Prevalence of urinary tract infection by multidrug resistant bacteria with special interest in extended spectrum beta lactamase producer in an urban hospital of Dhaka

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Abstract

Urinary tract infections (UTIs) caused by extended-spectrum beta lactamase (ESBL)-producing bacteria have become a growing problem worldwide. The aim of this study was to investigate the clinical and radiological findings in patients with community-acquired UTIs owing to ESBL-producing bacteria. Database of the patients that had UTI owing to ESBL-producing bacteria were evaluated. Urine samples were collected from UTI-suspected patients attended at Apollo Hospital in Dhaka. A total of 4210 OPD-patients and 2067 IPD-patients database were included in the study. Mid stream urine was collected and processed for culture. A total of 1222 isolates were found in UTI patients attended the hospital from January to June 2012, of which 404 isolates were ESBL positive. Among the ESBL isolates, E. coli (80%) and Klebsiella spp. (12%) were found to be the most prevalent organism in UTI patients. There was a temporal influx of ESBL containing bacteria associated UTI found in February and May in both IPD and OPD patients. Females are more vulnerable to ESBL infection than male. The patients of above 46 years were found to be relatively more vulnerable than the youth age group. Children below 15 years of age also have higher incidence of UTI. UTIs owing to ESBL-producing bacteria begin to replace UTIs owing to non-ESBL-producing bacteria. Thus, our future aim should be to evaluate the predisposing factors behind the fact to prevent infections owing to ESBL-producing bacteria.

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Introduction

The emergence and spread of antibiotic resistance in Enterobacteriaceae is a growing concern in human medicine. Enterobacteriaceae producing extended spectrum β-lactamases (ESBLs) have become efficient at inactivating β-lactam antibiotics especially the newer extended spectrum third generation cephalosporins (eg. ceftazidime, ceftriaxone, cefotaxime) and monobactams (eg. aztreonam). In addition, ESBL producing Enterobacteriaceae are frequently resistant to other groups of commonly used non β-lactam antibiotics such as fluroquinolones. Since its first observation in 1983, more than 170 β-lactamases have been recognized so far and produced by both gram positive and gram negative bacteria. Recently, the prevalence of ESBL procedures has been increasing and infections caused by these bacteria have become an emerging public health concern worldwide.

The etiology of most bacterial UTI is by the pathogens such as Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter faecalis, Pseudomonas aeruginosa, species of Enterobacter, Staphylococcus, Serratia and Acinetobacter. Among bacterial UTIs, E. coli is the most prevalent cause (Manikandan et al., 2011) accounting for greater than 80% of the infections. UTIs are just a few of the diseases that have become hard to treat with antibiotics (Foxman, 2010). The situation is of particular concern because self-medication and the use of antibiotics without medical guidance (Awad et al., 2007), which ultimately give rise to multidrug resistance. In Bangladesh, the prevalence of ESBLs has been reported since 1990s. A study carried out in BSMMU by Rahman (2007) demonstrated ESBL in 30% strains of the Gram negative bacteria. It is important to note that percentage of ESBL producing bacteria has been increased to 63% in last few years among Gram negative bacteria in Bangladesh (Ali Shah et al, 2004). In this study, we have aimed to demonstrate the prevalence of ESBL producing infectious agents commonly found in UTI patients in Bangladesh.

Materials and methods

Sample collection

A total 6277 number of urine samples were collected form UTIs suspected patients attended a private hospital in Dhaka, Bangladesh. Number of OPD (out patients department) samples were 4210 and IPD (in patients department) was 2067. Samples from OPD were collected by sample collection department and sample of IPD was collected by respective nursing station including several wards surgical intensive care unit, medical intensive care unit, neuro intensive care unit, neonatal intensive care unit, emergency department etc. Mid stream urine (MSU) samples were collected to sterile containers. Data about the patients were collected and recorded in a predesigned data sheet. The results of the experiments were recorded statistically.

Sample processing

Samples were transported to the microbiology laboratory of the hospital immediately after collection. Microbiological examinations were carried out as promptly as possible after collection to avoid unpredictable changes. Where there was a delay in examination of the samples, these were stored at a temperature of 4°C in a refrigerator.

