Patterns of Extended-Spectrum β-Lactamase Producing Uropathogens
Detection in Tertiary Care Hospital of Bangladesh


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ABSTRACT

It is a great concern that extended-spectrum β-lactamase (ESBL) and non-ESBL uropathogenic microorganisms have been worldwide illustrated to increase multidrug resistance. To study the prevalence and patterns of uropathogens, and antimicrobial susceptibilities profiles of ESBL and non-ESBL producing bacterial infection in a tertiary level health service center of Bangladesh. The prevalence of ESBL producing uropathogens and their antimicrobial resistance patterns were identified in 176 isolates from patients with UTI. ESBL and non-ESBL producing bacterial isolates and their antibiotic sensitivity and resistance patterns were distinguished from the 176 patients of suspected urinary tract infection. The Double-disc diffusion test was done to determine the presence of ESBL-producing bacterial strains. The most widely ESBL positive uropathogen was Escherichia coli (87%), followed by Pseudomonas aeruginosa (6.8%), Enterococcus spp (3.4%), Acinetobacter spp. (2.5%) and non-ESBL positive E. coli (41.4), Staphylococcus saprophyticus (25.9%), Pseudomonas aeruginosa (17.2%), Staphylococcus aureus (10.3%), and Klebsiella pneumoniae (5.2%). The current investigation found most frequently Escherichia coli in both ESBL and non-ESBL uropathogen group as 87% and 41.4% respectively. Generally, a large number of antibiotic resistance patterns and ESBL producing common bacterial isolates were found in this study which increases the public health problem. Therefore, for safe human life, we ought to be taking appropriate action against the threat.

Keywords: Prevalence, ESBL, Non-ESBL, Uropathogens, Tertiary region, and Antimicrobial patterns.

INTRODUCTION:

It has been alarmingly noticed that the infection of the urinary tract affects approximately. Around more than 150 million people each year are infected by bacterial pathogens in both genders. Urinary tract infection (UTI) is the second most common infection and is responsible for nearly seven million physician services per year (Akram et al., 2007). The patterns of the diseases and frequency of infection are related to age and sex. Among uropathogens, E. coli are found responsible for 80% of community-acquired UTI and 40% of healthcare-associated UTI. Other uropathogens include Proteus spp, Staphylococcus, and Klebsiella spp. Enterococcus and Acinetobacter (Goering et al., 2004). Astonishingly, uropathogens found to alternate their physiological characteristics to prompt multidrug...
resistance (Ronald et al., 2003). Enhancing the tendency of developing antibiotic resistance among uropathogens is a global problem (Mathai et al., 2000; Sarker et al., 2019). The uropathogens produce extended-spectrum β-lactamase (ESBL) enzymes through which they hydrolyze oxyimino β-lactam compounds and it is one of the prime factors contributing to highly decrease antimicrobial susceptibility against β-lactam antibiotics (Gniadkowski et al., 2001).

The rate of multidrug resistance increases due to improper treatment of UTI caused by the ESBL and non-ESBL uropathogenic bacteria (Du et al., 2002). For the therapy of infections with antibiotics due to ESBL and non-ESBL-producing uropathogens is still a great challenge to solve the risk factors, treatment options, and infection control measures for its infection (Paterson et al., 1999). It has been a considerable problem with appropriate antimicrobial therapy due to ESBL and non-ESBL positive uropathogens. The point of this investigation was to observe patterns and prevalence of antimicrobial susceptibility in patients of Khwaja Yunus Ali Medical College & Hospital in the tertiary region of Bangladesh.

MATERIALS AND METHODS:

Sample Collection - The study was carried on 589 non-consecutive and non-duplicate midstream clean-catch urine samples. The specimens were collected from the patients aseptically and processed at the microbiology laboratory of Khwaja Yunus Ali Medical College & Hospital Laboratory Services Department from January 2020 to June 2020.

For the isolation and identification of bacterial isolates, we conducted the following standard bacteriological technique (Ahmed et al., 2016). For these purposes, the following dehydrated culture media were used namely Blood agar, MacConky agar, XLD, TSI, Urea, and Citrate. Urine samples showing significant bacterial growth, of >105 colony-forming units (CFU/mL) with a single type of bacteria isolates, were considered uropathogens.

Testing for the ESBL Production - The identification of ESBLs production by 176 uropathogens out of 589 urine samples 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 Amoxicillin-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 ceftazidime disc towards the amoxicillin-clavulanic acid disc was considered ESBL production.

Antimicrobial Susceptibility Test - Antimicrobial susceptibility testing (AST) for all isolates was conducted on commercially available common antibiotics disc. Information on these antibiotics and their concentrations are shown in Table 1.

