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Original Article

Study of extended-spectrum β -lactamase-producing bacteria from urinary tract infections in Bangladesh

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ABSTRACT

Objective: Production of β -lactamase by pathogens causing urinary tract infection (UTI) has been demonstrated to increase resistance to antimicrobial agents. The current study showed the prevalence of uropathogens and their antimicrobial susceptibilities, based on extended-spectrum β -lactamase (ESBL) production, in Dhaka.

Materials and Methods: The prevalence of uropathogens and their antimicrobial resistance patterns were identified in 200 isolates from patients with UTI. Double-disc diffusion and E tests were performed to determine the presence of ESBL-producing strains.

Results: The most common pathogen was *Escherichia coli* (57%), followed by *Enterococcus* spp. (10.5%), *Klebsiella* spp. (11%), *Staphylococcus* spp. (4%), *Pseudomonas* spp. (10%), *Acinetobacter* spp. (5%), and *Enterobacter* spp. (9%). ESBL production occurred more frequently in *Klebsiella* spp. (72.7%) than *E. coli* (53.5%), and *Enterobacter* spp. (66.7%).

Conclusion: The current investigation found *E. coli* to be the most common uropathogen. Overall, the higher frequency of antimicrobial resistance as well ESBL production by the most common pathogens found in this study may demonstrate a public health threat and therefore, the community should be made aware of this problem.

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1. Introduction

Urinary tract infection (UTI) is known to affect approximately 150 million people each year. It is the second most common infection, and is responsible for approximately seven million doctor visits per year [1,2]. The frequency of infection varies especially with age and sex [3,4]. Among uropathogens, *Escherichia coli* is responsible for 80% of community-acquired UTI and 40% of healthcare-associated UTI. Other uropathogens include *Candida* spp., *Proteus mirabilis*, *Staphylococcus* and *Klebsiella* spp. [4–6]. Interestingly, uropathogens can change their physiologic features to induce antimicrobial resistance [7].

The increase in antibiotic resistance among uropathogens is a global problem [8].

The production of extended-spectrum β -lactamase (ESBL) enzymes, which are capable of hydrolyzing oxyimino β -lactam

compounds, is one of the factors contributing to high resistance against β -lactam antibiotics [9]. ESBLs demonstrate substrate specificity for most penicillins and first-, second-, and third-generation cephalosporins and aztreonam (not including cephamycins and carbapenems) [9,10]. ESBLs can hydrolyze these antibiotic agents but they can also be inhibited by β -lactamase inhibitors (clavulanic acid) [10]. The most common plasmid-mediated β -lactamases responsible for resistance to ampicillin are TEM-1, which can be found in Gram-negative bacilli such as *E. coli*, and *Klebsiella pneumoniae*-producing SHV-1 β -lactamase [10]. It is important to know the prevalence of ESBL in uropathogens for appropriate empirical treatment of UTI [9].

In developing countries, increased antibiotic resistance can be attributed to antibiotic abuse [11]. The current study was designed to show the distribution of uropathogens and the prevalence of ESBL-producing uropathogens.

2. Materials and methods

The study was carried out in the Department of Microbiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh from July 2010 to June 2011. Two hundred clinical specimens

Conflict of interest: none.

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(including urine, pus, sputum, and swabs) were collected from in-patients ($n = 121$) and outpatients ($n = 79$) at the hospital.

2.1. Isolation and identification of uropathogens

Uropathogens were identified through culture, microscopy, and biochemical tests. HighChrome chromogenic agar media, MacConkey agar, blood agar, chocolate agar, and Oxoid clarity agar media (Oxoid Ltd., Hampshire, UK) were inoculated with 100 μ L of urine from samples, and incubated at 37 °C for 24 hours. Bacterial identification was done by phenotypic examination of the culture, looking for typical characteristics, and by Gram staining, and a series of standard biochemical tests were also performed to identify the bacteria of interest [12,13].