Isolation of bacterial agent

All samples were inoculated in HiCrome UTI agar, 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 cfu was calculated.

All the isolates were preliminarily screened and identified by their colony morphology, pigment production and confirmed by motility and other relevant biochemical tests and Gram staining as per standard methods (Collee et al., 1996; Cheesbrough, 2000). Overnight cultures on Nutrient Agar were primarily screened by oxidase test. Oxidase- negative isolates were inoculated on Kligler Iron agar (KIA), Motility Indole Urease (MIU) agar, Simon’s Citrate media and incubated at 37°C for 24 h (if negative
results were observed, the incubation time was extended to 48 h) and observed for presumptive identification.

**Phenotypic screening of ESBL production**

All the Gram negative isolates were tested for detection of ESBL by Double disc synergy test as described by Jarlier *et al.* (1988). Antimicrobial discs (OXOID Antimicrobial discs) used were Ceftazidime (CAZ) 30 µg, Cefotaxime (CTX) 30 µg, Ceftriaxone (CRO) 30 µg and Aztreonam (ATM) 30 µg. The test is based on synergy between a cephalosporin and clavulanic acid. Mueller Hinton agar plates were prepared and inoculated with standardized inoculum of the test organism with sterile cotton swab.

Augmentin (20 µg amoxicillin and 10 µg clavulanic acid) disc was placed in the center of the inoculated plate. Third generation cephalosporin disc of ceftazidime, ceftriaxone, cefotaxime and aztreonem was placed about 20 mm distance from augmentin disc. The plate was incubated overnight at 37º C. Extension of the edge of the inhibition zone of ceftazidime, ceftriaxone, cefotaxime and aztreonem disc on the side exposed to the disc containing amoxicillin and clavulanic acid is positive for ESBL.

**Antimicrobial sensitivity test**

Mueller–Hinton agar media were used for antimicrobial susceptibility testing for the isolates. All the isolates were tested for antimicrobial sensitivity using disc diffusion technique by “Kirby- Bauer method” (Bauer *et al.*, 1966) against different antimicrobial agents. Antimicrobial agents used in the study were: Amoxiclave (AMC), Ciprofloxacin (CIP), Nitrofurantoin (F), Nalidixic acid (NA), Ceftriaxone (CRO), Gentamicin (CN), Ceftazidime (CAZ), Cefotaxime (CTX), Amikacin (AK), Aztreonam (ATM), Imipenem (IPM), Meropenem (MEM), Piperacillin (TZP), Ampicillin (AMP), Cefixime (CFM), and Cefepime (FEP).

Three to five isolated colonies of the organisms to be tested were picked from the pure culture plates by a sterile inoculating wire loop and suspended in 2-3 ml of sterile peptone water in a screw capped test tube. The turbidity was set to 0.5 McFarkland standard by spectrophotometer. The organisms were inoculated on the media with sterile cotton wool swab upon dipped into the bacterial suspension. The inoculated plate was left on flat level surface for 10 to 15 minutes with the lid closed for the absorption of excess moisture. Then with a disc dispenser; discs were placed on the surface of the inoculated plate with gentle pressure to ensure complete contact with the agar surface. The discs were placed evenly in such a way so that they were 15 mm away from the edge of the petridish and the distance between the centres of the two discs were approximately 24 mm. The plates were then incubated at 37ºC for 16 to 18 hours then reading was taken according to Cheesbrough (2000).

Zone of inhibition produced by each was considered into three susceptibility categories namely Sensitive (S), Intermediate (I), and Resistant (R) as described in Table (NCCLS, 1990).

