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Original Article

Prevalence of Extended Spectrum β-Lactamases (ESBLs) Producers Among Gram-Negative Bacilli in Urinary Tract Infections

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Abstract

Background: Antimicrobial resistance is now proclaimed as the most important challenge worldwide being faced by humanity in its fight against infectious diseases. Extended Spectrum β-Lactamases (ESBLs) producing organisms are increasing in number and causing more severe infections because of their continuous mutation and multidrug resistance property which make its treatment difficult. **Aims:** The present study was undertaken to detect the prevalence of the ESBLs producing bacteria in urinary tract infection. **Methods:** Isolated gram-negative bacteria initially screened by Minimum Inhibitory Concentration (MIC) ESBLs breakpoints. Then suspected ESBLs producers were confirmed by phenotypic confirmatory test. **Results:** 71 (59.17%) bacterial strains were isolated from 120 urine samples of patients of suspected urinary tract infection of which 66(92.96%) were gram-negative and 05(07.04%) were gram-positive. Among the isolated gram-negative bacteria 63(95.45%) were found suspected ESBLs producers of which 35(55.56%) were found as confirmed ESBL producers. The prevalence of ESBLs producing organisms in the present study were found to be 53.03% and Klebsiella spp. as most prevalent ESBLs producers. **Conclusion:** It is essential to report ESBL production along with routine sensitivity reporting, which will help the clinician in prescribing the proper antibiotics.

Key words : ESBLs; Gram Negative Bacilli; Urinary Tract Infection (UTI); Minimum Inhibitory Concentration (MIC).

INTRODUCTION

The accelerated emergence of antibiotic resistance among the prevalent pathogens is the most serious threat to the management of infectious diseases[1]. Infections by ESBL producing organisms are causing significant diagnostic and therapeutic problems in affected patient's[2]. ESBLs are mutant forms of β-lactamases enzymes coded by gens located on transferable plasmids, which can easily spread from one organism to another. The ESBL-producing organisms are often multi-drug resistant, as the plasmids producing ESBLs can carry resistance to other antibiotics[3].

Extended spectrum β-lactamases producing bacteria produce ESBL enzymes that mediate resistance to extended spectrum (third generation) cephalosporins (e.g. ceftazidime, cefotaxime, ceftrixone etc.) and monobactams (e.g. aztreonam) but do not affect cephamycins (e.g. cefoxitin and cefotatan) or carbapenems (e.g. meropenem or imipenem) and are inhibited by -lactamase inhibitors such as clavulanate, sulbactam and tazobactam[1, 4, 5].

The ESBL-producing bacteria are increasingly causing Urinary Tract Infection (UTI) becoming a major threat for patients in the hospital, long-term care facilities and community. The increasing drug resistance among these bacteria has made therapy of UTI difficult and has led to a greater use of expensive broad-spectrum antibiotics[6]. Inappropriate antibiotic selection in infections caused by these organisms is associated with treatment failures, poor clinical outcomes, prolonged hospital stay, increased morbidity, mortality and health care costs.

ESBLs have spread threateningly in many regions of the world and they presently comprise over 300 variants. The widespread use of the third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in these enzymes, which has led to the emergence of the ESBLs[7]. Drug resistance of this form is often difficult to recognize by using conventional antimicrobial susceptibility methods. Failure to identify ESBL producing organisms also contributes to their uncontrolled spread. Therefore, identification of the resistant phenotypes is important, particularly in developing countries where there is excessive use of antibiotics and lack of adequate antimicrobial resistance surveillance[8].

This study was designed to investigate the prevalence of ESBLs producing organisms among uropathogens which would guide clinicians and microbiologists for proper handling of these pathogens & prevent unnecessary use of antibiotics.

MATERIALS & METHODS

This cross sectional study was carried out in the department of Microbiology, Chittagong Medical College, during the period of June 2008 to May 2009. Samples were collected after taking informed written consent from both sexes and different age groups patients of indoor and outpatient department of Chittagong Medical College Hospital, Chittagong.

Inclusion criteria: Patients with clinical signs/symptoms of urinary tract infection.

Exclusion criteria: Pus cell <10/HPF in a centrifuged urine sample[9].

Using all aseptic precautions clean-catch mid-stream urine samples about 15-20ml were collected in sterile, wide mouth, tightly closed, leak-proof container by standard technique-for microscopy, culture and sensitivity test. After inoculating in Cystine Lactose Electrolyte Deficient (CLED) agar media by calibrated wire loop (0.01ml), identification of organisms were done as per standard laboratory methods of identification. A specimen was considered positive for UTI if a single organism was cultured at a concentration of 10^5 Colony Forming Unit/ml. Antimicrobial sensitivity of the isolates were tested by Kirby-Bauer disc diffusion technique against different antimicrobial agents, except imipenem, ceftriaxone, ceftazidime and cefotaxime which were tested for MIC by agar plate dilution method.

