Multidrug-Resistant ESBL-Producing Enterobacteriaceae Associated with Clinical Samples in a Tertiary Care Hospital, Sirajganj

Summiya Shamima Priti¹, Kakoli Akter¹, Md. Babul Aktar¹, Kaniz Mehzabin¹, Laila Jarin², Rasheda Yasmin Shilpi², Md. Farukh Faisal Ashrafi³, Abdullah Akhtar Ahmed¹, Jafrug Islam⁴, and Mohammad Zakerin Abedin¹,²*  

¹Department of Microbiology, Khwaja Yunus Ali University, Bangladesh; ²Microbiology Laboratory, Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh; ³Department of Microbiology, Asgar Ali Hospital, Bangladesh; and ⁴Department of Microbiology, Uttara Crescent Hospital, Bangladesh.  
*Correspondence: akkerin.abedin.mb@kyau.edu.bd (Mohammad Zakerin Abedin, Assistant Professor & Head, Department of Microbiology, Khwaja Yunus Ali University, Sirajganj, Bangladesh).

ABSTRACT

Extended-spectrum β-lactamase (ESBL) microorganisms have been shown to increase multidrug resistance globally, which is a great concern. The prevalence of ESBL-producing clinical pathogens and their antimicrobial resistance patterns were identified in 66 isolates from patients in Khwaja Yunus Ali Hospital with several clinical infections cultured on blood agar and MacConkey agar media. The most prevalent bacteria were Escherichia coli (80.3%), which were identified by the BD-Phonex automated identifier machine, followed by Pseudomonas spp. (6%), Klebsiella spp. (12.1%), and others (4.4%). This research was conducted from January 2023 to June 2023. Generally, a large number of antibiotic resistance patterns and ESBL-producing common bacterial isolates were found in this study, where most of the resistant percentage was found in third-generation cephalosporin antibiotics, which increases the public health problem. In this study, the most prevalent bacteria E. coli isolates were mostly resistant to penicillin (100%), ceftazidime, cefotaxime, and cefuroxime (98.4%). Besides, 95.4% resistance was shown against ceftiraxone. The double-disc synergy test was done to determine the presence of ESBL-producing bacterial strains. The most widely ESBL-positive isolate was Escherichia coli (83%). Among the 66 sample strains produced, the ESBL maximum (53.03%) belonged to female patients, while 46.97% belonged to male patients. This study focuses on the prevalence and patterns of clinical pathogens and the antimicrobial susceptibility profiles of ESBL-producing bacterial infections in a tertiary-level health service center in Bangladesh. Generally, a large number of antibiotic resistance patterns and ESBL-producing common bacterial isolates were found in this study, which increases the public health risk. Therefore, to save human life, we ought to be taking appropriate action against the threat.

Keywords: Extended-spectrum β-lactamase, Bacterial profiles, Multidrug-resistant, and Antibiotics.

INTRODUCTION:

Extended-spectrum beta-lactamase (ESBL)-producing bacteria have expanded dramatically globally, and they are one of the leading causes of morbidity and mortality in hospital-acquired infections (Kumar et al., 2014). This could be explained by the presence of the multidrug resistance in ESBL-producing isolates. Resistant bacteria are growing around the world as a challenge to the successful treatment of common diseases in both hospitals and the community (Prestinaci et al., 2015). The most frequent infections contracted in hospitals caused by Enterobacteriaceae include urinary tract, gastro-intestinal, and pyogenic infections (Atici et al.,
E. coli is the most commonly isolated species among the Enterobacteriaceae (Karlowsky et al., 2003; Shahen et al., 2019). Multidrug resistance in the E. coli is extensively documented (Karlowsky et al., 2003). Prolonged antibiotic exposure, hospitalization, severe sickness, unprecedented usage of third-generation cephalosporin (Kumar et al., 2014; Tewari et al., 2018) and increased use of intravenous devices or catheters are all risk factors for multidrug-resistant E.coli infection. Lactamase synthesis is possibly the single most important mechanism of penicillin and cephalosporin resistance (Tang et al., 2014). E. coli naturally produces chromosomally-mediated or the plasmid-mediated lactamases (Poirel et al., 2002). Penicillin-binding proteins are considered to have evolved into these enzymes (Hakenbeck, 1998). This evolution was most likely caused by the selection pressure applied by lactamase-producing soil organisms in the environment (Allen et al., 2009). Extended-spectrum beta-lactamases (ESBLs), enzymes that accelerate the hydrolysis of oxyimino-lactams such as cefotaxime, ceftriaxone, ceftazidime, and aztreonam, have recently been described (Jacoby, 1997).

