Faecal Carriage of Extended Spectrum Beta Lactamase Producing
Escherichia coli among Patients, Healthy Individuals and in Environment from a South Indian Tertiary Care Hospital

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Abstract

Aim: ESBL positive Escherichia coli isolated from faecal samples of inpatients, healthy individuals and the environment were analysed.

Methods: This is a prospective study conducted in the department of Microbiology, GSL Medical College and General Hospital, Rajahmundry in the months of July 2016 to June 2017.

Results: Out of 399 faecal samples processed (86 from inpatients; 187 from healthy individuals and 126 from environment), a total of 392 Escherichia coli strains were recovered. Among Escherichia coli strains obtained 141 isolates were found to be ESBL producers. Inpatients showed highest incidence (69.2%) of ESBL positive Escherichia coli compared to isolates from healthy subjects (34.8%). Lowest incidence (15.5%) was found from environmental strains. Among the inpatients higher carrier rate was noticed in patients admitted to ICU, catheterised patients, and in those with history of prior antibiotic use. ESBL producing E. coli recovered from inpatients also revealed an increase in the rate of resistance to other non-beta lactam group of antibiotics. The strains from the environment showed very little or no resistance. All ESBL positive E. coli obtained from different study groups were susceptible to carbapenems like meropenem.

Conclusion: Continued surveillance and monitoring of antimicrobial resistance in the hospital accompanied with strict infection control policies and restricted use of antimicrobials especially cephalosporins is the need of the hour.

Keywords: Extended Spectrum Beta Lactamases (ESBL); E. coli; Antibiotics; Kirby-Bauer's Method

Introduction

The widespread and inappropriate use of antibiotics has resulted in a significant increase in antibiotic resistant bacteria worldwide [1]. Production of inactivating enzymes, predominantly extended – spectrum beta lactamases (ESBL’s) had been implicated as a major mechanism in bacterial drug resistance. ESBL’s are plasmid mediated enzymes that can hydrolyse penicillins; first, second, third generation cephalosporins; and monobactams while beta lactamase inhibitors like clavulanic acid can inhibit such enzymes [2]. These enzymes are transferred via plasmids that result in spread of resistance both between the members of same species and also different species of bacteria [3,4]. Resistance to other non-beta lactam classes of antibiotics (amino glycosides and fluoroquinolones) is also disseminated by such plasmids [4]. Carbapenems are the preferred choice of treatment for serious infections caused by ESBL’s followed by fluoroquinolones.

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Faecal Carriage of Extended Spectrum Beta Lactamase Producing *Escherichia coli* among Patients, Healthy Individuals and in Environment from a South Indian Tertiary Care Hospital

Relentless use of beta-lactam antibiotics in the clinical practice has resulted in the appearance of beta-lactamases such as extended spectrum beta-lactamases (ESBLs) that are typically plasmid mediated and found mainly in Gram negative bacilli mostly *Escherichia coli* and *Klebsiella pneumoniae*. Most frequently reported ESBLs belong to TEM (temoneira), SHV (sulphhydryl variable) and CTX-M types. CTX-M type is spreading rapidly worldwide causing epidemics both in hospitals and community.

ESBL producers cause infections which pose challenges in treatment along with increased mortality and morbidity [5]. Infections due to ESBL producing bacteria are a major public health concern globally [6]. ESBL producing bacteria have been associated frequently with nosocomial infections. These drug resistant bugs are being reported with increasing frequency even from the community by several researchers [7]. Faecal carriage of ESBL producing bacteria by healthy subjects is implicated as a reservoir for dissemination of ESBL bacterial infections in the community. These bacteria were known to colonise the intestinal tract prior to infection, making asymptomatic faecal carriage with ESBL producing bacteria clinically significant. *Escherichia coli* and *Klebsiella pneumoniae* are frequently reported as pathogens. This necessitates continuous surveillance of ESBL producing bacteria among hospitalised patients, asymptomatic individuals in the community as well as from environment. This study aims to find out the incidence of faecal carriage of ESBL positive *E. coli* from different groups of humans (hospitalised patients and healthy subjects) and environment.

**Materials and Methods**

This is a prospective study conducted in the department of Microbiology, GSL Medical College and General Hospital, Rajahmundry in the months of July 2016 to June 2017.

Faecal samples were collected in sterile universal containers with screw caps from 86 inpatients, 54 from several intensive care units and 32 from different wards. Also faecal samples were obtained from 187 healthy individuals and infants (0 - 2 years). Swabs were collected from 126 different environmental sites such as sewage drains, public toilets, and market places including swabs from hospital wards, procedure rooms and intensive care units.