All ESBL and non-ESBL producing uropathogens were studied for antimicrobial sensitivity using disc diffusion technique by “Kirby-Bauer method” on the culture medium of Mueller-Hinton agar (Oxoid, UK) and interpretations were recorded according to the guidelines of clinical and laboratory standard institute (CLSI-2010) (Rahman et al., 2019; Manoharan et al., 2011).

The following commercially available antibiotics discs were used in the sensitivity test namely amoxicillin (20 µg), amoxiclav (30 µg), amikacin (30 µg), azithromycin (15 µg), ceftriaxone (30 µg), Ciprofloxacine (5 µg), gentamicin (30 µg), levofloxacin (5 µg), nitrofurantoin (30 µg), imipenem (10 µg), meropenem (10 µg). On each of the Mueller-Hinton agar plates was used only for five antibiotic disks and incubated aerobically at 37°C for 24 hours.

Table 1: Details on antibiotics used in this study

<table>
<thead>
<tr>
<th>Antibiotic group</th>
<th>Antibiotic name</th>
<th>Concentration (µg/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactam antibiotic</td>
<td>Amoxicillin: (AML)</td>
<td>20 µg</td>
</tr>
<tr>
<td>β-lactam/β-lactamase inhibitors combination</td>
<td>Amoxiclav (AMC)</td>
<td>20 µg + 10µg</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Amikacin (AK)</td>
<td>30 µg</td>
</tr>
<tr>
<td>Macrolide</td>
<td>Azithromycin (AZM)</td>
<td>15µg</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Third-generation cephalosporin</th>
<th>Ceftriaxone (CRO)</th>
<th>30 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporin</td>
<td>Cefuroxime (CXM)</td>
<td>30 µg</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Ciprofloxacin (CIP)</td>
<td>5 µg</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Gentamicin (GM)</td>
<td>30 µg</td>
</tr>
<tr>
<td>Fluoroquinolone</td>
<td>Levofloxacin (LEV)</td>
<td>5 µg</td>
</tr>
<tr>
<td>Nitrofurans</td>
<td>Nitrofurantoin (NI)</td>
<td>30 µg</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>Imipenem (IPM)</td>
<td>10 µg</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>Meropenem (MEM)</td>
<td>10 µg</td>
</tr>
</tbody>
</table>

RESULTS:
A total of 176 uropathogens from 589 urine sample cultures of inpatients were identified during this study period. Among these uropathogens 118 strains produced ESBL maximum 62 (52.5%) belonged to female patients while 56 (47.5%) belonged to male patients. While non-ESBL producing uropathogens shown no great variation in sexual point. With regard to the age categories of patients, a total of 96 (54.5%) samples were collected from adults, 66 (37.5%) samples belonged to the elderly population, and 14(8%) were obtained from children. Demographic characteristics of patients (Table 2).

Table 2: Demographic data of study population

<table>
<thead>
<tr>
<th>Age category</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESBL Positive</td>
<td>ESBL Negative</td>
<td>ESBL Positive</td>
</tr>
<tr>
<td>Children*</td>
<td>05</td>
<td>04</td>
<td>03</td>
</tr>
<tr>
<td>Adults**</td>
<td>22</td>
<td>14</td>
<td>43</td>
</tr>
<tr>
<td>Elderly***</td>
<td>29</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Total (%)</td>
<td>56 (47.5)</td>
<td>31 (53.4)</td>
<td>62 (52.5)</td>
</tr>
</tbody>
</table>

*Children: 0–17 years, **adults: 18–64 years, ***elderly: ≥65 years.

Table 3: Shows pattern of ESBL & non-ESBL producing uropathogenic bacteria

<table>
<thead>
<tr>
<th>ESBL</th>
<th>E. coli</th>
<th>K. pneumonia</th>
<th>P. aeruginosa</th>
<th>Acenatobacter sp.</th>
<th>Enterococcus sp. 04</th>
<th>S. aureus 06</th>
<th>S. saprophyticus 15</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>103 (87)</td>
<td>00 (6.8)</td>
<td>03 (2.5)</td>
<td>04 (3.4)</td>
<td>00</td>
<td>00</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>24 (41.4)</td>
<td>03 (5.2)</td>
<td>10 (17.2)</td>
<td>00</td>
<td>06 (17.2)</td>
<td>15 (25.9)</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
<td>03</td>
<td>18</td>
<td>03</td>
<td>04</td>
<td>06</td>
<td>15</td>
<td>176</td>
</tr>
</tbody>
</table>

The antibiotic susceptibility profile for ESBL-producing uropathogens is presented in Table 4. Among all antibiotics tested in this study, imipenem was found the most active agent as 98% against E. coli isolates. Out of the 112 ESBL producing E. coli isolates, 99% were resistant to ampicillin, 67% to