2.2. Antibiotic susceptibility testing

The agar disc diffusion assay was used to determine the antimicrobial susceptibilities of uropathogens. The discs used in this study included amikacin 20 μ g, imipenem 10 μ g, netilmicin sulfate 20 μ g, ciprofloxacin 5 μ g, nitrofurantoin 30 μ g, cloxacillin 5 μ g, amoxicillin 10 μ g, cephadrine 20 μ g, cotrimoxazole 25 μ g, nalidixic acid 30 μ g, mecillinam 30 μ g, ceftriaxone 30 μ g, cefotaxime 30 μ g, gentamicin 30 μ g, ceftazidime 30 μ g, cefuroxime 30 μ g, and aztreonam 10 μ g. The protocol for antibiotic susceptibility testing has been described previously [14,15]. The diameters of the zones of inhibition for individual antimicrobial agents were translated into susceptible, intermediate, and resistant categories according to National Committee for Clinical Laboratory Standards criteria [16].

2.3. Detection of ESBL by the double disc diffusion method

Positive (*K. pneumoniae* ATCC700603) and negative (*E. coli* ATCC 25922) control strains were inoculated onto Muller-Hinton agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India). An amoxicillin clavulanic acid (20 μ g + 10 μ g) disc was placed on the center of the plate. Ceftazidime (30 μ g), ceftriaxone (30 μ g), cefotaxime (30 μ g), and aztreonam (30 μ g) discs were placed peripherally away from the amoxicillin clavulanic acid disc [17]. After 24 hours of incubation at 37 °C, band formation between the amoxicillin clavulanic acid disc and any other disc was considered ESBL positive [17]. The ESBL positive strains were further subjected to phenotypic confirmatory tests using sensitivity discs, which contained third-generation cephalosporins both with and without clavulanic acid. The discs used included cefotaxime (30 μ g), cefotaxime + clavulanic acid (30 μ g + 10 μ g), ceftazidime (30 μ g), ceftazidime (30 μ g + 10 μ g), aztreonam (10 μ g), and aztreonam + clavulanic acid (30 μ g + 10 μ g). The differences in the zone of inhibition caused by the cephalosporins alone and when combined with clavulanic acid were recorded and if the difference was 5 mm or more, the strains were confirmed as ESBL-producing strains [17].

Table 1
Patterns of pathogenic isolates from UTI sample.

| Pathogenic bacteria | Total no. of organisms | Male | Female | ESBL positive | ESBL negative |
|----------------------------|------------------------|----------|----------|---------------|---------------|
| <i>Escherichia coli</i> | 114 (57%) | 56 (49%) | 58 (51%) | 61 (53.5%) | 53 (46.5%) |
| <i>Enterococcus</i> spp. | 21 (10.5%) | 5 (24%) | 16 (76%) | 1 (4.8%) | 20 (95.2%) |
| <i>Pseudomonas</i> spp. | 20 (10%) | 12 (60%) | 8 (40%) | 2 (20%) | 18 (80%) |
| <i>Enterobacter</i> spp. | 18 (9%) | 13 (72%) | 5 (28%) | 12 (66.7%) | 6 (33.3%) |
| <i>Klebsiella</i> spp. | 11 (5.5%) | 6 (55%) | 5 (45%) | 8 (72.7%) | 3 (27.3%) |
| <i>Acinetobacter</i> spp. | 10 (5%) | 6 (60%) | 4 (40%) | 2 (20%) | 8 (80%) |
| <i>Staphylococcus</i> spp. | 4 (2%) | 2 (50%) | 2 (50%) | 0 (0%) | 4 (100%) |
| <i>Citrobacter</i> spp. | 1 (0.5%) | 1 (100%) | 0 | 0 (0%) | 1 (100%) |
| <i>Proteus</i> spp. | 1 (0.5%) | 1 (100%) | 0 | 0 (0%) | 1 (100%) |

The experiments were in triplicates and the results were reproducible.

Table 2
Age distribution of ESBL-producing organisms (%).

| Age (y) | Total UTI sample | ESBL positive | ESBL negative |
|---------------|------------------|---------------|---------------|
| 0–10 | 20 | 50 | 50 |
| 11–20 | 18 | 44 | 56 |
| 21–30 | 44 | 30 | 70 |
| 31–40 | 30 | 26 | 74 |
| 41–50 | 37 | 51 | 49 |
| 51–60 | 25 | 56 | 44 |
| 61–70 | 11 | 55 | 45 |
| Older than 70 | 15 | 27 | 73 |

The ESBL screening E test strip (AB Biodisk, Solna, Sweden) is designed to detect the reduction in the ceftazidime minimum inhibitory concentration (MIC) in the presence of clavulanic acid with a fixed concentration of 2 mg/mL. A gradient of ceftazidime was created on one end and a gradient of combined ceftazidime and clavulanic acid was created on the other end. ESBL-producing strains were confirmed if the MIC in the presence of clavulanic acid was reduced by more than four dilution steps in comparison with ceftazidime alone [18].

