ETIOLOGY OF UTI AND FREQUENCY OF ESBL PRODUCING BACTERIA ISOLATED FROM PATIENTS OF DHAKA MEDICAL COLLEGE HOSPITAL WITH THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN

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Abstract:

Background: Urinary tract infections (UTIs) are the most common of all bacterial infections and occur at any time in the life of an individual. ESBL producing bacteria particularly Escherichia.coli is one of the most common causes of UTIs both in community and healthcare associated settings. Emergence of multidrug resistance (MDR) is quite alarming and cause failure of empirical treatment of UTIs. As a result increase the morbidity and mortality rate in the developing countries like Bangladesh.

Objective: The objective of this study was to find out the bacteria causing UTI from urine culture and detection of ESBL producing Esch.coli and K.pneumoniae with their anti-microbial susceptibility pattern.

Materials and Methods: A total of 1750 urine samples were collected from patients with symptoms and suspected UTI. Clean catch mid-stream urine samples were collected from indoor and outdoor patients of Dhaka Medical College Hospital during January 2015 to July 2015. Urine specimens were cultured in 5% Blood agar and MacConkeys agar media. The isolated bacteria were identified by gram staining and biochemical tests. Antimicrobial susceptibility and detection of ESBL were done by disc diffusion method.

Result: Out of 1750 urine samples, 403(23.03%) were positive by culture. Among the culture positive cases, 216 (53.59%) were female and 187 (46.41%) were male. The most common isolated bacteria were Esch.coli 295(73.20%) followed by Pseudomonas aeruginosa 85(21.09%), K.pneumoniae 10(2.48%), Proteus spp. 4(0.99%), Acinetobacter spp. 5(1.2%), Coagulase negative Staphylococcus (CONS) 4(0.99%). Among the isolated Esch.coli and K.pneumoniae, ESBL producing bacteria were 202 (68.47%) and 5 (50%) respectively. All the isolated bacteria showed low level susceptibility to all antibiotics that are used during the study period.

Conclusion: Treatment of UTIs is difficult when caused by multidrug resistant bacteria. Analysis of culture and sensitivity data should be done periodically to identify ESBL producing bacteria for proper treatment of UTIs.

Key words: Extended spectrum beta lactamases (ESBL), Multi drug resistant (MDR), urinary tract infections (UTIs), Dhaka Medical College Hospital.

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Introduction:

Urinary tract infections (UTIs) are common health problems both in the community and healthcare associated settings. Etiological agents vary according to geography and regions¹. In the world about 150 million urinary tract infections are reported per annum.² UTIs are more common in female than male. Approximately 1 in 3 women will require antimicrobial treatment for UTI before the age

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24 and 40%-50% of them develop UTI during their life time. UTIs may involve only the lower urinary tracts or both upper and lower tracts. Pathogen profile varies from region to region but Escherichia. coli remains the most common pathogen both in community acquired as well as hospital acquired UTI and contributing to approximately 80- 85% of infections. Other pathogens are K. pneumoniae, Proteus spp, Enterococcus fecalis, Pseudomonas aeruginosa, Enterobacter spp, Acinetibacter spp, Staphylococcus saprophyticus. Extended spectrum beta lactamase (ESBL) causing UTIs is the emerging problem over the last twenty five years. ESBL organisms produce enzymes that hydrolyze the beta lactam ring of beta lactam antibiotics like penicillins, cephalosporins and aztreonam rendering them ineffective. Beta lactamase producers are typically gram negative organisms mainly Escherichia. coli, Klebsiella pneumoniae spp. They are frequently multi drug resistant such as resistant to aminoglycosides, trimethoprimsulfamethoxazole and quinolones. As a result empirical therapy with cephalosporins and fluoroquinolones often fail to treat these patients. Infection with an ESBL producing organisms causing UTI is associated with treatment failure, delayed clinical response, higher morbidity and mortality. When UTI is suspected mid-stream urine should be collected for routine and microscopic urinary analysis, as well as culture and sensitivity. Urine culture and sensitivity results serve both to establish the definitive antimicrobial therapy and to provide surveillance data of antimicrobial resistance. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory.