ESBL Detection: The method recommended by Clinical Laboratories Standard Institute (CLSI) requires a two-step approach of initially screening for ESBL production and then performing confirmatory tests on screen positive isolates[10].

Screening for ESBL producers by dilution method

Agar dilution method: The screening for ESBL producers was done by agar dilution method as was recommended by CLSI. Any of the isolated organisms found to be grown at this stated screening antibiotics concentration (that is, MIC of the ceftriaxone, ceftazidime and cefotaxime >2 g/ml) according to CLSI, 2007 was considered as possible ESBL producers and spelled for the confirmatory tests. The use of more than one antimicrobial agent for screening improves the sensitivity of detection [10].

Detection of ESBLs by the confirmatory tests

Phenotypic confirmatory test: Confirmation of the ESBL-producing isolates was done by the phenotypic confirmatory test according to CLSI recommendation. In this test, third generation cephalosporin i.e. ceftazidime (30 µg) and cefotaxime (30 µg) disc alone and in combination with clavulanic acid (10 µg) were used. Ceftazidime, cefotaxime discs without clavulanic acid were placed on one side and ceftazidime, cefotaxime discs combined with clavulanic acid (30/10 µg) were placed on other side of the inoculated plate. After overnight incubation at 37°C, diameter of zone of inhibition was measured. A 5 mm or more increases in diameter of zone of inhibition for ceftazidime and cefotaxime tested in combination with clavulanic acid versus its zone when ceftazidime and cefotaxime tested alone confirms an ESBLs producing organism [10].

Reference strain for quality control used for ESBL detection

E. coli BB-32327 (CTX-M9) was used as positive control and E. coli ATCC (American Type Culture Collection) 25922 was used as negative control of ESBL detection test.

RESULTS

A total 120 urine samples from patients of suspected urinary tract infection were studied, of which 71 (59.17%) bacterial strains were isolated (Table-1).

Table 1 : Frequencies of urinary pathogens.

Culture Results	Number	Percentage
Culture Positive	71	59.17
Culture Negative	49	40.83
Total	120	100.0

Among the 71 bacterial isolates, of which majority were *E. coli* 45(63.38%), followed by *Klebsiella* species 14(19.72%), *Enterococci* species 05(07.04), *Acinetobacter* species 03(04.22%), *Proteus* species 02(02.82%) & *Pseudomonas* species 02(02.82%) (Table-2).

Table 2 : Distribution of bacterial species among the isolates (n = 71).

Name of bacterial species	Number(n=71)	Percentage
Gram-negative organism		
E. coli	45	63.38
Klebsiella species	14	19.72
Acinetobacter species	03	04.22
Proteus species	02	02.82
Pseudomonas species	02	02.82
Total	66	92.96
Gram-positive organism		
Enterococci species	05	07.04
Grand Total	71	100.00

It appears from Table-3 that total 66 isolated gram-negative bacteria were screened for suspected ESBLs producers on the basis of MIC breakpoints by agar dilution method, out of which 63(95.45%) were found suspected ESBLs producers & 03(4.55%) gave negative result.

Table 3 : Detection of ESBL producing bacteria on the basis of MIC (screening test) by agar-dilution method (n = 66).

ESBL by agar-dilution method	Number of isolates	Percentage (%)
Positive	63	95.45
Negative	03	04.55
Total	66	100.0

Screening positive 63 spected ESBLs producing bacteria were subjected to phenotypic confirmatory test, 35(55.56%) were found as confirmed ESBL producers & 28(44.44%) showed negative result (Table-4).

Table 4 : Detection of ESBL producing organisms on the basis of phenotypic confirmatory test (n = 63).

Phenotypic confirmatory test	Number of isolates	Percentage (%)
Positive	35	55.56
Negative	28	44.44
Total	63	100.0

Out of 66 gram-negative bacteria 35(53.03%) were found to ESBLs producer. Higher rate of ESBLs was observed in Klebsiella species 08 (57.14%) out of 14, followed by E. coli 24(53.33%) out of 45, Proteus species 01(50.00%) Pseudomonas Species 01(50.00%) & Acinetobacter species 01(33.33%) out of 03 (Table-5).

Table 5 : Distribution of ESBLs producers among gram-negative bacteria (n=66).

Name of bacteria	Total no. of gram-negative bacteria	Number of ESBL producers
E. coli	45	24(53.33)
Klebsiella species	14	08(57.14)
Acinetobacter species	03	01(33.33)
Proteus species	02	01(50.00)
Pseudomonas species	02	01(50.00)
Total	66	35(53.03)

• Figures within parentheses indicate percentages

DISCUSSION

In the present study, a total of 120 urine samples from patients of different age groups were collected from which (culture positive) urinary isolates were 71(59.17%). This result is closely related to that of Rahman in BSMMU, Dhaka, who found 53.57% culture positive isolates from urine samples[11].