**Inclusion criteria**

1. Faecal samples were collected from inpatients who are on antibiotic therapy and with a minimum four day period of hospitalisation.
2. Stool samples were obtained from healthy individuals and infants without any history of recent antibiotic usage from the past three months.
3. Both male and female patients and all age groups were included in the study.
4. Environmental samples included swabs from both public places and hospital.

**Exclusion criteria**

1. Inpatients within first 3 days of admission are excluded.
2. Healthy adults or infants with recent usage of antibiotics (in past 3 months) were excluded.
3. The study did not include outpatients.

Faecal samples and swabs were inoculated directly on to MacConkey agar plates and incubated at 37°C for 18 to 24 hours. All the colonies with different morphology were further identified by means of biochemical tests; enterobacterial isolates were then processed further. Plates which showed no primary growth after 24 hours were further incubated up to 48 hours. *E. coli* isolates were identified as gram negative bacilli, motile, lactose fermenting with methyl red test positive, indole production test positive, vogues proskauer test negative and citrate test negative, nitrate reduction test positive.

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Antimicrobial susceptibility of all faecal *E. coli* strains was determined by CLSI disk diffusion method (modified Kirby-Bauer) using ampicillin, ciprofloxacin, cefotaxime, ceftazidime, cefepime, gentamicin, amikacin, doxycycline, cotrimoxazole, imipenem, meropenem. All isolates showing a decreased susceptibility to at least one third generation cephalosporin or cefepime were confirmed for ESBL phenotype using double disk synergy test.

All the *E. coli* strains are recovered from different study groups were further tested for ESBL production using double disc method as per clinical laboratory standard institute (CLSI) guidelines. Antibiotic discs - ceftazidime and ceftazidime-clavulanic acid; cefotaxime and cefotaxime-clavulanic acid were used. Production of ESBL was indicated if the difference in zone diameter between antibiotic and antibiotic – inhibitor combination is greater than or equal to 5 millimetres. Positive and negative controls used were *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 respectively [9]. Antiograms of ESBL positive *E. coli* isolates from different study groups were analysed with respect to cephalosporins, carbapenems and other non-beta lactam classes of antimicrobials.

### Results

A total of 399 samples, faecal (n = 266; 86 from patients, 187 from healthy individuals) and 126 from environment were collected. A total of 392 *E. coli* strains recovered from different study groups (78 obtained from patients; 198 from healthy subjects and 116 from environment) were further tested for ESBL production. Out of 392 *E. coli* strains isolated, 141 were found to be ESBL producers. Of the groups studied, the frequency of ESBL producing *E. coli* was found to be 69.2% among patients, 34.8% among healthy individuals, and 15.5% among the isolates from environment (Table 1).

<table>
<thead>
<tr>
<th>S. no</th>
<th>Study group (n = 86)</th>
<th>No. of isolates</th>
<th>No. of <em>E. coli</em> (%)</th>
<th>No. of ESBL <em>E. coli</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Patients (n = 86)</td>
<td>107</td>
<td>78 (72.9)</td>
<td>54 (69.2)</td>
</tr>
<tr>
<td>2</td>
<td>Healthy subjects (n = 187)</td>
<td>224</td>
<td>198 (88.4)</td>
<td>69 (34.8)</td>
</tr>
<tr>
<td>3</td>
<td>Environment (n = 126)</td>
<td>144</td>
<td>116 (80.6)</td>
<td>18 (15.5)</td>
</tr>
</tbody>
</table>

Table 1: Distribution of ESBL *E. coli* among different study groups.

A significantly higher incidence of ESBL *E. coli* was noticed among inpatients compared to healthy subjects and environment. Environment revealed a very low rate of ESBL *E. coli* (15.5%) compared to human subjects (44.57%).

*E. coli* strains (n = 78; 51 from ICU patients and 27 from ward patients) obtained from inpatient sampling were analysed for the presence of ESBL production. A total of 54 *E. coli* strains were found to be ESBL positive. A higher incidence of ESBL *E. coli* strains was found from ICU (84.3%) compared to ward patients (40.7%) (Table 2).

<table>
<thead>
<tr>
<th>S. no</th>
<th>Study group (n = 86)</th>
<th>No. of <em>E. coli</em> (n = 78)</th>
<th>No. of ESBL <em>E. coli</em> (n = 54) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ICU (54)</td>
<td>51</td>
<td>43 (84.3)</td>
</tr>
<tr>
<td>2</td>
<td>Wards (32)</td>
<td>27</td>
<td>11 (40.7)</td>
</tr>
</tbody>
</table>

Table 2: Distribution of ESBL *E. coli* between ICU and ward patients.

*E. coli* strains (n = 78; 40 from catheterised patients and 38 from non-catheterised patients) obtained from inpatients were analysed for the presence of ESBL production. Among them 54 were ESBL producers. Catheterised patients showed higher frequency of ESBL *E. coli* (90%) compared to non- Catheterised (47.3%) patients (Table 3).

