ESBL producing *Escherichia coli* – its prevalence and antibiogram

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Abstract

**Introduction:** *E. coli* exhibits resistance to commonly used beta-lactam antibiotics by production of β-lactamases. Hence, creating awareness regarding these enzymes and its detection becomes necessary for better patient care. The present study was undertaken to detect extended-spectrum β-lactamases (ESBL) producers.

**Materials and Method:** A total of 101 *E.coli* isolated from different clinical samples were studied. ESBL production was detected by using combination disk method.

**Results:** Of 101 *E.coli* isolates tested, 77.2% were found to be ESBL producers. They showed highest susceptibility to amikacin (91%) followed by gentamicin (69.2%), meropenem (62.8%), ceftriaxone (37.1%), ceftoxitin (30.7%), ciprofloxacin (19.2%), amoxicillin-clavulanic acid (16.6%), cefepime (10.2%) and ampicillin (3.8%).

**Conclusions:** Reporting of ESBL producers along with routine sensitivity pattern will help the clinicians in prescribing the appropriate antibiotics. Amikacin, gentamicin and imipenem which showed highest sensitivity are helpful for the treatment of infections which are caused by ESBL producing *E.coli*.

**Keywords:** *E.coli*, ESBL, Combination disk test

Introduction

*Escherichia coli* (*E. coli*) is a very diverse species of bacteria that forms a major part of the normal intestinal flora.(¹) It mainly causes urinary tract infection, diarrhea, pyogenic infection, sepsis and are also common in community acquired infections.(²) Antibiotic resistance has become a major concern of the 21st century. β-lactams are among the commonly used classes of antibiotics. However, resistance to β-lactams has emerged and that resistance is mainly mediated by the mechanism of production of β-lactamases is.(³) Also resistance in *E. coli* and other members of family Enterobacteriaceae is commonly due to beta-lactamase enzyme that is encoded by the *bla* gene either in the chromosome or plasmid.(⁴) These enzymes hydrolyze the amide bond of the four-membered characteristic β-lactam ring, thus rendering the antimicrobial ineffective.(⁵)

ESBL producers pose challenges to clinical microbiologists, clinicians and infection control professionals.(⁶) They mediate resistance to penicillins, oxymino-cephalosporins, such as cefotaxime, aztreonam, ceftazidine and monobactams and can be inhibited by clavulanate, sulbacam or tazobactam.(⁷,⁸) It was first discovered in Germany in 1983 in strains of Klebsiella spp. Once they are commonly seen in Escherichia coli and Klebsiella spp., but now it is also prevalent in other members of the Enterobacteriaceae and in non-fermenters e.g., Pseudomonas aeruginosa and Acinetobacter baumannii.(⁹) Recently, increasing number of ESBL producers has been noted in outpatient settings which reduce the treatment options to a limited number of antibiotics.(¹⁰) The result of which includes emergence of multidrug resistant strains, treatment failure and hence increased mortality.(¹¹)

Since many years, carbapenemases are used for treatment of infections caused by ESBL and AmpC producers.(¹²) But extensive and sometime unnecessary use of the carbapenems, poor sanitation and large population has facilitated the emergence of carbapenem resistant bacteria and hence the effectiveness of this antibiotic group was challenged.(¹³) Many reports of different beta-lactamases produced by different mutant genes are known from all over the world. But the data available is very less since many laboratories do not identify and report them. Its identification is very important for proper treatment and effective infection control in hospitals.(¹⁴) Its detection also helps to reduce increasing resistance in pathogens.(¹⁵)

Materials and Method

A retrospective analysis of *E.coli* isolates, isolated at Subbaiah institute of medical sciences, Shimoga was performed. The sex and age of patients, number of *E.coli* isolated and the antimicrobial susceptibility patterns were collected from the registration records. The data was then analyzed by entering into Excel. As the study was based on secondary data there were no ethical issues.

A total of 101 *E.coli* isolates isolated from various samples were studied. The samples were inoculated onto MacConkey agar and blood agar which were then incubated aerobically at 37°C for 18-24 hrs. The isolates were identified by standard procedures.(¹⁶) Antibiotic sensitivity was done on Mueller Hinton agar by Kirby Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) guidelines.(¹⁷) Antibiotic discs used were: ampicillin (10µg), amoxyclav (20/10µg), gentamicin (10µg), amikacin (30µg), ciprofloxacin (10µg), cotrimoxazole.
(1.25/23.75µg), cefoxitin (30µg), cefepime (30µg), meropenem (30µg).

**Criteria for selection of ESBL producing strains:**
The isolates showing zone of inhibition of ≤ 22 mm for Ceftazidime, ≤ 27 mm for cefotaxime and ≤ 25 mm for Ceftriaxone by disc diffusion method were considered to be suspicious for ESBL production.

**Detection of ESBL:** This was performed as per the recommendations of CLSI. The ceftazidime (30 µg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 µg discs) were used. An increase of ≥ 5 mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer.

**Results**

Of 101 E.coli isolates, 78 (77.2%) were ESBL producers. Of these 78 ESBL producers, 47(60.3%) were from females and 31(39.7%) from males and 40(51.3%) isolates were from outpatient department and 38(48.7%) from inpatient. The age of > 45 years 40(51.3%) followed by age 18-45 years 31(39.7%) were the most affected groups. Majority of the isolates were susceptible to amikacin (91%) followed by gentamicin (69.2%), meropenem (62.8%), cotrimoxazole (37.1%), cefoxitin (30.7%), ciprofloxacin (19.2%), amoxicillin-clavulanic acid (16.6%), cefepime (10.2%) and ampicillin (3.8%).