**Materials and Methods:**
This study was done in the microbiology department of Dhaka Medical College from January 2015 to July 2015. Total 1750 urine samples were collected from patients from outpatient and inpatient departments. Data regarding age and sex of the patients were collected and recorded in a predesigned data collection sheet. The results of the tests were recorded systematically.

**Samples collection**
Freshly voided mid stream urine specimens were collected in to sterile containers from patients of all age groups with clinical suspicion of UTI. Samples were transported to the microbiology laboratory immediately after collection.

**Samples processing**
Microbiological examinations were carried out as promptly as possible after collection to avoid unpredictable changes. The samples were cultured for isolation and identification of the pathogenic bacteria. Using a calibrated wire loop of loop diameter 4mm, 10 µl of un-centrifuged specimens were transferred into agar plates.

**Isolation of bacterial agent**
All samples were inoculated into 5% Blood agar and MacCokey agar media (Oxoid), semi quantitative streaking was used for quantification of bacterial load in urine. The inoculated plates were incubated at 37°C aerobically. After overnight incubation, plates were examined for growth and colony forming units (cfu) were calculated. A specimen was considered positive for UTI if a single organism was cultured at a concentration of >10^5 cfu/ml.

All the isolates were preliminarily screened and identified by their colony morphology, pigment production, hemolysis on blood agar and confirmed by Gram staining, oxidase test, motility test and other biochemical tests as per standard methods.

**Phenotypic screening of ESBL production.**
All the gram negative isolates were tested for detection of ESBL by double disc synergy test as described before. Antimicrobial discs (Oxoid) ceftazidme(CAZ) 30µg, cefotaxime (CTX) 30 µg, ceftriaxone (CRO) 30µg were used. Mueller Hinton agar plates were prepared and inoculated with standardized inoculums of the organism with sterile cotton swab. Disc containing 20µg amoxicillin and 10µg clavulanic acid was placed in the center of the inoculated plate. Third generation cephalosporin disc of ceftazidme, ceftriaxone, and cefotaxime was placed about 20mm distant from amoxicillin-clavulanate disc. The plate was incubated overnight at 37°C.
c. Extension of the inhibition zone of ceftazidime, ceftriaxone and cefotaxime disc on the side exposed to the disc containing amoxicillin and clavulanic acid was considered positive for ESBL.

Operational definition of ESBL producers: Organisms which are resistant to penicillin, first, second and third generation of cephalosporin, aztreonam (but not cephemycins or carbapenem) and inhibited by â-lactamases inhibitors such as clavulanic acid are considered as ESBL producers (CDC, 2010).

According to Clinical and Laboratory Standards Institutes (CLSI) guidelines zone of inhibition for ceftazidime <22mm and cefotaxime < 21mm are presumptively taken to indicate ESBL production. ESBL production was confirmed if there was 5mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone (CLSI, 2015).

Control strain - Standard strain of Klebsiella pneumoniae ATCC 700603 was used as ESBL positive control and Escherichia coli ATCC 25922 was used as ESBL negative control.

Antimicrobial sensitivity test:
Mueller-Hinton agar media were used for antimicrobial susceptibility test by using disc diffusion technique by Kriby Bauer method against different antimicrobial agents. According to CLSI guidelines the following antimicrobial agents were used in the study amoxiclave, ciprofloxacin nalidixic acid, amikacin, gentamicin, ceftriaxone, ceftazidime, cefotaxime, imipemem, nitrofuratoin, cotrimoxazole and doxacyclin. Three to five isolated colonies of the organisms to be tested were picked from the pure culture plates by a sterile wire loop and suspended in 2-3 ml of sterile normal saline in a screw capped test tube. In a good light the turbidity of suspension was matched to 0.5 McFarland standards. The organisms were inoculated on the media with sterile cotton wool swab upon dipped into bacterial suspension. The inoculated plates were left on the flat surface for 10-15 minutes. Then the antibiotic discs were placed on the inoculated plates. The plates were then incubated at 37°C for 24 hours and reading was taken. Zone of inhibition produced by each was considered into susceptibility categories namely Sensitive(S), Intermediate(I), and Resistant(R) (CLSI, 2015).