They are members of the Ambler molecular class A and the Bush-Jacoby functional group 2be (Sawa et al., 2020). These enzymes have been identified in considerable quantities in diverse places and have been found to be abundant in several E. coli strains (Sawa, Kooguchi et al., 2020). They have also been discovered in other Enterobacteriaceae members, including Klebsiella spp, Citrobacter spp, Enterobacter spp, Proteus spp, and non-lactose fermenters such as Pseudomonas aeruginosa (Abbott, 2011). Over 200 distinct ESBLs have been described to date (Kumari, 2017). Major epidemics of these resistant organisms in the several members of the Enterobacteriaceae and Pseudomonas spp. have been documented all over the world, resulting in a lack of therapeutic choices (Chaudhary, 2004).

ESBL-producing bacteria are likely more common than is currently known because they frequently go unnoticed by standard susceptibility testing methods (MP et al., 2010). Resistance to other non-lactam antibiotics, such as aminoglycosides and chloramphenicol, has been linked to ESBL strains (Pitout et al., 1997). Another characteristic of these ESBL strains is that they may exhibit a falsely sensitive zone of inhibition when tested using the Kirby-Bauer disk diffusion method (Kumar et al., 2014). To comprehend the disease burden and take the appropriate precautions to avoid its spread, current knowledge of the prevalence of ESBL generation by commonly isolated pathogens such as E. coli is required (Kumar et al., 2014). As a result, the current investigation was carried out with the goal of determining the prevalence of ESBL-producing Enterobacteriaceae and their antimicrobial resistance profile in order to develop an effective antibiotic strategy and plan.

MATERIALS AND METHODS:

Sample collection
Bacterial isolates from clinical samples such as pus, urine, blood, wound swab, throat, sputum, ear swab, and other bodily fluids were obtained in the department of microbiology at Khwaja Yunus Ali Medical College and Hospital (KYAMCH). The research was conducted from January 2023 to June 2023. Khwaja Yunus Ali University's Ethics Committee granted ethical permission.

Isolation and identification
20 ml of urine samples collected in a universal container and one loopful (0.002 ml) were inoculated into the chromogenic UTI agar, MacConkey agar, and blood agar plates. For the blood sample, we used a Bactec™ BD-M15 (USA) automated machine along with a positive signal, which was then sub-cultured on MacConkey and Blood Agar media. Other specimens, such as sputum, body fluids, and swabs, were collected in sufficient amounts and then inoculated on the blood agar and MacConkey agar plates using an inoculating loop. All inoculated media were incubated aerobically overnight at 37°C. On the basis of colony morphology, the organisms were identified, and biochemical analyses were performed using oxidase test, Kligler Iron Agar stain, motility indole urea, and Simon citrate reaction, etc.

Antimicrobial Susceptibility Test
Antimicrobial susceptibility testing (AST) for all isolates was conducted on commercially available common antibiotics disc. All ESBL and non-ESBL producing clinical pathogen were studied for antimicrobial sensitivity using disc diffusion technique by "Kirby-Bauer method" on the culture medium of Mueller-Hinton agar (HiMedia, India) and interpretations were recorded according to the guidelines of clinical and laboratory standard institute.
Testing for the ESBL Production

The identification of ESBLs production by 66 positive clinical bacterial pathogens was conducted by a modified double-disc synergism test (Ahmed et al., 2017). Bacterial suspension of 0.5 McFarland standards was plated in Muller-Hinton agar with the Amoxycillin-clavulanic acid (30 µg) disc in between and 20 mm apart from Ceftazidime (30 µg) and Ceftriaxone (30 µg) discs. Expansion of the zone of inhibition around Ceftriaxone and/or ceftazi
dime disc towards the amoxicillin-clavulanic acid disc was considered ESBL production.