**Table 1: Number of ESBL producers**

<table>
<thead>
<tr>
<th>No. of E.coli isolates</th>
<th>ESBL producers No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>78 (77.2)</td>
</tr>
</tbody>
</table>

**Table 2: Age, sex and OP/IP distribution of ESBL producing E. coli**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. of isolates</th>
<th>Sex</th>
<th>OP No. (%)</th>
<th>IP No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 18</td>
<td>7(9)</td>
<td>M</td>
<td>2(2.6)</td>
<td>5(6.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td>4(5.1)</td>
</tr>
<tr>
<td>18 - 45</td>
<td>31(39.7)</td>
<td>M</td>
<td>9(11.5)</td>
<td>22(28.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td>20(25.7)</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>40(51.3)</td>
<td>M</td>
<td>20(25.6)</td>
<td>20(25.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td>16(20.5)</td>
</tr>
<tr>
<td>Total</td>
<td>78(100)</td>
<td>M</td>
<td>31(39.7)</td>
<td>47(60.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td>40(51.3)</td>
</tr>
</tbody>
</table>

**Table 3: Antibiotic Sensitivity Pattern of ESBL producing E. coli**

<table>
<thead>
<tr>
<th>AMP</th>
<th>AMC</th>
<th>G</th>
<th>AK</th>
<th>CIP</th>
<th>COT</th>
<th>CN</th>
<th>CPM</th>
<th>MRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1(1.3)</td>
<td>5(6.4)</td>
<td>29(37.2)</td>
<td>38(48.7)</td>
<td>7(8.9)</td>
<td>17(21.8)</td>
<td>11(14.1)</td>
<td>3(3.8)</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>2(2.5)</td>
<td>8(10.2)</td>
<td>25(32)</td>
<td>33(42.3)</td>
<td>8(10.3)</td>
<td>12(15.3)</td>
<td>13(16.6)</td>
<td>5(6.4)</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>3(3.8)</td>
<td>13(16.6)</td>
<td>54(69.2)</td>
<td>71(91)</td>
<td>15(19.2)</td>
<td>29(37.1)</td>
<td>24(30.7)</td>
</tr>
</tbody>
</table>

AMP – Ampicillin, AMC – Amoxiclav, G – Gentamicin, AK – Amikacin, CIP – Ciprofloxacin, COT – Cotrimoxazole. CN – Cefoxitin, CPM – Cefepime, MRP – Meropenem

**Discussion**

Multi drug-resistant (MDR) pathogens are increasing in frequency in tertiary health centre’s which are associated with high morbidity and mortality. The problem of ESBLs has become serious. These enzymes are detected in many strains of pathogenic bacteria with a potential for dissemination. The spread of ESBL-encoding genes within the microbial genome is due to their common localization on self-transmissible or easily movable broad-range plasmids. Some of the risk factors for infection with ESBL producers are continuous antibiotic exposure, ICU stay for longer duration, severe illness, common usage of ceftazidime and other third generation cephalosporin’s, instrumentation or catherization.

In our study, ESBL was detected in 78 (77.2%) E.coli isolates. Our findings are similar to that of Nachimuthu Ramesh et al. and MS Kumar et al. who reported a high prevalence of ESBLs among E. coli. Overuse of third generation cephalosporin’s and other beta-lactams to treat gram negative infections is one of the prime factors responsible for increased resistance to this class of antibiotics. Initially ESBLs were associated with nosocomial outbreaks but recently studies have shown its significant increase in community isolates. This study showed very less sensitivity to cefepime 8(10.2%). A marked decrease in the susceptibility to cefepime (33%) from the reported rate of 96% in the past decade by Villanueva et al. (2003) was also noted. This could be interpreted as an extended activity to fourth-generation cephalosporin. The only antibiotics that can effectively be used for ESBL-associated infections are aminoglycosides and carbapenems. Our study showed maximum sensitivity to amikacin 71(91%) followed by gentamicin 54(69.2%) and meropenem 49(62.8%). Similar findings have been reported from study done by R. Thakur et al. For the treatment of infections with ESBL producers carbapenems may still be considered. Among the non beta-lactam antibiotics, the higher resistance rate was observed for ciprofloxacin 15(19.2%) and cotrimoxazole 29(37.1%) which is similar to study done by A Bora et al. However, the percentage of isolates...
resistant to cotrimoxazole was higher in outpatients, possibly due to the increased use of this drug in the outpatient setting.\(^{(27)}\)

The accurate detection of extended-spectrum β-lactamases is a major clinical problem, particularly in invasive infections. Failure of which has contributed to their dissemination and also to therapeutic failures.

**Conclusion**

The present study has shown very high percentage of ESBL producing *E. coli*, which has created a challenge for the microbiologists and clinicians. Simple test like combination disk method can be routinely done to detect ESBL producers. Hence routine practice of ESBL detection and its reporting will help in appropriate treatment of the patients and also prevent further development of bacterial drug resistance which is very important.

**References**


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