3. Results

Among 200 samples, *E. coli* was the predominant pathogenic isolate (114 of 200), followed by *Enterococcus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Klebsiella* spp., *Acinetobacter* spp., *Staphylococcus* spp., *Citrobacter* spp., and *Proteus* spp. (Table 1). *E. coli* was more likely to cause UTIs in females whereas *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacter* spp. were more likely in males. The prevalence of UTIs caused by *Klebsiella* and *Staphylococcus* were similar in males and females. Both *Citrobacter* and *Proteus* were seen only in one male patient each. This study found most UTI cases occurred in those 21–30 years old (44%), followed by those 41–50 years old (37%). However, UTI patients 41–50 years old (19%) were more likely to have ESBL-producing uropathogens. Those between the ages of 21–30 (31%) and 31–40 (22%) years were more likely to have ESBL negative strains.

3.1. Prevalence of ESBL-producing organisms

Identified isolates were further tested for the production of ESBL (Table 1). Sixty-one of the total 114 *E. coli* identified were positive for ESBL. Twenty of the 21 *Enterococcus* isolates were negative for ESBL; 18 of 20 *Pseudomonas* spp. samples were negative and 12 of the 18 samples of *Enterobacter* spp. were positive. Eight of the 11 *Klebsiella* spp. samples were ESBL positive and eight of 10 samples of *Acinetobacter* spp. were negative. All four *Staphylococcus* spp., one *Citrobacter* spp., and one *Proteus* spp. samples were found to be ESBL negative. The prevalence of ESBL was higher in those 41–60 years old and was lowest in those older than 70 years (Table 2).

In this study, ESBL positive isolates were mainly identified in *E. coli* (53.5%), *Klebsiella* spp. (72.7%), and other members of the Enterobacteriaceae family such as *Acinetobacter* spp. and *Enterobacter* spp.

3.2. Antibiogram profile of the uropathogens

Among all the isolates tested, *E. coli* and *Staphylococcus* spp. showed the lowest levels of resistance against most of the antibiotic agents. *E. coli* showed 1.1% resistance against imipenem, 2% resistance against nitrofurantoin, 7.1% resistance against amikacin, and 7.7% resistance against amoxicillin (Table 3). Of four samples of *Staphylococcus* spp. tested, no resistance was observed against cloxacillin, cefotaxime, cefuroxime, amikacin, and imipenem. *E. coli* and *Staphylococcus* spp. showed the highest levels of resistance against amoxicillin (92.3% and 100%, respectively). *Enterobacter* spp. showed 100% sensitivity only to imipenem. It also showed high levels of sensitivity to amikacin and netilmicin sulfate (92.9% and 91.7%, respectively). *Enterobacter* spp. showed 100% resistance against amoxicillin, ceftazidime, and aztreonam. *Klebsiella* spp. showed the lowest level of resistance against imipenem and the highest level of resistance against amoxicillin, aztreonam, cefotaxime, ceftazidime, and cefuroxime. Only one sample of *Enterococcus* spp. was tested for susceptibility to cloxacillin and was found to be resistant. However, the pathogen was highly sensitive to imipenem (88.2%) and nitrofurantoin (80%), whereas it was 100% resistant against nalidixic acid, ceftazidime, cefuroxime, and aztreonam.

High levels of resistance against most of the antibiotic agents was observed in cases of nonfermentative isolates (Table 4). *Pseudomonas* spp. showed 100% resistance against mecillinam and cefuroxime. Resistance against all other drugs was above 50%. *Acinetobacter* showed the highest levels of resistance against amoxicillin, cephadrine, cotrimoxazole, and aztreonam. *Acinetobacter* spp. and *Pseudomonas* spp. showed low levels of resistance to imipenem (10% and 33.3%, respectively).

