Antibiotic Sensitivity Pattern and Esbl Detection among Clinical Isolates of Escherichia Coli

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Abstract

Background: ESBL producing bacteria poses a major problem for clinical therapeutics. The ESBL producing isolates of E.coli among other clinical isolates has been increasing over the past few years resulting in limitation of therapeutic options.

Materials and methods: A total number of 20 clinical specimens of E.coli were obtained from different clinical samples. They were subjected for the antibiotic susceptibility pattern by Kirby bauer disc diffusion method. The 3rd and 4th generation cephalosporins resistant isolates will be detected for ESBL production.

Results: In our isolates, we have found increased percentage 14/20 (70%) of isolates showed sensitivity to amikacin followed by gentamicin, which showed sensitivity of 9/20 (45%). 80-90% of E.coli isolates showed resistance to cephalosporin group of drugs. All E. coli isolates 20/20 (100%) were found to be extended spectrum beta lactamase producers using Ceftazidime (30µg), ceftazidime/clavulanic acid (30/10µg) and 11/20 (55%) of isolates were shown to be positive for ESBL using cefotaxime (30µg), cefotaxime/clavulanic acid (30/10µg).

Conclusion: The study reveals higher percentage of isolates were resistant to different drugs and ESBL producers were more. We conclude that resistant to cephalosporins were due to extended spectrum beta lactamase production in our isolates.

Keywords: E.coli, antibiotic sensitivity testing, ESBL

INTRODUCTION

Urinary tract infection is one of the most important causes of morbidity and mortality. Escherichia coli is the most common urinary pathogen isolated from 50-90% of all the uncomplicated urinary tract infections. Extended spectrum beta lactamases (ESBL) were documented globally. The advent of ESBL producers as posed a great problem to the use of many antibiotics particularly cephalosporin.

MATERIALS AND METHODS

Bacterial isolates
A total of 20 non repetitive clinical isolates of E.coli were collected from Saveetha Medical College, Chennai. They were processed for a battery of standard biochemical tests and confirmed. Isolates were preserved in semisolid Trypticase soy broth stock and stored at 4°C until further use.

Antibiotic susceptibility testing
Antibiotic susceptibility test was determined for these isolates to routinely used antibiotics such ampicillin, amoxicillin, amikacin, norfloxacin, cefazidime, cefotaxime, ciprofloxacin and gentamicin as by Kirby Bauer disc diffusion method.

Detection of ESBL in E. coli
E.coli producing ESBL were detected by disc potentiation test. Overnight culture suspensions of E.coli isolates were adjusted to 0.5 McFarland standards. Sterile Mueller Hinton agar plates were lawn inoculated with the culture suspensions. Ceftazidime (30µg), ceftazidime/clavulanic acid (30/10µg), cefotaxime (30µg), cefotaxime/clavulanic acid (30/10µg), (Hi Media, Mumbai) were placed over the lawn inoculated Mueller Hinton agar plates and incubated at 37°C for 18 hours. >5mm increase in zone diameter in the cephalosporins with clavulanic acid than cephalosporins tested alone areas considered as positive for ESBL production.

RESULTS

Sample wise distribution of clinical isolates of E.coli
Of the 20 clinical isolates of E.coli, 12/20 (60%) were from urine, 4/20 (20%) from stool, 3/20 (15%) and 1/20 (5%) were from wound swab and pus respectively. Figure 1 depicts the sample wise distribution of clinical isolates of E.coli

Antibiotic susceptibility testing
In our isolates, we have found increased percentage 14/20 (70%) of isolates showed sensitivity to amikacin followed by gentamicin, which showed sensitivity of 9/20 (45%). 80-90% of E.coli isolates showed resistance to cephalosporin group of drugs. However, we have observed an elevated level of resistance to other routinely used antibiotics. The detailed resistant pattern of E.coli isolates were showed in table 1.
Figure 1: Sample wise distribution of clinical isolates of *E. coli*

