ABSTRACT

Introduction: ESBL producing organisms set unique challenges to clinical microbiologists, clinicians, and infection control professionals. ESBL producing organisms are also now emerging as uropathogens adding to the problem of drug resistance.

Study design: cross sectional study.

Place and duration of study: Index Medical College Hospital and Research Centre, Jan 2012-Dec 2013

Methodology: All members of Enterobacteriaceae isolated from urinary samples of female patients were included and identified using standard biochemical tests. Detection of ESBL was carried out by double disk diffusion technique.

Results: Out of total 200 samples examined, 152 showed growth. From 152 samples, total of 132 isolates of Enterobacteriaceae were identified during the study period. Of those 65.15% of isolates were found to be ESBL producing. ESBL positivity within individual organism group was highest in Klebsiella species 86.36%, followed by Escherichia coli 64.51%, Proteus species 31.25% and Enterobacter species 30%. Overall ESBL production rate was 43%. ESBL producers were sensitive to Amikacin, Nitrofurantoin, chloramphenicol and carbapenem, while they are resistant to commonly used antimicrobials.

Conclusion: A high frequency of ESBL producing organisms especially Klebsiella species and Escherichia coli amongst the hospital obtained urinary isolates was documented. As the available treatment options are limited, antimicrobial control policies together with the implementation of infection control measures remain of high importance.

Key words: Extended- spectrum beta-lactamase. Enterobacteriaceae, Klebsiella, E. coli, Antibiogram, Carbapenem, Fluoroquinolone, Amikacin
Plasmids containing genes encoding for ESBLs often contain resistance determinants for other classes of antimicrobial agents and are readily transmissible from strain to strain and between different species of enteric Gram negative bacilli. Production of β-lactamases is the most important mechanisms of resistance to β-lactam antibacterials. ESBL positive strains are associated with increased mortality as compared to the ESBL negative strains. There is great concern regarding ESBL spread in hospitals and failure to treat infections caused by ESBL positive organisms. In country like India, where microbiological diagnosis is not available to most of the populations, clinicians prescribe more than one antimicrobial, which results in drug resistance. Knowledge of local microorganism pattern and their antibacterial sensitivity pattern is essential for effective low cost treatment.

This study was carried out as no study has been done regarding prevalence and drug susceptibility of ESBL producing bacteria causing Urinary tract infection especially in female patients in this region and will provide information regarding prevalence of ESBL production among uropathogen and their susceptibility patterns. In order to ensure rational treatment of highly resistant pathogens, the occurrence of ESBL and its primary studies may serve as a base for further research and findings.

**MATERIALS AND METHODS**

**Study design:** The study was carried out in Index Medical College Hospital and Research Centre and was a cross sectional study.

**Patient inclusion criteria:** The study included 200 female IPD patients from obstetrics and gynaecology ward, clinically suspected urinary tract infection (high fever, burning micturition, frequency of micturition), during January 2012 to December 2013, a period of 2 years. Female patients were included as they are more prone to UTI than male patients and IPD patients were also included as it is a risk factor for acquisition of ESBL producing isolates.

**Specimen collection:** Wherever possible, clean voided early morning midstream urine specimens were collected in a sterile container, before starting antibiotics. If not early morning at least mid stream samples were included.

**Microbiological methods:** All samples were examined by wet mount and cultured on MacConkey and blood agar plates. All suspected colonies were identified by Gram staining, colony characteristics, motility and biochemical reactions. Kass criteria were followed for significant bacteriuria. Antimicrobial Susceptibility Testing (AST) was performed using disk diffusion method as described by the Clinical and Laboratory Standard Institute (CLSI) using Kirby-Bauer method (CLSI,2012). E. coli ATCC 25922 was used as a standard quality control strain. The Optical Density (O.D.) of the cultures were adjusted to 0.1 (at 530 nm) and swabbed on Mueller-Hinton (MH) Agar plates for further studies.

ESBL screening and Phenotypic Confirmatory Disc Diffusion Test (PCDDT) for ESBL detection:

All the isolates showing resistance to third generation cephalosporins, namely Ceftazidime, Ceftriaxone and Cefotaxime, were further tested for confirmation of β-lactamase production by phenotypic methods. The screening was done as per CLSI guidelines (CLSI, 2012) mentioned in Table 1.

**Table 1: Standard zone sizes of antibiotics for ESBL screening as per CLSI guidelines (CLSI, 2012)**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Strength</th>
<th>Zone size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aztreonam</td>
<td>30 mcg</td>
<td>&lt;27 mm</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30 mcg</td>
<td>&lt;26 mm</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>30 mcg</td>
<td>&lt;22 mm</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>30 mcg</td>
<td>&lt;27 mm</td>
</tr>
</tbody>
</table>
For confirmation, Ceftazidime (30 mcg) was used alone as well as in combination with Clavulanic acid (10 mcg). Both the disks were placed on MH Agar plates pre-swabbed with the respective cultures and incubated at 37°C for 24 hrs. An increase in the zone diameter for Ceftazidime-Clavulanic acid by ≥5 mm was considered positive for ESBL production (CLSI, 2010). [17] Double disk synergy test (DDST) was carried out using 5 antibiotics, namely Amoxicillin-Clavulanic acid (20/10 mcg), Aztreonam (30 mcg), Cefotaxime (30 mcg), Ceftriaxone (30 mcg) and Ceftazidime (30 mcg). The disks were placed at a distance of 1.5 cm from a centrally-placed Amoxicillin-Clavulanic acid disk. Enhancement of the zone of inhibition towards the Clavulanate disk after 24 hrs incubation at 37°C was considered indicative of a potential ESBL producer (Jarlier et al., 1988). [18]

RESULTS
In the current study, out of 200 samples processed, 152 were positive for culture. And out of 152 isolated organisms 132 were from Enterobacteriaceae family. Among Enterobacteriaceae isolates, 86 (65.15%) were ESBL (Table 2).

