RISING TREND OF ESBL PRODUCTION IN UROPATHOGENIC E. COLI CAUSING WORRY? - A STUDY IN A TERTIARY CARE HOSPITAL

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\textbf{ABSTRACT}

\textbf{BACKGROUND}
Urinary Tract Infection (UTI) caused by Escherichia coli (E. coli) has become a significant global public health problem. The resistance to \(\beta\)-lactam antibiotics in E. coli further adds to the problem. The pattern of drug resistance among E. coli undergoes considerable variation and consequently the treatment of Urinary Tract Infection (UTI) requires constant updating of the antibiotic sensitivity profile.

The study was thus undertaken to know resistance pattern of E. coli isolates with special reference to production of extended spectrum \(\beta\)-lactamases (ESBLs) in urinary samples.

\textbf{MATERIALS AND METHODS}
This is a three-year (2015 - 2017) retrospective descriptive study undertaken in Srinivas Institute of Medical Sciences and Research Centre, Mukka, Mangalore. Standard culture techniques for urine samples were followed. Antibiotic sensitivity test was done by Kirby-Bauer disc diffusion method and interpretation was done following Clinical and Laboratory Standards Institute (CLSI) guidelines. ESBL detection was done by NCCLS phenotypic confirmatory combination disc diffusion method using ceftriaxone (30 \(\mu\)g) alone and ceftriaxone + clavulanic acid (30 \(\mu\)g/ 10 \(\mu\)g).

\textbf{RESULTS}
E. coli is the most frequent isolate found in UTI (65.4\% of total isolates) throughout three years. The overall resistance pattern of E. coli exhibited highest resistance against ampicillin (95\%) and amoxycyl (83.3\%) with rising trend in resistance to Cephalsporins and Quinolones in three successive years. The rising trend in ESBL production rate was observed from 27.3\% (2015) to 39.4\% (2017) with least resistance against meropenem (9.9\%), piperacillin/ tazobactam (10\%), nitrofurantoin (11.4\%) and amikacin (12.1\%). It was also observed that > 50\% of ESBL producing E. coli isolates exhibited resistance against co-trimoxazole and Quinolones.

\textbf{CONCLUSION}
The incidence of the ESBL producing E. coli has been steadily increasing over the past few years. The knowledge on the resistance pattern in a geographical area will help in guiding the appropriate and the judicious use of antibiotics.

\textbf{KEY WORDS}
Uropathogens, ESBL, E. coli.

\textbf{HOW TO CITE THIS ARTICLE:}

\textbf{BACKGROUND}
Urinary Tract Infection (UTI) is a spectrum of disease caused by microbial invasion of the genitourinary tract that extends from the renal cortex of the kidney to the urethral meatus.\textsuperscript{1} Approximately, 40\% of women and 12\% of men experience at least one symptomatic infection during their lifetime.\textsuperscript{2}

It also contributes as the most common nosocomial infection in many hospitals and accounts for approximately 35\% of all hospital-acquired infections. This burden causes serious impact on the socioeconomic life of individuals and also leads to a large proportion of antibacterial drug consumption.\textsuperscript{3} UTIs having E. coli as aetiological agent are common infections with an estimated annual global incidence of at least 250 million cases.\textsuperscript{4}

Studies indicate that uropathogens are becoming increasingly resistant to the antibiotics used for the treatment of UTI. As an example, SENTRY- an Antimicrobial Surveillance Program that monitored UTI worldwide over a 4-year period between 1997 and 2000 showed an increase in resistance among UTI. The term uncomplicated UTI refers to the invasion of a structurally and functionally normal urinary tract by a non-resident infectious organism.\textsuperscript{5} A complicated UTI is an infection associated with a condition such as a structural or functional abnormality of the genitourinary tract or the presence of an underlying disease that interferes with host defence mechanisms, which increases the risk of acquiring infection or of therapeutic failure. This could include the presence of an indwelling catheter or urinary stent, the presence of an obstructive uropathy of any aetiology or urinary tract modifications such as an ileal loop or pouch.\textsuperscript{6} Most cases of UTI are caused by Gram-negative...
bacilli with *E. coli* accounting for over 90% of uncomplicated UTIs. Uncomplicated infections can be treated with short courses of antibiotics, while complicated UTIs require longer and more intensive courses of antibiotics. However, resistance to the commonly used antibiotics is increasing and making treatment more difficult. An important mechanism of antibiotic resistance among *E. coli* is through ESBL production. In particular, ESBL producing *E. coli* are emerging worldwide. The ESBL producing strains are particularly feared as they are resistant to all Penicillins, Cephalosporins (including third and fourth generation agents) and to aztreonam.