Results:
Out of 1750 urine samples, 403 (23.02%) were positive by culture. Among the culture positive cases 216(53.59%) were female and 187 (46.41%) were male. Out of 403 positive culture the most common isolated bacteria were Escherichia coli 295(73.20%), followed by Pseudomonas aeruginosa 85(21.09%), K. pneumoniae 10(2.48%), Proteus spp 4(9.99%), Acinetobacter spp 5(1.2%), Coagulase negative Staphylococcus (CONS) 4(9.99%) (Table-I).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esch. Coli</td>
<td>295</td>
<td>73.20</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>85</td>
<td>21.09</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>10</td>
<td>2.48</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>04</td>
<td>0.99</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>05</td>
<td>1.2</td>
</tr>
<tr>
<td>Cogulase negative Staph (CONS)</td>
<td>04</td>
<td>0.99</td>
</tr>
<tr>
<td>Total</td>
<td>403</td>
<td>100</td>
</tr>
</tbody>
</table>

Highest 175 (43.42%) culture positive cases were belonged to the age group between 46-90 years followed by 150 (37.22%) in the age group between 19-40 years. Among the 295 isolated Escherichia coli, 202 (68.45%) were ESBL producer and among the 10 isolated k.pneumoniae, 5(50%) were ESBL producer (Table-II).

None of the antimicrobial agents were shown to be 100% effective in this study. ESBL producing bacteria were comparatively less sensitive to antimicrobial agents than non ESBL producer. Regarding sensitivity pattern of Isolated ESBL producing Esch. coli, 56.95% were sensitive to amikacin, 7.8% to amoxicillin-clavulanic acid, 12.08% to ceftriaxone, 10.11% to ceftazidime, 33.9% to doxyciline, 31.5% to cotrimoxazole, 40.83% to gentamicin, 34.9% to mecillinam, 47.50% to imipenem, 10.8%) to nalidixic-acid, 29.83% to ciprofloxacin, 32.22% to nitrofuratoin and 16.2% to cefotaxime (Table -III)
Table-II

**ESBL and non-ESBL-producing bacteria among the isolated Esch.coli and Klebsiella pneumoniae (n=300)**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>ESBL-producing n(%)</th>
<th>Non-ESBL-producing n(%)</th>
<th>Total n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esch. coli</td>
<td>202(68.47)</td>
<td>88(41.53)</td>
<td>290</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>5 (50.00)</td>
<td>5 (50.00)</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>207(69.00)</td>
<td>93(31.00)</td>
<td>300</td>
</tr>
</tbody>
</table>

Table-III

**Distribution of Antibiotic susceptibility pattern of isolated gram negative bacteria (n=399)**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AK</td>
</tr>
<tr>
<td>Esch. coli (n=297)</td>
<td>56.95</td>
</tr>
<tr>
<td>Non-ESBL(n=88)</td>
<td>76.88</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>48.22</td>
</tr>
<tr>
<td>Proteus spp(n=4)</td>
<td>25.00</td>
</tr>
<tr>
<td>Acinetobacter spp</td>
<td>20.00</td>
</tr>
</tbody>
</table>

Amoxiclave (AMC), Ciprofloxacin (CIP), Nalidixic acid (NA), Amikacin (AK), Gentamicin (CN), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefotaxime (CTX), Imipenem (IPM), Nitrofuratoin (F), Cotrimoxazole (COT) and Doxycilin(DO).