<table>
<thead>
<tr>
<th>S. no</th>
<th>Study group (n = 86)</th>
<th>No. of <em>E. coli</em> (n = 78)</th>
<th>No. of ESBL <em>E. coli</em> (n = 54) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Catheterised (n = 42)</td>
<td>40</td>
<td>36 (90)</td>
</tr>
<tr>
<td>2</td>
<td>Non-catheterised (n = 44)</td>
<td>38</td>
<td>18 (47.3)</td>
</tr>
</tbody>
</table>

Table 3: Distribution of ESBL *E. coli* between catheterised and non - catheterised patients.

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The incidence of ESBL producing *E. coli* among catheterised patients was higher than the non-catheterised patients.

*E. coli* strains (n = 78; 44 from patients with history of prior antibiotic use and 34 from patients with no definite history of antibiotic use) obtained from inpatients were analysed for the presence of ESBL production. Out of 54 ESBL producing *E. coli* recovered, the frequency of ESBL producing *E. coli* was found to be 39 (88.6%) from patients with history of prior antibiotic use and 15 (44.1%) from patients with no definite history of antibiotic use (Table 4).

<table>
<thead>
<tr>
<th>S. NO</th>
<th>Study group (n = 86)</th>
<th>No. of E. coli (n = 78)</th>
<th>No. of ESBL E. coli (n = 54) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Patients with history of prior antibiotic use (n = 46)</td>
<td>44</td>
<td>39 (88.6)</td>
</tr>
<tr>
<td>2</td>
<td>Patients with no definite history of antibiotic use (n = 40)</td>
<td>34</td>
<td>15 (44.1)</td>
</tr>
</tbody>
</table>

*Table 4: Distribution of ESBL E. coli among patients with or without prior history of antibiotic use (past 14 days).*

The frequency of ESBL producing *E. coli* among patients with history of prior antibiotic use was found to be higher when compared to patients with no definite history of antibiotic use.

All ESBL *E. coli* showed resistance to ampicillin and all cephalosporins other than Cefepime.

Strains from Environmental samples showed greater susceptibility (88.9%) to cefepime followed by 33.3% for healthy individuals. The least (9.26%) susceptibility was shown by inpatient strains.

Isolates from environmental samples (88.9%) and healthy individuals (50.7%) showed good and moderate sensitivity to ciprofloxacin respectively while low susceptibility (18.5%) was shown from inpatient isolates. Strains from Healthy individuals showed 100% susceptibility to gentamicin followed by 75.4% for environmental strains and 66.7% to isolates from inpatients (Table 5).

<table>
<thead>
<tr>
<th>S. No</th>
<th>Study group</th>
<th>No. of ESBL positive E. coli</th>
<th>Cefepime (%)</th>
<th>Ciprofloxacin (%)</th>
<th>Gentamicin (%)</th>
<th>Piperacillin-Tazobactam (%)</th>
<th>Amikacin (%)</th>
<th>Meropenem (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inpatients</td>
<td>54</td>
<td>5 (9.26)</td>
<td>10 (18.5)</td>
<td>36 (66.7)</td>
<td>46 (85.19)</td>
<td>44 (81.48)</td>
<td>54 (100)</td>
</tr>
<tr>
<td>2</td>
<td>Healthy individuals</td>
<td>69</td>
<td>23 (33.3)</td>
<td>35 (50.7)</td>
<td>52 (75.4)</td>
<td>61 (88.4)</td>
<td>62 (89.9)</td>
<td>69 (100)</td>
</tr>
<tr>
<td>3</td>
<td>Environment</td>
<td>18</td>
<td>16 (88.9)</td>
<td>16 (88.9)</td>
<td>18 (100)</td>
<td>18 (100)</td>
<td>17 (94.4)</td>
<td>18 (100)</td>
</tr>
<tr>
<td>4</td>
<td>All</td>
<td>141</td>
<td>44 (31.2)</td>
<td>61 (43.3)</td>
<td>106 (75.2)</td>
<td>125 (88.7)</td>
<td>123 (87.2)</td>
<td>141 (100)</td>
</tr>
</tbody>
</table>

*Table 5: Antimicrobial susceptibility profiles of ESBL E. coli by study group.*

*E. coli* from Environmental samples yielded strains that showed 100% susceptibility to piperacillin-tazobactam followed by 88.4% for healthy individuals and 85.19% for inpatients. Strains from Healthy individuals revealed 94.4% sensitivity to amikacin followed by 89% from inpatients while 81.48% was shown from environmental samples. All the isolates from different study groups were found to be susceptible to carbapenems like meropenem (100%).
Several scientific reports have been published in INDIA on the prevalence of ESBL's in recent years. Our study revealed 141 out of 392 E. coli strains as ESBL positive accounting to 35.97% of isolates as ESBL producers. In the present study, the rate of faecal carriage of ESBL E. coli was higher among inpatients than healthy patients or environment. The excessive use of antibiotics could explain the higher prevalence of faecal carriage of ESBL producing organisms in the hospital compared with the rate in environment. This study correlates with another south Indian study from vellore by George and co-workers which showed a higher frequency of ESBL E. coli among the patients than strains from environment [10].