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitivity (20)(%)</th>
<th>Intermediate (20)(%)</th>
<th>Resistant (20)(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>5</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>5</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>10</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>5</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>Amikacin</td>
<td>70</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>45</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>15</td>
<td>15</td>
<td>70</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>20</td>
<td>5</td>
<td>75</td>
</tr>
</tbody>
</table>

Table 1: Results of antibiotic susceptibility pattern of *E. coli*

Figure 2: Representative picture for antibiotic susceptibility testing of *E. coli*
DETECTION OF ESBL
All isolates were subjected for ESBL detection by disc potentiation test. All E. coli isolates 20/20 (100%) were found to be extended spectrum beta lactamase producers using Ceftazidime (30µg), ceftazidime/clavulanic acid (30/10µg) and 11/20 (55%) of isolates were shown to be positive for ESBL using cefotaxime (30µg), cefotaxime/clavulanic acid (30/10µg).

DISCUSSION
Sarma et al., 2010 have observed 41% of ESBL producers among Enterobacteriaceae group of bacteria isolated from orthopedic patients in United Kingdom. [7]
George et al., 2014 have found that among the healthy subjects, 46.1% strains from adults and 25% strains from infants were ESBL producers. [8] The overall frequency of occurrence of ESBL producing E. coli was much higher in the human subjects (43.9%) when compared to that of the environment (10.5%) in Vellore district of Tamil nadu. Shukla et al., 2004 have shown that, 120 isolates of K. pneumoniae were included which were resistant to at least three antibiotics that was tested in Uttar Pradesh, Indian. [9] 88.3% of the isolates were moderately sensitive and were studied for ESBL production. 72% of the isolates which was included in the study were resistant to all the three 3GC antibiotics. All the strains were sensitive to imipenem but among the non beta lactam antibiotics ciprofloxacin and amikacin were most effective drugs and 89.63 and 73.86% of the strains were sensitive to these drugs. Sarma et al., 2010 have shown that ESBL was detected in almost every species of Enterobacteriaceae in the study mainly dominated by K. pneumoniae and E.coli. Patients at high risk for developing colonisation or infection with extended spectrum beta lactamase producing organisms are often seriously ill patients with prolonged hospital stays and in whom invasive medical devices are present for a prolonged duration. Shukla et al., 2004 have shown that ESBL producing K. pneumoniae was isolated in the hospital. The routine antimicrobial sensitivity tests may fail to detect ESBL mediated resistance against 3GC which in turn lead to treatment failure particularly when cephalosporins were used. Since all the isolates were sensitive to imipenem and there were large number of isolates from blood it can be the drug of choice for the treatment of infections due to extended spectrum beta lactamase producing K. pneumoniae strains in seriously ill patients. George et al., 2014 have shown that there have been several reports on the prevalence of extended spectrum beta lactamase in recent years in Indian. In this study, out of the 363 E. coli isolated from different human and environmental sources, 122 were found to produce ESBL. The usage of antimicrobials is one of the major etiological factors for colonisation with ESBL producing Enterobacteriaceae. The carrier rate was found to be 25% and 48% among the infants and healthy adults respectively. Several research studies have suggested that the acquisition of ESBL harbouring isolates may be mediated by contaminated food and water. Environment showed a very low rate (10.5%) of ESBL E.coli. A high rate of dissemination of E. coli in the environment, but not the ESBL E. coli indicates a loss of resistance by losing their plasmids in the environment.

CONCLUSION
We have found that an increased percentage of isolates were resistant to most of the routinely used antibiotics. However, a good sensitivity was observed to aminoglycosides. Most of the isolates were resistant to cephalosporin group of antibiotics. These isolates were found to exhibit extended spectrum beta lactamase. We conclude that our e.coli isolates were ESBL producers and we suggest the clinicians to screen for ESBL in clinical settings in routine practice to avoid any misusage of antibiotics.
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REFERENCES