4. Discussion

The current study was conducted to identify various microorganisms that may be involved in the development of UTI and determine their antibiotic resistance patterns as well as their ESBL

Table 4

Antibiotic susceptibility pattern of nonfermentative isolates (%).

| Organisms Antibiotics | <i>Pseudomonas</i> spp. | | <i>Acinetobacter</i> spp. | |
|--------------------------|-------------------------|------|---------------------------|------|
| | R | S | R | S |
| AMO (10 µg) | ND | ND | 100 | 0 |
| CLO (5 µg) | ND | ND | ND | ND |
| CEPH (20 µg) | ND | ND | 100 | 0 |
| CTX (25 µg) | ND | ND | 100 | 0 |
| CIP (5 µg) | 65 | 35 | 90 | 10 |
| NIT (30 µg) | ND | ND | ND | ND |
| NLA (30 µg) | ND | ND | ND | ND |
| MCL (30 µg) | 10 | 90 | ND | ND |
| CEF (30 µg) | 81 | 19 | 90 | 10 |
| GEN (30 µg) | 72.7 | 27.3 | 90 | 10 |
| CPX (30 µg) | 80 | 20 | 80 | 20 |
| CFZ (30 µg) | 80 | 20 | 80 | 20 |
| CFX (30 µg) | 100 | 0 | 87.5 | 12.5 |
| AMI (20 µg) | 50 | 50 | 33.3 | 66.7 |
| AZT (10 µg) | 95 | 5 | 100 | 0 |
| IMI (10 µg) | 33.3 | 66.7 | 10 | 90 |
| NTS (20 µg) | 60 | 40 | 33.3 | 66.7 |

The experiments were in triplicate and the results were reproducible. AMI = amikacin; AMO = amoxicillin; AZT = aztreonam; CEF = ceftriaxone; CEPH = cephradine; CFX = cefuroxime; CFZ = ceftazidime; CIP = ciprofloxacin; CLO = cloxacillin; CPX = cefotaxime; CTX = cotrimoxazole; GEN = gentamicin; IMI = imipenem; MCL = mecillinam; ND = not defined; NIT = nitrofurantoin; NLA = nalidixic acid; NTS = netilmicin sulfate; R = resistant; S = sensitive.

activity. The prevalence of uropathogens found in this study was in agreement with similar studies conducted in India and Sudan [1,19]. Those studies and another study in Pakistan indicated that the most common cause of UTI in Asian countries is *E. coli* [20]. Previous studies and the current study identified *Pseudomonas* spp., *Klebsiella* spp. and *Enterobacter* spp. as other common causes of UTI [21,22]. Studies in Jordan found Enterobacteriaceae was the most common cause of UTI [23]. Previous studies conducted in Dhaka were also in agreement with the current results, confirming *E. coli* as the most common uropathogen in Bangladesh [24,25]. Studies in Nigeria also claimed that *E. coli* and *Pseudomonas* spp. are the most common uropathogens [26].

ESBL-producing organisms are known to exhibit important therapeutic implications as they show resistance against third-generation cephalosporins, broad-spectrum ampicillin, and monobactams. In our study, *E. coli* showed higher resistance against

Table 3

Antibiotic susceptibility pattern of the pathogenic isolates (%).

| Pathogens | Gram negative | | | | | | Gram positive | | | |
|--------------|--------------------------|------|-----------------------------------|------|---------------------------------|------|-----------------------------------|------|------------------------------------|------|
| | <i>E. coli</i> (n = 114) | | <i>Enterobacter</i> spp. (n = 18) | | <i>Klebsiella</i> spp. (n = 11) | | <i>Enterococcus</i> spp. (n = 21) | | <i>Staphylococcus</i> spp. (n = 4) | |
| Antibiotics | R | S | R | S | R | S | R | S | R | S |
| AMO (10 µg) | 7.7 | 92.3 | 100 | 0 | 100 | 0 | 31.6 | 68.4 | 100 | 0 |
| CLO (5 µg) | ND | ND | ND | ND | ND | ND | 0 | 100 | 0 | 100 |
| CEPH (20 µg) | 55.6 | 44.4 | 80 | 20 | 62.5 | 37.5 | 31.6 | 68.4 | 50 | 50 |
| CTX (25 µg) | 48.6 | 51.4 | 36.4 | 63.6 | 33.3 | 66.7 | 75 | 25 | 33.3 | 66.7 |
| CIP (5 µg) | 65.2 | 34.8 | 50 | 50 | 60 | 40 | 65 | 35 | 66.7 | 33.3 |
| NIT (30 µg) | 2 | 98 | 12.5 | 87.5 | 50 | 50 | 20 | 80 | ND | ND |
| NLA (30 µg) | 85.9 | 14.1 | 60 | 40 | 60 | 40 | 100 | 0 | ND | ND |
| MCL (30 µg) | 24.7 | 75.3 | 44.4 | 55.6 | 50 | 50 | 95 | 5 | ND | ND |
| CEF (30 µg) | 41.9 | 58.1 | 55.6 | 44.4 | 80 | 20 | 85 | 15 | 25 | 75 |
| GEN (30 µg) | 25.9 | 74.2 | 41.7 | 58.3 | 66.7 | 33.3 | 57.1 | 42.9 | 25 | 75 |
| CPX (30 µg) | ND | ND | 85.7 | 14.3 | 100 | 0 | 42.9 | 57.1 | 0 | 100 |
| CFZ (30 µg) | ND | ND | 100 | 0 | 100 | 0 | 100 | 0 | ND | ND |
| CFX (30 µg) | ND | ND | 83.3 | 16.7 | 100 | 0 | 100 | 0 | 0 | 100 |
| AMI (20 µg) | 7.1 | 92.9 | 7.1 | 92.9 | 14.3 | 85.7 | 68 | 32 | 0 | 100 |
| AZT (10 µg) | ND | ND | 100 | 0 | 100 | 0 | 100 | 0 | ND | ND |
| IMI (10 µg) | 1.1 | 98.9 | 0 | 100 | 10 | 90 | 11.8 | 88.2 | 0 | 100 |
| NTS (20 µg) | ND | ND | 7.7 | 92.3 | 20 | 80 | 46.3 | 53.7 | 100 | 0 |