Similar results were shown by a Saudi Arabian study with higher rate of ESBL E. coli from faecal samples of inpatients compared to healthy persons. Environment revealed a very low rate of ESBL E. coli. Distribution of E. coli but not ESBL E. coli in the environment indicates a loss of resistance by losing their plasmids. Indiscriminate use of antibiotics is implicated as a major risk factor for colonisation with ESBL E. coli [11].

In the present study, distribution of ESBL producing E. coli between ICU and ward patients showed that the rate of faecal carriage of ESBL producing E. coli among inpatients was higher from ICU than ward patients. This study correlates with Indian study [12]. Admission to ICU and high dependency areas is considered a risk factor for colonisation with ESBL enterobacteriaceae [13-15].

The present study also showed that the rate of faecal carriage of ESBL positive E. coli was higher among catheterised patients than non-catheterised patients. This study correlates with a Spanish study by a Jesus Rodriguez-Bano and co-workers [14]. Presence of foreign devices is a risk factor for colonisation with ESBL E. coli [13]. According to the present study observations, increased frequency of ESBL E. coli was recovered from faecal samples of patients with history of prior antibiotic use (in past one month) compared to inpatients with no history of prior antibiotic use. Similar observations with a higher faecal carriage of ESBL enterobacteriaceae was mentioned in several studies [12].

Antimicrobial susceptibility profile of ESBL E. coli recovered from different study groups showed resistance to ampicillin and all cephalosporins except cefepime. ESBL producers isolated from patients revealed an increase in resistance rate to other non-beta lactam group of antibiotics compared to healthy subjects. This may be related to increased load of antibiotic pressure in hospital environment. ESBL producers recovered from healthy subjects revealed higher susceptibility rates to other non-beta lactam antibiotics and carbapenems compared to patients. All ESBL E. coli were sensitive to meropenem in vitro. Carbapenems are preferred drugs for infections caused by ESBL E. coli followed by amikacin and piperacillin-tazobactam. Cefepime is the only cephalosporin active against ESBL E. coli. Among inpatients (46 out of 86) 53.49% had received prior therapy with third generation cephalosporins and/or fluoroquinolones. The excessive use of these antibiotics explains the higher rate of faecal carriage of ESBL producers in the hospital.

In the present study fluoroquinolones resistance was high among strains recovered both from inpatients (44 out of 54) (81.5%) and healthy individuals (34 out of 69) (49.3%). Although no clear documentation was there regarding recent exposure to antibiotics among healthy individuals, the unrestricted (over the counter) sale of antibiotics in developing countries is likely to create general pool of resistant organisms in the population. Oral formulation of amoxicillin-clavulanate and fluoroquinolones (e.g. ciprofloxacin and levofloxacin) are some of the antibiotics frequently used without prescriptions.

Environmental strains showed good susceptibility to other non-beta lactam antibiotics including Cefepime. Carbapenems remain preferred drug for infections caused by ESBL E. coli followed by piperacillin-tazobactam among beta lactam drugs; amikacin being an active non-beta lactam antibiotic.
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**Conclusion**

A high rate of faecal carriage of ESBL *E. coli* was noted among inpatients in our study. Colonisation of ESBL *E. coli* in healthy individuals indicate reservoir of infections as carriers.

Environment showed a very low rate of ESBL *E. coli*. Present study emphasises the need and importance of regular surveillance and monitoring of local resistance patterns to restrict infections caused by ESBL *E. coli*.

Although clinical laboratories perform routine screening to detect ESBL producers in individuals with community onset infections the results are unlikely to indicate the actual prevalence of ESBL producers in the community and asymptomatic carriers may remain unnoticed for long periods. The existence of ESBL *E. coli* in the gut has clinical implications, intestinal colonisation is a prerequisite for infection by them. Henceforth infectious disease physicians and clinical microbiologists need to be aware that ESBLs are not only confined to hospital environment but also in the community and should deal accordingly.

The limited availability of treatment options for infections caused by ESBL- producers necessitates restricting the use of antimicrobials along with implementation of prompt infection control measures.

Also over the counter sale of antibiotics without prescription needs to be prohibited to control the high rate of carriage for these organisms and to increase awareness among the public regarding hazards of taking antibiotics without medical consultation.

**Bibliography**

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