Statistical analysis

Chi-square test is used for statistical analysis of the data. A ‘P value’ less than 0.05 were considered statistically significant.

RESULTS:

During the research period, 66 clinical pathogens were detected in various clinical samples of inpatients. In 66 sample isolates, phenotypic identification of ESBL production revealed that E. coli was the most common (80.3%), followed by Klebsiella spp. (12.1%), P. aeruginosa (3%), and others (4.5%). The bulk (45%) of the 66 sample strains developed belonged to female patients, while the other 55% belonged to male patients. In terms of patient age groups, females over the age of 49 had the highest prevalence (47%), followed by those aged 13-24 years (3%), and those aged 37-48 years (19%). In men, the age group was 37-48 years (33%), the age group was 25-36 years (17%), and the remaining 13% were in the age group of 13-24 years (Table 1).

In this study, the most prevalent bacteria, namely E. coli isolate, showed mostly resistance to penicillin (100%), ceftazidime, cefotaxime, and the cefuroxime (98.4%). Besides, the 95.4% resistance was shown against ceftriaxone presented in the Table 3. The resistance rates for the aminoglycoside and carbapenem groups of antibiotics were quite less than those for the third generation of antibiotics, where amikacin, gentamicin, imipenem, and meropenem, and nitrofurantoin were 16.7%, 57.5%, 39.4%, 33.3%, and 31.8%, respectively.

Table 1: Demographic data of study population.

<table>
<thead>
<tr>
<th>Age range</th>
<th>Female (%)</th>
<th>Male (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-12</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>13-24</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>25-36</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>37-48</td>
<td>19</td>
<td>33</td>
</tr>
<tr>
<td>&gt;49</td>
<td>47</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Number and Frequency (%) of ESBL producing bacteria.

<table>
<thead>
<tr>
<th>Biological samples</th>
<th>E. coli (n=53)</th>
<th>Klebsiella spp. (n=8)</th>
<th>Salmonella enterica (n=1)</th>
<th>Staphylococcus spp.</th>
<th>Streptococcus spp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Pus</td>
<td>Blood</td>
<td>Sputum</td>
<td>Swab</td>
</tr>
<tr>
<td>E. coli</td>
<td>33(50%)</td>
<td>11(16.7%)</td>
<td>3(4.5%)</td>
<td>1(1.5%)</td>
<td>5(7.6%)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>4(6%)</td>
<td>-</td>
<td>-</td>
<td>2(3%)</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>2(3%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1(1.5%)</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1(1.5%)</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1(1.5%)</td>
</tr>
</tbody>
</table>

Table 3: Results of Antibiotic Susceptibility pattern of bacteria.

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Pattern</th>
<th>AK</th>
<th>AMC</th>
<th>CPM</th>
<th>AMP</th>
<th>CAZ</th>
<th>CTR</th>
<th>CXM</th>
<th>CTX</th>
<th>ATM</th>
<th>GEN</th>
<th>IPM</th>
<th>MEM</th>
<th>NIT</th>
<th>P</th>
<th>SXT</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (n=53)</td>
<td>S</td>
<td>79.25</td>
<td>75.48</td>
<td>1.9</td>
<td>9.44</td>
<td>1.89</td>
<td>5.66</td>
<td>1.89</td>
<td>3.78</td>
<td>18.36</td>
<td>56.6</td>
<td>51</td>
<td>58.5</td>
<td>56.4</td>
<td>0</td>
<td>26.42</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>20.75</td>
<td>24.52</td>
<td>98.1</td>
<td>90.56</td>
<td>98.11</td>
<td>94.34</td>
<td>98.11</td>
<td>96.22</td>
<td>81.14</td>
<td>43.4</td>
<td>49</td>
<td>41.5</td>
<td>43.4</td>
<td>100</td>
<td>73.58</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>S</td>
<td>87.5</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
<td>12.5</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>75</td>
<td>75</td>
<td>62.5</td>
<td>62.5</td>
<td>63</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>12.5</td>
<td>75</td>
<td>75</td>
<td>87.5</td>
<td>87.5</td>
<td>75</td>
<td>100</td>
<td>100</td>
<td>25</td>
<td>25</td>
<td>37.5</td>
<td>37.5</td>
<td>38</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>S</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
DISCUSSION:
For physicians around the world, extended-spectrum β-lactamase-expressing multidrug-resistant clinical pathogens provide major obstacles to the therapeutic management of clinical cases of urinary tract infection. The goal of the current investigation was to show that an ESBL-producing bacterium could be isolated from clinical samples of patients in a major hospital in Bangladesh. It was found that 66% of pathogens produced ESBLs. E. coli (80.3%) produced ESBLs more frequently than Pseudomonas spp. (3%), or Klebsiella spp. (12%). This figure was reported in a study completed in Khartoum State hospitals by Mekki et al. (2010) who recorded ESBL production among E. coli and Klebsiella species isolates as 66%. Similarly, a few numbers of ESBL-producing E. coli (36%), Egypt, occurred during 2013-14 (ElSayed et al., 2023). The high ranges of 41.0 to 63.6 percent in E. coli were reported for the prevalence of ESBL production in other studies in India (Grover et al., 2006). The most well-known pathogens, E. coli, are thought to be part of the normal flora in the genitourinary and digestive tracts. However, they can ascend the urethra and enter the urinary tract. E. coli has explicit virulence characteristics that allow it to adhere to and attack host cells, create toxins, consume supplements, and evade the host’s immune system (Abedin et al., 2020).