**Result**

A total of 6277 urine samples were collected from microbiology laboratory of the hospital under study. Among those, 4210 (67.07%) were from OPD patients and 2067 (32.92%) from IPD patients. The urine samples were streaked on HiCrome UTI agar, which formed characteristic species-specific appearance upon 24 h incubation. Based on the colony characteristics of the isolates (Table 1), those were preliminary screened and then identified by biochemical test. The biochemical tests and respective profile of each bacterial isolates are given in Table 2. According to the cultural and biochemical characterization, the isolates were identified, which constituted mostly of Gram negative Enterobacteriaceae family. *Escherichia coli* is the most predominant isolates among the UTI cases, which followed by *Klebsiella* spp. A number of other bacterial species were also isolated. The overall isolated agents have been shown in Fig. 1. The ratio of ESBL positive isolates among the total isolates are also shown in Fig. 2. *E. coli* have highest proportion of ESBL producers compared to its total isolates. The ESBL cases are more prevalent in female (231) than male (173) patients.
The etiological agents sometimes coexisted in a particular patient. These mixed infection have high potential to acquire genetic heterogeneity by inter species horizontal gene transfer. In most of the cases, the clinical features were imposed by any one of the members. In this study, a significant number of cases were found to have multiple etiological agents co-infected a patient.

**Table 1.** Colony morphological characteristics of different UTI isolates on HiCrome agar media.

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<tbody>
<tr>
<td>Size</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Large</td>
<td>Moderate</td>
<td>Large</td>
<td>Moderate</td>
</tr>
<tr>
<td>Shape</td>
<td>Circular</td>
<td>Circular</td>
<td>Irregular</td>
<td>Circular</td>
<td>Irregular</td>
<td>Circular</td>
</tr>
<tr>
<td>Margin</td>
<td>Entire</td>
<td>Entire</td>
<td>Undulate</td>
<td>Entire</td>
<td>Serrate</td>
<td>Undulate</td>
</tr>
<tr>
<td>Elevation</td>
<td>Convex</td>
<td>Convex</td>
<td>Flat</td>
<td>Convex</td>
<td>Flat</td>
<td>Raised</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Pink</td>
<td>Blue</td>
<td>Pale green or yellow</td>
<td>Cream colour</td>
<td>Orange to brown to Blue to blue green</td>
<td></td>
</tr>
</tbody>
</table>

Among the ESBL infected patients an age distribution chart also been built and found to have significant pattern (Fig. 3). The patients aged below 15 or above 45 are more vulnerable to ESBL infection. The overall results have been summarized in Fig. 4. The limitation of the result is the deduction of mixed culture as etiology.

**Table 2.** Biochemical Characteristics of different UTI isolates.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Oxidase</th>
<th>Citrate</th>
<th>MIU Medium</th>
<th>KIA Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mortality</td>
<td>Indole</td>
<td>Urea</td>
<td>Slope</td>
</tr>
<tr>
<td>E. coli</td>
<td>- +</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>- +</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>+ +</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>- +</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>- d</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>- +</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>- +</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>- +</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Providencia spp.</td>
<td>- +</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

R= Red pink (alkaline reaction), Y= Yellow (acid reaction).

**Discussion**

Extended-spectrum beta-lactamases (ESBLs) constitute a growing class of plasmid mediated beta-lactamases which confer resistance to broad spectrum beta-lactam antibiotics. They are commonly expressed by *Enterobacteriaceae* but the species of organisms producing these enzymes are increasing and this is a cause for great concern. The prevalence of ESBL producing organisms is increasing worldwide and several outbreaks have been reported. Serious infections with these organisms are associated with high mortality rate as therapeutic options are limited. The emergences of ESBLs create a real challenge for both clinical microbiology laboratories and clinicians because of their dynamic evolution and epidemiology, wide substrate specificity with it therapeutic implications, their significant diagnostic challenges and their prevention and infection control issues (Al-jasser, 2006).
In addition to increasing resistance to cephalosporins, resistance to other commonly used antibiotics such as fluoroquinolones is increasing. Clinical microbiology laboratories play a vital role in the detection and control of ESBL-producing Gram negative bacilli. However, many laboratories are not fully aware of the importance of ESBL producing organisms and how best to detect them. In Europe it is estimated that 35% of ESBL producing organisms are incorrectly reported as susceptible to cephalosporins. ESBLs demonstrate low level resistance in vitro. Routine disc susceptibility tests performed by laboratories may therefore fail to detect ESBL positive strains because these strains can be interpreted as sensitive to the extended spectrum cephalosporins (Svard, 2007).