Among the bacterial isolates, 66(92.96%) were gram-negative and 05(07.04%) were gram-positive in our study (Table-2). Similar to present study Rahman and Alim of BSMMU, Dhaka found 90% gram-negative & 10% gram-positive and 90.21% gram-negative & 9.79% gram-positive isolates respectively[11,12].

Amongst the isolates in our study, E. coli (63.38%) was the highest urinary isolates followed by Klebsiella (19.73%), Enterococci (7.04%), Acinetobacter (4.22%), Pseudomonas (2.82%) & Proteus spp. (2.82%). Our results are also in agreement with those of Rahman and Alim in BSMMU, Dhaka who also found E. coli followed by Klebsiella spp. as the most prevalent urinary isolates[11,12]. Most common agents causing UTIs are gram negative bacilli. E coli, a normal flora of gastrointestinal tract, is the commonest causative organism of UT I[13].

The reason of high ratio for E. coli is the presence of this bacteria in the feces, thus it cause autoinfection. In addition, after gaining entry to the bladder, E. coli are able to attach to the bladder wall and form a biofilm that resists the body's immune response[14].

As of now, no country wide study has been conducted for the detection of the prevalence of ESBL production in Bangladesh, individual studies which were done in different parts of the country showed a varying prevalence, based on various risk factors and local reasons.

In the present study, we found 63(95.45%) suspected ESBLs producers from 66 gram-negative isolates, based on MIC, ESBLs screening breakpoints (Table-3). As using more than one antibiotic increase the sensitivity, we used three third generation cephalosporins (ceftriaxone, ceftazidime & cefotaxime) for the screening[10]. Our finding is closely related to that of Metri et al. in North Karnataka, India, who found 91.74% suspected ESBLs producers by screening test[7]. When these 63 screening positive isolates were subjected to the confirmatory tests, 35(55.56%) were confirmed as ESBL producers by Phenotypic Confirmatory Test (PCT) (Table-4).

The prevalence of ESBLs producing organisms in the present study were found (Table-5) to be 53.03%, which is higher than that of Alim 23.19% and Rahman 30.90% both in BSMMU but lower than that of Biswas of BSMMU 80% and Yasmin of Mymensingh 71.30%. Ullah et al. (2009), in Pakistan found 58.7% ESBL producers[11,12,15,16]. The prevalence of ESBLs producers in India ranges from 6.6% to 91%, in Europe from 23-25% for Klebsiella spp. and 5.4% for E. coli and in United States from 0 to 25%, depending on the institution[3,7].

The variation on ESBL positivity might be due to the number of isolates studied, variation in institution to institution, geographic location and also country to country[5,8]. The prevalence of ESBL production is high in the referral centers and the intensive care unites where the patients are referred from the peripheral centers and where the antibiotic use is profuse[7]. The higher prevalence compared to western countries can be explained by the fact that western countries have strict infection control policies and practices, efficient and effective antibiotic audit systems, shorter average hospital stays, better nursing barriers, and other important health care measures which substantially decrease the chances of acquisition and spread of ESBLs strains. The uncontrolled use of 3rd generation cephalosporins at our hospital could be a leading contributory factor to the high ESBLs prevalence observed in this study[17].

ESBLs are most commonly recognized in Klebsiella spp. and E. coli and most prevalent in Klebsiella pneumoniae[18,19]. We also found Klebsiella spp. (57.14%), as the leading ESBLs producers followed by E. coli (53.33%), Proteus (50.00%), Pseudomonas spp .01 (50.00%) and Acinetobacter spp. (33.33%) in our study, which correlates with those of Alim & Rahman in BSMMU, Yasmin at Mymensingh, Metri et al. & Giriyapur et al. in India who also found Klebsiella spp. as the most common ESBL producers[7,8,10,11,16].

The high occurrence of ESBLs in Klebsiella spp. is of great concern since infections caused by this bacterium are very common and resistance of the organism may be due to the presence of capsule that gives some level of protection to the cells, presence of multidrug resistance efflux pump, easy spreading nature, pathogenic and efficient at acquiring and disseminating resistance plasmid.

Klebsiella spp. has some virulence factor like hyper-viscosity, polysaccharide capsule and production of endotoxin, carbapenemases, which make it more resistant[20,21].

CONCLUSION

Existing of extended spectrum β-lactameses in bacteria and their potential multidrug resistance will create serious problems in the future as their continuous mutation and limited therapeutic option. Indiscriminate use of antibiotics especially 3rd generation cephalosporins and monobactams should be avoided. The regular detection of ESBLs producing organisms should be carried out in every laboratory.

DISCLOSURE

All the authors declared no competing interest.

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