Major risk factors for colonisation or infection with ESBL-producing organisms are long-term antibiotic exposure, prolonged hospital stays, residence in an institution with high rates of third-generation cephalosporins use, and in whom invasive devices (Urine catheters, endotracheal tubes and central venous lines) are present for a prolonged duration. The frequency of detection of ESBL producing strains (Including *E. coli*) is quite variable, but is consistently increasing. *E. coli* is one of the main ESBL-producing pathogen. ESBL production is transmitted from one bacterium to another through plasmids. These plasmids can carry multiple drug-resistant genes against aminoglycosides, quinolones and sulfamethoxazole at the same time. The phenomenon of multidrug resistance has brought tremendous difficulties to the clinical treatment of infection due to limited therapeutic options.

**MATERIALS AND METHODS**

This is a three-year (2015 - 2017) retrospective descriptive study undertaken in Srinivas Institute of Medical Sciences and Research Centre, Mulka, Mangalore. All the Culture and Sensitivity (C/S) reports of urine samples, maintained in the record registers of Microbiology laboratory during the study period were included and analysed. A total of 22,055 urine specimens obtained from outpatients and inpatients were analysed. Urine culture was done by standard loop method, a semi-quantitative method. The organisms isolated from urine culture were identified by conventional biochemical tests. Only the *E. coli* isolates were considered for this study. Antimicrobial susceptibility test was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and the interpretations were carried out according to the Clinical and Laboratory Standards Institute guidelines. Antibiotics against which sensitivity was tested included ampicillin (10 μg), amoxycyclav (20 μg/10 μg), ceftazidime (30 μg), ceftriaxone (30 μg), aztreonam (30 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), co-trimoxazole (25 μg), norfloxacin (10 μg), nitrofurantoin (300 μg), piperacillin-tazobactam (100 μg/10 μg) and meropenem (10 μg). Quality control of media and discs were performed using ATCC *E. coli* control strain 25922. ESBL production in *E. coli* was detected routinely by NCCLS phenotypic confirmatory combination disc diffusion method using ceftazidime (30 μg) and cefazidime/clavulanic acid (30 μg/10 μg). An increase in the inhibition zone diameter of ≥ 5 mm for a combination disc versus ceftazidime disc alone confirmed ESBL production. Whole of the data were collected and compiled in the year wise manner and compared year wise to see the trend in the resistance pattern.

**RESULTS**

Over a period of three years (2015 - 2017), a total of 7127 (32.3%) urine samples showed significant growth, in which 4,658 (65.4%) isolates were *E. coli* as expected *E. coli* was the most frequent isolate. The next most frequently isolated bacteria were *Klebsiella spp.* (15.8%). However, isolates other than *E. coli* were not considered in our study. Out of 4,658 *E. coli* isolates 2,705 (58%) of the isolates were obtained from female patients.

The year wise distribution of urine samples, number of cultures with significant growth and number of isolated *E. coli* are given in Table 1.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of Urine Samples</th>
<th>No. of Cultures with Significant Growth</th>
<th>No. of E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>4,850</td>
<td>1,697 (35%)</td>
<td>1,052 (62%)</td>
</tr>
<tr>
<td>2016</td>
<td>7,125</td>
<td>2,280 (32%)</td>
<td>1,527 (67%)</td>
</tr>
<tr>
<td>2017</td>
<td>10,080</td>
<td>3,150 (31%)</td>
<td>2,079 (66%)</td>
</tr>
<tr>
<td>Total</td>
<td>22,055</td>
<td>7,127 (32.3%)</td>
<td>4,658 (65.4%)</td>
</tr>
</tbody>
</table>

*Table 1*

All the 4,658 *E. coli* isolates were subjected to antimicrobial susceptibility testing and ESBL detection. The overall resistance pattern of *E. coli* was highest against ampicillin (95%) and amoxycyclav (83.3%). There was a rising trend in resistance to cephalosporins (58.6% - 70.6% in ceftriaxone, 60% - 68% in ceftazidime) and ciprofloxacin (57.6% - 65.5%) in three successive years. More than 50% of isolates exhibited resistance against co-trimoxazole throughout three years. However, the resistance to nitrofurantoin, amikacin, piperacillin-tazobactam and meropenem was least (Table 2 and Graph 1).