Table 2: Spectrum of isolated organisms:

<table>
<thead>
<tr>
<th>Isolated Organisms</th>
<th>Frequency</th>
<th>ESBL Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>62(31%)</td>
<td>40(64.51%)</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>32(16%)</td>
<td>38(86.36%)</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>12(6%)</td>
<td></td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>10(5%)</td>
<td>3(30%)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>8(4%)</td>
<td>5(62.5%)</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>8(4%)</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>2(1%)</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>10(5%)</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>8(4%)</td>
<td></td>
</tr>
<tr>
<td>No growth</td>
<td>48(24%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>86 (43%)</td>
</tr>
</tbody>
</table>

Fig. shows the total no. of enterobacteriaceae isolates and total no. of them which shows presence of ESBL production. Among Klebsiella spp., E.coli, Enterobacter spp. and Proteus spp. Percentage of ESBL production were 86.36%, 64.51%, 30% and 31.25% respectively.

In comparison to non ESBL producers, ESBL producers showed high resistance to commonly used antibiotics. (Fig 2)
DISCUSSION

Although since 1990s, prevalence of ESBL has been constantly reported in many studies done throughout world including India; [19-23] very few studies have been done in central India regarding ESBLs. In this study, among urine cultures of 200 clinically suspected UTI female patients 152 (76%) samples showed growth. From 152 organisms isolated, 132 organisms were from Enterobacteriaceae family and again from 132 organisms, 86(65.15%) were ESBLs producing strains. Mohanty et al and Hadi Mehrgan et al reported overall prevalence of ESBL producers in 68.78% and 77.7% respectively which is also similar to our study. [24-25] Very high prevalence of ESBL production by uropathogens in our study, i.e. in our hospital setting may have been caused by the excessive use of broad spectrum antibiotics in our hospital and to a higher level in our community setting, together with lack of attention to laboratory screening of ESBL production by clinical isolates. A study done in Indore reported only 36.8% of ESBL production which is much less than ours. [26] This may be due to inclusion of only IPD patients in our study, which is a known risk factor for ESBL acquisition. [12,13] Other countries, in comparison with India, showed a reduced incidence of ESBL producers. A study has shown that the prevalence of ESBL producing isolates of E.coli is 13.3% in Lebanon, 9.2% in Korea, 10.3% in Arabia and 17% in Turkey. [27] The reports presented by different authors clearly indicate that the prevalence of ESBL producing organisms among clinical isolates vary greatly geographically and rapidly changing over time. [28,29]

Spectrum of uropathogens isolated from urinary samples in current study is not too different from that reported in the literature. Spectrum of organisms grown was E.coli (31%), Klebsiella spp. (44%), S.aureus (10%), Enterobacter spp. (10%), Proteus spp. (16%), S.saprophyticus (8%) and Pseudomonas spp. (1%). Among these, percentage of ESBL producing organisms were Klebsiella spp. (86.36%), E.coli(64.51%), proteus spp. (31.25%) and Enterobacter spp. (30%). The SENTRY surveillance programme from Asia Pacific and South Africa also reported that most common ESBL producer was Klebsiella spp. similar to our study. [30] Two studies from India reported a high frequency of ESBL producing organisms, Klebsiella spp. emerged as the top most ESBL producing organism. Khurana et al reported 38.5% of Klebsiella followed by 24.7% of E.coli in urinary isolates while Mathur et al reported 80% of Klebsiella spp. as most frequent ESBL producing organism. [31,32]

According to phenotypic data generated, Antibiogram of ESBL producing organisms has shown high resistance to commonly used antibiotics. ESBL producers showed resistance to beta-lactam antibiotics as well as non-beta-lactam antibiotics. ESBL producers have shown higher degree of resistance to Fluroquinolones (ciprofloxacin, Norfloxacin, Ofloxacin) which are commonly prescribed drugs for UTI. Such a high degree of resistance clearly shows the misuse of antibiotics by healthcare professionals. Low degree of resistance to Amikacin and Nitrofurantoin was observed in ESBL producers and hence may be helpful in combating severe infections. Reduced in vitro susceptibility to aminoglycosides in the isolates (<30%) has voided their use in the treatment of infections. Similar findings have been documented previously. [32,33] With moderate activity against urinary ESBL-producing isolates, Nitrofurantoin might be useful in the therapy of lower urinary tract infection, at least in patients who could take oral medications. Feizabadi et al. reported higher resistance (74%) of ESBL-producing
K. pneumoniae to this antibiotic. All ESBL producers were sensitive to carbapenems which are used as last resort drugs. These results are in accordance with the findings in a tertiary care hospital based in South India.

Briefly, as the available treatment options are limited, new challenges presented by the changing nature and distribution of these enzymes, clinicians need to be familiar with the clinical significance of these enzymes, and clinical microbiology laboratories require adopting a technique most appropriate to them for their detection.

CONCLUSION

Increasing resistance to valuable antibiotics in uropathogens, mediated principally by beta-lactamases, has become a major concern. This is suggestive of a need for regular screening and surveillance for ESBL producing organisms in our region. Patients with these organisms should be nursed with contact precautions to avoid cross infections among patients admitted in hospital and also physicians should be regularly informed about the changing pattern of Antibiogram for common organisms. People should be sensitized about danger of irregular and incomplete dosage of antibiotics leading to selection and spread of resistant organisms.

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