The experiments were in triplicate and the results were reproducible. AMI = amikacin; AMO = amoxicillin; AZT = aztreonam; CEF = ceftriaxone; CEPH = cephradine; CFX = cefuroxime; CFZ = ceftazidime; CIP = ciprofloxacin; CLO = cloxacillin; CPX = cefotaxime; CTX = cotrimoxazole; GEN = gentamicin; IMI = imipenem; MCL = mecillinam; ND = not defined; NIT = nitrofurantoin; NLA = nalidixic acid; NTS = netilmicin sulfate; R = resistant; S = sensitive.

ciprofloxacin, nitrofurantoin, nalidixic acid, mecillinam, and gentamicin than isolates previously tested in Europe [21], thus revealing a trend of increasing antibiotic resistance of UTI-causing pathogens in our country, as found previously [27]. Compared with previous studies, the current investigation found higher resistance rates for Gram-negative pathogens against nalidixic acid, cefuroxime, ceftazidime, and amoxicillin [22]. This increasing drug resistance demands coordinated monitoring of drug activity and usage.

Antibiotic resistance in nonfermentative isolates was determined to be due to the rare but progressively rising rates of resistance [23]. Moreover, nonfermentative isolates are commonly found in soil and water and are able to infect immunocompromised individuals [23]. In this study, nonfermentative isolates were found to be highly resistant against cefotaxime, ceftazidime, cefuroxime, and aztreonam. An important point in our study was that in a clinical setting, imipenem would be the drug of choice because it showed high levels of sensitivity (92.6%). Consequently, drugs to be avoided include cefotaxime, ceftazidime, and cefuroxime. This study was in agreement with Health Protection Agency guidelines, which suggest that nitrofurantoin is one of the first-line drugs for UTI [28]. Similar studies in London have also identified nitrofurantoin as the most effective treatment choice [29].

Bangladesh has a large amount of antibiotic abuse due to the easy availability of antibiotic agents without physician prescriptions. Notably, similar studies in developed countries including the United States and European countries have revealed lower levels of resistance against most commonly used antibiotic agents, because cases of abuse of antibiotic agents are unusual there [29,30]. Developing countries including Bangladesh, Pakistan, and India have problems with abuse of antibiotic agents [31]. Studies of antibiotic resistance patterns in India, Pakistan, and some African countries have revealed resistance rates similar to those observed in our investigation [20,31,32].

Because of abuse of antibiotic agents, antibiotic resistance starts earlier in Bangladesh compared with developed countries [25]. This clinical complication not only remains a challenge for UTI eradication but also for public health, and threatens the lives of individuals. The high resistance rate of pathogens to commonly used antibiotic agents as found from our study samples from UTI patients puts patients at high risk. Hence, routine screening for antibiotic susceptibility is recommended.

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Appendix A. Supplementary material

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.tcmj.2013.01.008>.

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