***Table 2. (Year Wise Resistance Pattern of E. coli Isolates)***

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>2015 R (%)</th>
<th>2016 R (%)</th>
<th>2017 R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>976 (92.8%)</td>
<td>1442 (94%)</td>
<td>2001 (96.2%)</td>
</tr>
<tr>
<td>Amoxycyclav</td>
<td>849 (80.7%)</td>
<td>1288 (84.3%)</td>
<td>1742 (83.8%)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>616 (58.6%)</td>
<td>1005 (65.8%)</td>
<td>1468 (70.6%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>632 (60%)</td>
<td>985 (64.5%)</td>
<td>1414 (68%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>606 (57.6%)</td>
<td>908 (59.5%)</td>
<td>1362 (65.5%)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>515 (49%)</td>
<td>888 (58%)</td>
<td>1170 (56.3%)</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>553 (52.6%)</td>
<td>790 (51.7%)</td>
<td>1096 (52.7%)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>450 (42.8%)</td>
<td>683 (44.7%)</td>
<td>1000 (48.1%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>419 (39.8%)</td>
<td>736 (48.2%)</td>
<td>692 (33.3%)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>130 (12.4%)</td>
<td>169 (11%)</td>
<td>211 (10.1%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>121 (11.5%)</td>
<td>161 (10.5%)</td>
<td>179 (8.6%)</td>
</tr>
<tr>
<td>Piperacillin-Tazobactam</td>
<td>109 (10.4%)</td>
<td>132 (8.6%)</td>
<td>185 (8.9%)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>75 (7.1%)</td>
<td>138 (9%)</td>
<td>168 (8%)</td>
</tr>
</tbody>
</table>

The results of ESBL producing E. coli were analysed year wise, which showed that 287 (27.3%) were ESBL producers out of 1,052 isolated E. coli in the year 2015, 537 (35.2%) out of 1,527 E. coli in the year 2016 and 819 (39.4%) out of 2,079 E. coli in 2017 showing rising trend in ESBL rate which increased significantly from 27.3% to 39.4% between 2015-2017 (Table 3 and Graph 2).

Table 3. Occurrence of ESBL in E. coli in the Years 2015 - 2017

<table>
<thead>
<tr>
<th>Year</th>
<th>E. coli No.</th>
<th>ESBL producing E. coli</th>
<th>ESBL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>1,052</td>
<td>287</td>
<td>27.3%</td>
</tr>
<tr>
<td>2016</td>
<td>1,527</td>
<td>537</td>
<td>35.2%</td>
</tr>
<tr>
<td>2017</td>
<td>2,079</td>
<td>819</td>
<td>39.4%</td>
</tr>
</tbody>
</table>

Graph 1. (Year Wise Resistance Pattern of E. coli Isolates)

DISCUSSION

Infection of the urinary tract is one of the most common infectious diseases and it would affect all age groups including men, women and children worldwide. A total of 22,055 non-repetitive urine specimens were analysed during the study period (2015 - 2017). Of these, 7,127 (32.3%) urine samples showed significant growth. Similar growth rates were reported (30.8%) in a study by Baral P et al. The growth rate was higher (41.7%) compared to our study in a study done by Bhatt C et al. Data analysis revealed a much higher percentage of women (58%) to be suffering from UTI as compared to men (42%). The men are usually less prone to UTI as compared to females owing to the longer course of the urethra and bacteriostatic properties of prostate secretions.

The antimicrobial susceptibility patterns have changed overtime, but the spectrum of agents causing UTI has remained relatively constant with E. coli being the most common isolate. Earlier studies from different regions of India and from other countries have reported that the most prevalent UTI pathogen was E. coli followed by Klebsiella spp. Similarly, in our study E. coli (65.4%) is the most common pathogen isolated in both inpatients and outpatients of both sexes followed by Klebsiella spp. (15.8%), which goes in accordance with the study done by Chauhdhri et al.

