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Original Article

Prevalence and antibiogram of ESBL producing gram negative bacilli isolated from urine in Dhaka Medical College Hospital, Bangladesh

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ABSTRACT

Extended spectrum ß-lactamase (ESBL) producing strains are resistant to a wide variety of common antimicrobials and become a major clinical concern worldwide that has complicated treatment strategies. The current study has been carried out to detect ESBL producing gram negative bacilli with their antimicrobial susceptibility pattern from urine collected over a period of 12 months from July 2011 to June 2012 from Dhaka Medical College Hospital. Samples were cultured in blood agar and MacConkey's agar media and organisms were identified by different biochemical tests such as oxidase test, reaction in MIU and simmon's citrate media and different sugar fermentation tests. ESBL producers were detected by double-disk synergy test (DDST). From total of 300 urine samples, 157 (52.33%) gram negative bacilli causing UTI were isolated and most of them were *Escherichia coli* (71.34%) followed by *Enterobacter aerogenes* (13.38%). Among the isolates, 45 (28.66%) ESBL producers were detected. The highest of ESBL producer was observed in Escherichia coli (32.14%) followed by *Klebsiella pneumoniae* (28.57%), *Enterobacter aerogenes* (23.80%) and *Acinetobacter spp* (20%). ESBL producers were significantly more resistant to ciprofloxacin, gentamycin, doxycycline, amoxiclav and co-trimoxazole than non-ESBL producers. All the ESBL producing strains were sensitive to imipenem. The result of this study provides insight into the high proportion of highly resistant ESBL producing organisms and more effective strategies are needed to control the spread of these resistant organisms.

Key words: Antimicrobial resistance, Bangladesh, ESBL, Gram negative bacilli.

INTRODUCTION

Extended spectrum ß-lactamase (ESBL) isolates were first detected in Western Europe in the mid-1980s and since then, their incidence has been increasing[1]. In recent years the emergence of ESBLs in gram negative bacteria has increased, which has produced a global concern regarding treatment of bacterial infections[2,3].

In several surveillance studies showed a relatively high prevalence rate of ESBL producers in the Asia-pacific area, though the prevalence varies with geographical areas and time[4,5]. Previous studies in Bangladesh reported the prevalence of ESBL producers ranging from 23.3% to 80%[6-8]. ESBL enzymes are plasmid-mediated and capable of inactivating the effects of penicillin, first-, second-, third-generation cephalosporins and monobactams but do not affect cephamycin or carbapenem[9]. At present, more than 300 different ESBLs have been identified in gram-negative bacilli and blaCTX-M is considered as the most frequent type of ESBLs worldwide[10].

The present study was undertaken to determine the prevalence of ESBL producers from urine samples in Dhaka Medical College hospital and also the antimicrobial resistance patterns among ESBL and non-ESBL producers.

MATERIALS AND METHODS

Study design and population: It was a cross-sectional study. Urine samples were collected from the outdoor and indoor patients from Dhaka Medical College Hospital. Clean catch mid stream urine samples were collected in sterile containers.

Bacterial isolates: A total of 300 urine samples were collected from suspected patients of UTI during July 1, 2011 to June 30, 2012 in the Department of Microbiology, Dhaka Medical College, Bangladesh. Approval was obtained from research review committee (RRC) and ethical review committee (ERC) of Dhaka Medical College according to declaration of Helsinki and national and institutional standards. Written consent was obtained from all participants. Gram-negative bacteria were isolated from the samples and they were examined phenotypically for ESBLs production.

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Isolation of gram-negative bacteria: All the samples were inoculated on blood agar and MacConkey agar media and incubated at 37ºC aerobically for 24 hours. The incubated plates were examined for bacterial growth and the organisms were identified by colony morphology, hemolytic criteria, staining character, pigment production and biochemical tests such as oxidase test, reaction in MIU and simmon's citrate media and different sugar fermentation tests [11].

Antimicrobial susceptibility testing and screening of ESBL producers: The antimicrobial susceptibility pattern was determined by Kirby Bauer disk-diffusion method on Mueller-Hinton agar using commercially available antibiotic discs (Oxiod, Hampshire, UK) according to CLSI guidelines[12]. The antibiotic disk used in antibiogram for all the gram-negative bacteria were co-trimoxazole (1.25/23.75 µg), gentamycin (10 µg), ciprofloxacin (5 µg), doxycycline (30 µg), azithromycin (30 µg), amoxiclav (20+10 µg), ceftriaxone (30 µg), ceftazidime (30 µg) and imipenem (10 µg). *Escherichia coli* ATCC 25922 was used for quality control. ESBL producers were screened by disk-diffusion method using ceftazidime and ceftriaxone. If the isolates were resistant to any of these drugs, they are considered as suspected ESBL producers[9].