ESBL Prevalence and Antimicrobial Susceptibility Profiles
Phenotypic identification of ESBL production of 176 uropathogens isolates showed that 118 (67%) of all bacterial isolates were confirmed to be ESBL producers, while 58 (33%) isolates were non-ESBL producers. The E. coli was the most frequent uropathogen (72%) followed by P. aeruginosa (17.2%) and Staph. saprophyticus (8.5%). 10 of 18 Pseudomonas spp. samples were negative, 04 of samples of Enterobacter spp. and 03 Acinetobacter spp. were positive. All 06 Staphylococcus aureus, 15 Staphylococcus saprophyticus and 03 Klebsiella spp samples were found to be ESBL negative (Table 3).
amoxicillin-clavulanic acid, and 36% to gentamicin. The resistance rates for amikacin, azithromycin, ceftriaxone, and levofloxacin were 17%, 82%, 82%, and 53%, respectively. Additionally, 2% and 23% of the isolates were least resistant to imipenem and meropenem, respectively.

**DISCUSSION:**

Multidrug-resistant uropathogens expressing extended-spectrum \( \beta \)-lactamase pose worldwide serious challenges to clinicians for the therapeutic management of clinical cases in urinary tract infection (UTI). In our study, both males and females were the commonest pervasive of bacterial infections and 35% of sound healthy women were warning indications of UTIs (Rezwana et al., 2015).

The current study was embraced to demonstrate the presence of ESBL-producing uropathogen isolates in clinical samples of patients in the tertiary medical clinic of Bangladesh. The predominance of ESBL-producing pathogens was found to be 67%. ESBL production occurred more frequently in *E. coli* (87%) than *P. aeruginosa* (6.8%), *Enterococcus spp.* (3.4%) and *Acinetobacter spp.* (2.5%). The non-ESBL production occurred more frequently also in *E. coli* (41.4%) than *Staph saprophyticus* (25.9%), *P. aeruginosa* (17.2%), *S. aureus* (10.3%) and *Klebsiella spp.* (5.2%).

This figure is high contrasted with the figure reported in a study completed in Khartoum State hospitals by Mekki et al., who recorded ESBL production among *E. coli* and *Klebsiella* species isolates as 53%. Similarly, a few numbers of ESBL producing *E. coli* (36%) and a greater number of non-ESBL producing *E. coli* (64%) were distinguished in Egypt during 2013-14 (Elsayed et al., 2017). The high ranges of 41.0 to 63.6 percent in *E. coli* were reported for the prevalence of the ESBL production in other studies in India (Grover et al., 2006).

*E. coli* are the most well-known uropathogens and considered as normal flora of the gastrointestinal and distal urogenital, but they can ascend the urethra and get access to the urinary tract. Explicit harmfulness factors found in *E. coli* permit it to hold fast to and attack have cells, produce poisons, use have supplements, and avoid the host’s immune system (Abedin et al., 2020). ESBLs constitute a serious threat to the \( \beta \)-lactam therapy. Because of the difficulty in their detection by the current clinical methods, many of these bacterial isolates have been falsely reported to be susceptible to the widely used broad-spectrum \( \beta \)-lactams (MacKenzie et al., 2002). We found such an associated resistance with gentamicin (36%) and the flouroquinolones (67%). Varsha et al., reported 91.17% and 94.91% resistances respectively to gentamicin and ciprofloxacin in the ESBL producers (Gupta et al., 2007).

Compared with our previous studies done at Khwaja Yunus Ali Medical College & Hospital in 2013, the current investigation found lower resistance rates for the majority of isolated *E. coli* were resistant to Meropenem (41.4%) and Amikacin (42.3%) followed by gentamicin (47.4%), amoxiclav (56.9%), Ciprofloxacin (57.8%), (Ahmed et al., 2016). This decreased drug resistance indicates successfully coordinated monitoring of drug activity and usage. Overall, these findings indicate that ESBLs production in bacterial species differs greatly all over the world, and it rapidly changes from time to time and place to place.

**CONCLUSION:**

This study was illustrated to find out the prevalence and patterns of ESBL and non-ESBL producing uropathogens in clinical isolates from the tertiary level hospital of Bangladesh against commonly prescribed antibiotics. The prevalence of ESBL producing *E. coli* is 87%. Multidrug resistance patterns were discovered to be altogether higher in ESBL (67%) producer isolates when contrasted with non-ESBL (33%) producer isolates. This finding highlights the importance of constant observing programming of multidrug resistance in our hospitals. It also showed the need for developing attempts to decrease the prevalence of ESBL producing uropathogens. ESBLs are clinically significant and patients infected with ESBL-producing uropathogens are vitally important to treat with appropriate antibiotics.

Finally, many studies had varied findings according to place and time casting doubt on their quality and making comparisons among them difficult. Subsequently, there is a requirement for normalization of observation methodology and routine surveillance in the country, alongside appropriate activities to abate the overall rate of resistance.
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CONFLICTS OF INTEREST:

No conflict of interest among the authors.

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