ESBLs pose a significant risk to β-lactam treatment. Many of these bacterial isolates have been incorrectly reported to be susceptible to the widely used broad-spectrum beta-lactams because they are difficult to detect using existing clinical procedures (MacKenzie et al., 2002). We found such an associated resistance with gentamicin (36%) and the fluoroquinolones (67%). Gupta et al. (2007) reported 91.17% and 94.91% resistance, respectively, to gentamicin and ciprofloxacin in the ESBL producers. Compared with our previous studies done at Khwaja Yunus Ali Medical College & Hospital, the current investigation found lower resistance rates for the majority of the isolated E. coli, which were resistant to Meropenem (50%) and Amikacin (66%), followed by gentamicin (34%), amoxiclav (40%), and Ciprofloxacin (57.8%) (Ahmed et al., 2016). This decreased drug resistance indicates successful coordinated monitoring of drug activity and usage. Overall, these results show that ESBL production in bacterial species varies significantly over the world and changes quickly over time and space.

Study limitations
This study carries several basic limitations. This study analyzed only a few ESBL producing bacteria and the use limited β-lactam antibiotics. The small sample size was also a limiting factor in performing fully powered statistical analyses. However, our results were generated from a Resource - limited setting and maintained internal validity by repeating independent experiments where necessary.

CONCLUSION:
In this investigation, the incidence and trends of ESBL and the non-ESBL-producing uropathogens against routinely prescribed antibiotics in clinical isolates from tertiary institutions in Bangladesh were examined. This discovery emphasizes how crucial it is to continuously monitor and program antibiotic resistance in our hospitals. It also demonstrated the necessity of creating strategies to lessen the occurrence of clinical infections that produce ESBLs. Patients infected with ESBL-producing bacteria must be treated with the proper antibiotics since ESBLs are clinically significant. Finally, the results of numerous studies differed depending on location and time, raising concerns about their validity and
making it challenging to draw comparisons between them. In order to reduce the overall rate of resistance, it is necessary for observation methods and routine surveillance to be normalized throughout the nation. These results call for immediate surveillance and action to prevent the introduction of ESBL-producing bacteria on a national and international scale.

ACKNOWLEDGEMENT:
We are grateful to the Chairman of the Trusty Board and the Director of Khwaja Yunus Ali Medical College Hospital for carrying out this study. We express gratitude toward Md. Abdul Karim and Mazharul Haque for assisting in laboratory work at the Department of Laboratory Services of Khwaja Yunus Ali Medical College & Hospital, Sirajganj, Bangladesh.

CONFLICTS OF INTEREST:
There is no conflict of interest among the authors.

REFERENCES:
https://doi.org/10.7860/JCDR/2010/748


https://doi.org/10.1016/s0002-9343(97)00044-2


https://doi.org/10.34104/ejmhs.0190109


https://www.msjonline.org/index.php/jrms/article/view/4604

https://doi.org/10.34104/ejmhs.024.044049