Detection of ESBL producers by double-disk synergy test (DDST): ESBL producers were further confirmed by DDST as described previously[13]. Mueller Hinton agar was inoculated with standardized inoculum (corresponding to 0.5 McFarland tube) using sterile cotton swab. An amoxiclav disk was placed on the center of the plate and third generation cephalosporins (ceftriaxone, ceftazidime and cefotaxime) and aztreonam disks were placed at 20 mm distance (center to center) from the amoxiclav disk. After incubation at 37ºC for 24 hours, a clear extension of the edge of inhibition zone of any of the four drugs toward amoxiclav disk was interpreted as ESBL producer (Fig 1)

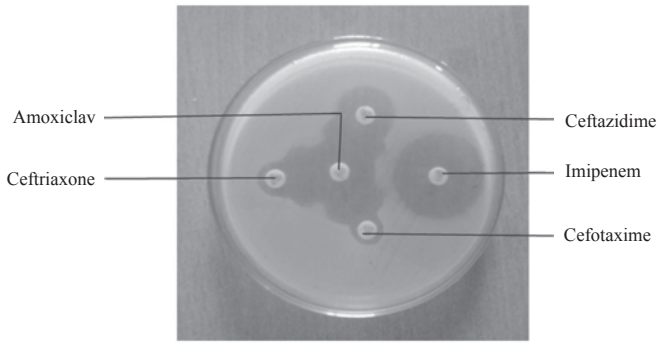

Figure 1: Double-disk synergy test

Data analysis: Data were analyzed using Microsoft Excel (2007) and comparisons were performed using chi-square test.

RESULTS

Out of the 300 urine samples 157 (52.33%) gram-negative bacilli causing UTI were isolated, of which *Escherichia coli* was the most predominant bacteria followed by *Enterobacter aerogenes*, *Acinetobacter* species, *Klebsiella pneumoniae, pseudomonas aeruginosa, Proteus vulgaris and Citrobacter freundii*. Among the various gram-negative bacteria 45 (28.66%) were ESBL producers and highest ESBL production was observed in *Escherichia coli* (32.14%) followed by *Klebsiella pneumoniae* (28.57%) (Table 1).

In present study, the female group constituted majority of the patients, which was 62.42% and 37.58% was male. *Escherichia coli, Klebsiella pneumoniae* and *Acinetobacter* species was more likely to cause UTI in females where as *Enterobacter aerogenes, Pseudomonas aeruginosa* and *Proteus vulgaris* were more predominant pathogen in males (Table 1).

Table 1. Patterns of isolated gram-negative bacteria from urine

Isolated organism	Total number (%)	Male n (%)	Female n (%)	ESBL positive strains n (%)
Escherichia coli	112 (71.34)	35 (31.25)	77 (68.75)	36 (32.14)
Enterobacter aerogenes	21 (13.38)	13 (61.90)	8 (38.10)	5 (23.80)
Acinetobacter species	10 (6.37)	4 (40.00)	6 (60.00)	2 (20.00)
Klebsiella pneumoniae	7 (4.46)	2 (28.57)	5 (71.43)	2 (28.57)
Pseudomonas aeruginosa	3 (1.91)	2 (66.67)	1 (33.33)	0 (0.00)
Proteus vulgaris	2 (1.27)	2 (100.00)	0 (0.00)	0 (0.00)
Citrobacter freundii	2 (1.27)	1 (50.00)	1 (50.00)	0 (0.00)
Total	157 (100.00)	59 (37.58)	98 (62.42)	45 (28.66)

This study found that most (46.5%) of UTI cases were in 21-30 years followed by in the age group of 41-50 years (36.3%) and 31-40 years (13.38%). However most of the ESBL producing uropathogens were found in the age group of 31-50 years and lowest in the age group of 11-20 years with male and female distribution being 40% and 60% respectively (Table 2).