The traditional susceptibility methods lack sensitivity and/or specificity and this issue has prompted the search for an accurate test to detect the presence of ESBLs (Datta et al., 2004). Significant members of ESBL are also missed by Double disc synergy test. In this study a total of 1264 Gram negative isolates were studied, among them 32% of the isolates were found ESBL producer. A study carried out in BSMMU by Rahman (2007) who found, ESBL in 30.90% strains of the Gram negative bacteria. It is important to note that percentage of ESBL producing bacteria has been increased from 30% to 32% in last few years among Gram negative bacteria in Bangladesh. The reason of this higher percentage of ESBL in present study may be due to random use of 3rd generation cephalosporin unnecessarily. Extensive use of 3rd generation cephalosporin has contributed to the evolution of ESBL (Jacoby and Medeiros, 1991). In India, research also showed different prevalence pattern among ESBL in Enterobacteriaceae isolates ranging from 12.6 (Datta et al., 2004) to 68% (Mathur et al., 2002). Research explain this low prevalence due to their hospital infection control cell, which advise periodic antibiotic rotation every 6 months.

Among the 768 isolates of E. coli, 322 (41.92%) were ESBL producer, among the 254 Klebsiella spp, 50 (19.68%) were ESBL producer and among the 66 Pseudomonas species, 17(25.75%) were ESBL producer. Among 50 Proteus species, 51 Acinetobacter species, 41 Enterobacter species, and 34 others species, ESBL producer were 7(14.0%), 5(9.80%), 1(2.43%), and 2(3.58%) respectively. Rahman (2007) in BSMMU showed ESBL producer in E. coli in 35.38% strain, Klebsiella spp in 43.47%, Enterobacter spp in 31.25%, Proteus spp 27.11%, Acinetobacter spp 26.32% and Pseudomonas spp in 17.07% strains. Increase number of ESBLs producer is probably due to previously treated with beta-lactam.
drugs, bed retention, immune suppression, association with other diseases, temporary or permanent urinary catheter (Chlebicki and Oh, 2004).

In the present study drug resistance of all ESBL producers to most of the antibiotics (Amoxycillin, ciprofloxacin, ceftriaxone, ceftazidime, nalidixic acid) were found higher. This implies that ESBL producing organisms are multidrug resistant. Genes that code for ESBL are linked to other resistance genes (Ahmed, 2002). In this study, ESBL producing isolates were 100% sensitive to imipenem. According to CDC (1999), ESBLs are defined as enzymes which hydrolyze 3rd generation cephalosporin but sensitive to cephaparin and imipenem.

Treatment of infection caused by ESBL producing organism can be done by carbapenem e.g. imipenem, meropenem, etrapenem. ESBL producing organisms are sensitive to 2nd generation cephalosporins in vitro but not recommended for treatment according to NCCLS as may not be effective in vivo. NCCLS recommends when ESBL production is confirmed, results be reported as resistance to all penicillins, cephalosporins excluding cephaparin and aztreonem (CDC, 1999).

Infections caused by ESBL-producers are associated with high rates of morbidity and mortality, prolonged hospital stay heavy expenses. This is due to the increased rate of treatment failure of these infections. Infections caused by ESBL can not be treated with cephalosporins but since ESBLs are difficult to detect and many clinical laboratories are not aware to them, cephalosporins are often used anyway. Several studies have reported that carbapenems are the best alternative for treating serious infections caused by ESBL-positive isolates (Colodner, 2005). These antibiotics are high stable to β-lactamases, however, they are very expensive and the uses should be limited. It has been reported that ESBL-positive isolates can become resistant to carbapenems due to loss of porin proteins in the outer membrane (Sturenburg and Mack, 2003). Plasmids responsible for ESBL production frequently carry genes encoding resistance to other groups of commonly used antibiotics and therefore, the quinolones and aminoglycosides may also be unsuitable for use.

Several studies have shown that by limiting the use of these agents alone, or in combination with infection control measures, the frequency of ESBLs isolates can be reduced substantially. Educational programs for medical staff to increase awareness should also be developed. Furthermore, the implementation of monitoring programs is an important part of the prevention strategy against the development of such resistance.

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