceutical market particularly an important class of antibiotics, members of which contain a β-lactam ring that inhibits peptidoglycan syntheses by covalent binding to the active site serine of penicillin binding protein. β-lactam sub-class includes carbapenems, cephalosporin, penicillin, monobactam and clavams. This predicament is further accentuated by the emergence of ESBL producing *E. coli* strains. The earlier known β-lactamases that were active against first generation β-lactams, were followed by ESBLs that have the ability to hydrolyze oxyimino-cephalosporins. It is important to note that the genetic determinants of divers ESBLs and carbapenemases including imipinase, veronaintegron encoded metallo β-lactamase (VIM), *K. pneumonia* carbapenemase (KPC), oxacillinase (oxa) and NDM enzyme in gram negative bacteria like *K. pneumoniae*, *E. coli*, *P. engerinosa* and *A. baumannii* has supported the emergence of isolates that demonstrate resistance to all β-lactam antibiotic. This has seriously hampered the treatment of savior infections, particularly among hospitalized patients. Whatever may be the mechanism, the most startling feature has been the rapid spread an expansion of emergence of resistance demonstrated by the pathogens.[24] The present study clearly brings forth that there is a high prevalence of ESBL producing *E. coli* strains in urine collected from UTI patients visiting Al-shifa multispecialty hospital situated in south east Delhi and therefore, they showed resistance to commonly prescribed antibiotics. *E. coli* is the causative organism in 58% of the cases of UTI. It has been reported that production of ESBL varying from 28% to 84% in previous studies from India.[25] In adult women, UTI has been noted as the most commonly encountered infection the world over. High incidence of community acquired UTIs are reported from Asia, Pacific, Denmark, India, Russia, Japan and the
USA. Among all of these regions incidence of UTIs by ESBL producing *E. coli* was found to be highest in India (60%) followed by Hongkong (48%) and Singapore (33%). Out of 241 *E. coli* strains, 106 strains (44%) were found to be showing ESBL type of resistance. Such a high number for a particular type of resistance (ESBL) is of considerable significance because ESBL have the ability to render all β-lactam rings containing antibiotics ineffective. A major observation that needs to be emphasized in this study is that most of the isolated strains were show resistance to 3-5 antibiotics which included the first and second generation of cephalosporins. Very high resistance for ampicillin particularly (98%) in this research corroborates previous studies conducted in our lab, nalidixic acid (94%), cefotaxime (78%), ceftriaxone (75%), ceftazidime (73%), and showing sensitivity to streptomycin (13%), clavulanic acid combination with ceftazidime and cefotaxime (7%) and imipenem (5%). Imipenem and clavulanic acid combination with β-lactam antibiotic shows least resistance which may be a result of lesser use of this drug as it is least prescribed by doctors. Clavulanic acid is a β-lactamase inhibitor, has been isolated from *Streptomyces spp*. It contains a β-lactam ring and binds strongly to β-lactamase at or near its active site, thereby hindering its enzymatic activity.

Antibiotic resistance to causative agents of UTI is increasing at a rapid pace, even imipenem and clavulanic acid combination with β-lactam antibiotic, which are prescribed as a last resort, have started showing its ineffectiveness (Graph 1). Since most of the conventional antibiotics have started showing greater degree of resistance towards most of the pathogen, physicians are compelled to prescribe these last resort drugs to treat infections. Although these drugs are very effective in dealing with the infections, but future is not far away when even these drugs will be rendered ineffective. Interaction of bacteria with these drugs would lead to development of resistance and enhanced incidence of ESBL. In other studies, this is also observed that microorganisms show antibiotic resistance even in the absence of exposure of commercial antibiotic, since these microorganisms have been already exposed to similar naturally occurring antibiotics. Therefore it is a frightening situation that needs immediate attention of health administration of the world in general and of India in particular.
5. Conclusion:

After the introduction of beta-lactam drug as antimicrobial in 1940s, the CTX-M enzymes, like TEM and SHV type ESBL emerged in 1980s. Although they were not prevalent until 1995 but in recent times, it has become a hazard all over the world. Their genes are the descendants of chromosomal \textit{bla} genes of \textit{Klebsiella} sp. and family of enterobacteriaceae.

Among females, UTI is more prevalent compared to males due to their wider urethra. After comparing our results with the previous studies, an increased incidence of UTI was found in females belonging to Delhi. This increase may be due to retention of urine in the bladder for a long time which could be attributed to lack of adequate number of public conveniences for females in Delhi. Further it was also observed that the people belonging to the age group of 16-30 years are more affected with UTI indicating that highly sexually active age group is more vulnerable to urinary tract infection. The study also infers that \textit{E. coli} is still the most prevalent causative organism of UTI and the ESBL type of drug resistance is now found in 44\% of \textit{E. coli} isolates from UTI patients. Here also, in comparison to previous studies done in UTI patients, the ESBL type of resistance is increasing in \textit{E. coli} isolates.

The results in this study are a part of larger study that we have carried out. Since the presence of ESBL type of drug resistance are constantly evolving and are under dynamic flux, hence, it poses a global threat to public health programs, there is a necessity for regular antimicrobial sensitivity surveillance not only for the presence and spread of ESBL genes both urban and rural populations but also for more informed treatment.

Acknowledgement:

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References:


Table 1: CLSI guidelines regarding ESBL producer

<table>
<thead>
<tr>
<th>Antibiotic disc</th>
<th>Considered ESBL positive if</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Cefotaxime</td>
<td>CTX 30 µg</td>
<td>Inhibition zone ≤ 27 mm</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>CTR 30 µg</td>
<td>Inhibition zone ≤ 25 mm</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>CAZ 30 µg</td>
<td>Inhibition zone ≤ 22 mm</td>
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Table 2: Age wise distribution of UTI patients

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total No of patients</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 15 yrs</td>
<td>67</td>
<td>25</td>
<td>42</td>
</tr>
<tr>
<td>16-30 yrs</td>
<td>192</td>
<td>38</td>
<td>154</td>
</tr>
<tr>
<td>31-45 yrs</td>
<td>72</td>
<td>17</td>
<td>55</td>
</tr>
<tr>
<td>≥ 46 yrs</td>
<td>83</td>
<td>21</td>
<td>62</td>
</tr>
<tr>
<td>Total</td>
<td>414</td>
<td>101</td>
<td>313</td>
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</tbody>
</table>

Table 3: Age wise distribution of UTI patients harboring ESBL producing *E. coli*

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total No <em>E. coli</em> positive</th>
<th>ESBL producer</th>
<th>Non ESBL producer</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 15 yrs</td>
<td>44</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>16-30 yrs</td>
<td>114</td>
<td>39</td>
<td>75</td>
</tr>
</tbody>
</table>
Table 4: Age and sex wise distribution of UTI patients harboring ESBL producing *E. coli*

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total No of ESBL positive patients</th>
<th>ESBL positive Male</th>
<th>ESBL positive Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 15 yrs</td>
<td>24</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>16-30 yrs</td>
<td>39</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>31-45 yrs</td>
<td>21</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>≥ 46 yrs</td>
<td>22</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>32</td>
<td>74</td>
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