Table 2. Age and sex distribution of patients with ESBL positive strains

Age (year)	Male n (%)	Female n (%)	Total n (%)
11-20	0 (0.00)	1 (2.22)	1 (2.22)
21-30	3 (6.67)	6 (13.33)	9 (20.00)
31-40	5 (11.11)	7 (15.56)	12 (26.67)
41-50	6 (13.33)	10 (22.22)	16 (35.55)
>50	4 (8.89)	3 (6.67)	7 (15.56)
Total	18 (40.00)	27 (60.00)	45 (100.00)

All the ESBL producers were resistant to aztreonam, cefotaxime, ceftriaxone and ceftazidime. The proportion of resistance to the other antimicrobials was 91.11% for amoxiclav, 93.33% for co-trimoxazole, 93.33% for ciprofloxacin, 91.11% for doxycycline and 82.22% for gentamycin (Table 3). All strains were sensitive to imipenem. Resistance rate of ESBL producers was higher against amoxiclav ($p<0.001$), Co-trimoxazole ($p<0.001$), ciprofloxacin ($p<0.01$), doxycycline ($p<0.05$) and gentamicin ($p<0.1$) when compared to non-ESBL producing gram-negative bacteria (Table 3).

Table 3. Antimicrobial resistance pattern of ESBL (n=45) and non-ESBL (n=112) producing gram-negative bacilli.

Antibiotic	ESBL positive strains n (%)	ESBL negative strains n (%)	Chi-square value	Difference (p)
Amoxiclav	41 (91.11)	72 (61.28)	12.84	$p<0.001$
Co-trimoxazole	42 (93.33)	74 (66.07)	12.63	$p<0.001$
Ciprofloxacin	42 (93.33)	82 (73.21)	9.45	$p<0.01$
Doxycycline	41 (91.11)	85 (75.89)	4.68	$p<0.05$
Gentamycin	37 (82.22)	77 (68.75)	2.92	$p<0.1$

DISCUSSION

In present study, *Escherichia coli* was the most predominant (71.34%) gram-negative bacilli found in urine followed by *Enterobacter aerogenes* (13.38%), *Acinetobacter baumannii* (6.37%), *Klebsiella pneumoniae* (4.46%) which correlates with the studies conducted in Bangladesh, India and Pakistan[14-16]. Previous studies conducted in Dhaka also showed *Escherichia coli* as the most common uropathogen in Bangladesh[17,18]. In our study, the percentage of female (62.42%) UTI patients were more compared to male (37.58%) which correlates with the study of other authors[19] and the prevalence was common in the age group of 21-30 years whereas less bellow 20 years which is similar with previous study[20]. Forty-five (28.66%) ESBL producing gram-negative bacteria were detected in this study. Previous studies in Bangladesh reported 23-31% ESBL producing gram negative bacteria[6,8], which is similar to the present study. However in another study, 80% ESBL producers were reported[7]. In a previous study it was observed that the higher prevalence of ESBL producers in Asia than in Europe and America[21]. The discrepancy of the findings of different studies may be due to the prevalence of ESBL producers vary with time as well as from country to country, city to city and even hospital to hospital in one city. In this study, *Escherichia coli* (32.14%) was the most predominant ESBL producers which in agreement with the reports of other authors[6,8]. This study revealed a higher occurrence of ESBL producing uropathogens in the age group of 31-50 years which coincide with a previous report[22].

In this study, antibiotic resistance in the urinary isolates was found to be high against the commonly used antibiotics. Increasing pattern of resistance of urinary pathogens in Bangladesh have been reported by other researchers[6,23]. ESBL producers are known to exhibit important therapeutic implications as they show resistance against third-generation cephalosporins, broad-spectrum ampicillin and monobactams. Present study observed significant resistance against ceftriaxone, ciprofloxacin, aztreonam, co-trimoxazole, gentamycin and doxycycline compared to non-ESBL producers. In Bangladesh, ESBL producers were not detected routinely because of resources and facilities deficiency in most of the laboratories. This increasing antimicrobial resistance is alarming and suggests that ESBL producers should be detected routinely in most of the microbiology laboratories. ESBL production co-exists with resistance to several other antibiotics as ESBL genes are located in plasmid that can be transferred from one organism to another easily and can incorporate genetic material coding for resistance to other antimicrobial classes[24,25]. So, simultaneous resistance to co-trimoxaxole, ciprofloxacin, gentamycin and doxycycline was frequent. A high prevalence of ESBL producers reduces therapeutic options and creates a need to report them routinely in laboratories because ESBL producers are significantly resistant to both ß-lactams and non ß-lactams than non-ESBL producers. In present study, regarding antimicrobial susceptibility imipenem was the most effective antibiotic against ESBL producers which correlates with the other studies[6,26].

CONCLUSION

In present study, all the ESBL strains isolated were highly resistant to commonly used antimicrobial agents and retained their sensitivity against imipenem. In view of this emerging drug resistance the practice of routine ESBL testing along with conventional antibiogram should be done which will help in the proper treatment of the patients and also prevent further development of bacterial drug resistance.

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