Discussion:

Urinary tract infection is one of the most commonly diagnosed infections and typically most easily treated infections in young and healthy individuals. UTI caused by extended-spectrum beta-lactamase (ESBL)-producing organism have become a growing problem worldwide. Infection with ESBL-producing bacteria raises mortality and it prolongs hospital stay along with an increase treatment cost. ESBL production has been observed mostly in the Enterobacteriaceae mainly in Esch. coli and Klebsiella pneumoniae but all other clinically relevant Enterobacteriaceae species are also common ESBL producer.

In this study 23% urine samples yielded growth, which is similar to data of Pakistan (21.8%). In the present study the most predominant isolates were gram negative bacilli (99.6%) and ESBL producing Esch. coli and K. pneumoniae were 68.47% and 50% respectively. Isolation rate of ESBL producing bacteria was very high in this study and in the line with study done in Saudi Arabia where it was reported that ESBL producing Esch. coli were 57.4% and K. pneumoniae were 71.7%. Similarly in Pakistan and South India it was reported that ESBL producing Esch. coli were 56% and 39.66% respectively. In the present study comparatively low level of sensitivity were observed to all antibiotics in ESBL producing bacteria than the non ESBL producing bacteria and higher numbers of ESBL producing bacteria were multi drug resistant. An ESBL producing organism was considered multidrug resistant if it was additionally resistant to all three classes of the following antimicrobials: fluoroquinolones, trimethoprim-sulfamethoxazole and aminoglycosides. In this study 20.82% ESBL producing Esch. coli were sensitive to ciprofloxacin, 21.5% to cotrimoxazole and 40.83% to gentamicin. These findings were...
similar to the findings of Ranjini et al. (2015) and by Sharma et al. (2012) that maximum numbers of ESBL were multidrug resistant and poor sensitivity to ciprofloxacin (5.63%), cotrimoxazole (11.26%), gentamicin (40.84%) \(^{25}\) and highly resistant to ciprofloxacin (89%), norfloxacin (82%), cotrimoxazole (90%). \(^{25,27}\) Also from Central Saudi Arabia and Bangladesh it was reported that ESBL producing \textit{Esch. coli} \(^{28,6}\) were highly resistant to ciprofloxacin, gentamicin and cotrimoxazole. \(^{28,6}\) Ahmed and Salma in 2002 reported that multidrug resistant ESBL producing bacteria carry genes encoded for ESBL that are linked to other resistance genes. \(^{29}\) In this study, only 7.8% and 10% ESBL producing \textit{Esch. coli} and \textit{K. pneumoniae} were sensitive to amoxicillin-clavulanic acid respectively. Similar findings were noted by Ahmed et al. (2015) that amoxicillin-clavulanic acid were the least potent antibiotic for ESBL producing \textit{Esch. coli} and \textit{K. pneumoniae}. \(^{22}\) The significance of reporting ESBL producing bacteria is that all ESBL producers are resistant to penicillins, cephalosporins and aztreonam. \(^{30,27}\) Comparing with the previous studies present study observed that ESBL producing \textit{Esch. coli} were moderately sensitive to imipenem (47.50%), nitrofurantoin (32.22%) and amikacin (56.95%). Regarding the highest level of resistance to quinolons, cefalosporins, amoxicillin-clavulanic acid and cotrimoxazole, it was advocated that these drugs should not be used in empirical therapy for UTIs \(^{30}\). In future empirical treatment for UTIs should be reformulated, as poor sensitivity to all antibiotics was observed for all isolates in this study. The findings suggest that persistent increasing trend of antibiotic resistance and proportion of ESBL production is rising day by day. This indicates need to focus on regular surveillance and proper antibiotic administration in order to decrease the MDR and ESBL frequency. Also, further molecular studies are recommended to elucidate the basis of this multidrug resistance and ESBL production.

**Conclusion:**

UTIs caused by ESBL is a major problem now in the field of medicine throughout the world. Multi drug resistant bacteria causing UTIs are difficult to treat. As low level sensitivity to most of the antibiotics was observed, so regular culture and sensitivity test should be done to modify the empirical treatment of UTIs.

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**References**