Among all 4,658 E. coli isolates highest resistance was noticed against ampicillin (95%) and amoxyclav (83%). There was a rising trend in resistance to cephalosporins from 58.6% (2015) to 70.6% (2017) for ceftriaxone, 60% (2015) to 60% (2017) for ceftazidime in three successive years, which could be due to frequent usage of these antibiotics for prophylactic and therapeutic purposes.
other infections in the institution. A trend of increasing resistance to ciprofloxacin (57.6% - 65.5% from 2015 - 2017) was found in our study. Fluoroquinolones have a wide variety of indications, permeate most body compartments and are ubiquitously prescribed, accounting for the emergence of their resistance.16 Similar increasing trend in resistance to cephalosporins and quinolones are found in the study done by Jeyasekharan DD et al.17 On an average resistance to co-trimoxazole in our study was 52.4%, which could be attributed to their wide usage for a variety of other indications. Resistance to gentamicin (39.7%) was higher than amikacin (9.9%) in our study, which goes in accordance with the study done by Kulkarni SR et al18 with 90.89% sensitivity to Amikacin (9.11% resistance) and 59.2% sensitivity to Gentamicin (40.76% resistance). This is in contrast to study done by Revathy Saravanam et al19 in which isolates were more sensitive to gentamicin compared to amikacin. This supports the possibility of changing sensitivity pattern with time difference. The decreasing trend in resistance to amikacin (11.5% - 8.6%) was noticed in our study. This could be due to being injectables the use of aminoglycosides are restricted in the community care setting and hence might have shown better sensitivity rates. The overall resistance to nitrofurantoin was least (11%) throughout the three years. Re-emergence of E. coli sensitivity to nitrofurantoin is probably due to non-usage of the drug for a long period of time. Our findings are similar to other Indian studies, which have also demonstrated nitrofurantoin as an appropriate agent for first-line treatment of community-acquired UTIs. Given the fact that nitrofurantoin has no role in the treatment of other infections, it can be administered orally and is highly concentrated in urine. It may therefore be the most appropriate agent for empirical use in uncomplicated UTI.

Our study exhibited the rising trend in ESBL production rate from 27.3% (2015) to 39.4% (2017). Such rising trend in ESBL rate is of considerable significance, because ESBLs have the ability to render all β-lactam rings containing antibiotics ineffective. Studies from different parts of India reported the different ESBL rates with 24.7% ESBL rate in a study done by Khurana et al.20 31.8% ESBL rate in a study by Purohit M et al.21 34% in a study conducted by Poovendran et al.22 and 41% of ESBL rate in a study done by Baby Padmanab et al.23 These ESBL positivity rates in urinary isolates of E. coli correlate with figures of our study.

ESBL producing strains are creating significant therapeutic problems, since these pathogens are resistant to a wide range of β-lactams including third generation cephalosporins. In our study, it was found that resistance to cephalosporins in ESBL producing E. coli was coexisting with resistance to other antibiotics. The resistance to co-trimoxazole was between 59.8% - 63.2% resistance to ciprofloxacin was between 67.2% - 74.8%, resistance to norfloxacin was between 47.2% - 55.6% and gentamicin was between 39.2% - 44.5% in all the ESBL producers in the years 2015 - 2017. The reason for this resistance is that ESBLs are encoded by plasmids, which also carry resistance genes for other antibiotics leading onto multidrug resistance. Multidrug resistance may be due to a number of factors like inappropriate self-medication, lack of prescribing regulations, substandard or falsified medicines and agricultural use of antibiotics.24

There was absolute resistance to beta-lactam-lactamase inhibitor combination drug amoxicillin-clavulanic acid. This is likely to be due to overuse of this drug. However, pipercillin-tazobactam was effective against ESBL producing E. coli with resistance rate ranging between 9.6% and 11.2% in the years 2015 - 2017. carbapenems work by the inhibition of cell wall synthesis and are highly resistant to the beta-lactamase enzyme. In our study, meropenem was the most effective antibiotic against ESBL-producing E. coli. The resistance to Meropenem in ESBL producing E. coli was ranging between 9.4% - 10.6% in our study. The lower resistance rate to carbapenems could be due to its usage pattern in our institution. It is most commonly used in critically ill patients where the administration of appropriate doses for the full duration is ensured. Surprisingly, resistance to nitrofurantoin in ESBL producing E. coli remains minimal in our study. It may be related to the fact that it has multiple mechanisms of action, hence may demand the organisms to develop more than a single mutation to concur resistance.25

CONCLUSION
ESBL producing E. coli has become the most worrisome causative agent in UTI. One of the most worrisome aspects of ESBL positive E. coli concerns the high rate of resistance to non-β-lactam antibiotics, particularly quinolones, co-trimoxazole and aminoglycosides. The rising trend of ESBL production in E. coli is quite alarming as it limits the choices and outcome of antimicrobial treatment indicating the requirement of continuous monitoring systems and effective infection control measures.

REFERENCES


