DETECTION OF IMIPENEM RESISTANT EXTENDED SPECTRUM β-LACTAMASES (ESBLS) PRODUCERS AMONG GRAM-NEGATIVE BACILLI IN CHITTAGONG MEDICAL COLLEGE HOSPITAL, CHITTAGONG

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Abstract

Background: Extended-Spectrum β-lactamases (ESBLs) producing bacteria are increasing in number and causing more severe infections because of their continuous mutation and multidrug resistance property which make its treatment difficult. The emergence of carbapenem (Imipenem) resistantant ESBLs will worsen the management of infections and increases the mortality rates. The present study was undertaken to detect the imipenem resistantant ESBLs producing bacteria in patients attending Chittagong Medical College Hospital. **Materials and methods:** All the isolates from different clinical samples were identified by standard procedure of identification & antibiotic sensitivity pattern were done accordingly. Isolated gram-negative bacteria were initially screened by Minimum Inhibitory Concentration (MIC) ESBLs breakpoints & then confirmed by Phenotypic Confirmatory Test (PCT). **Results:** In the present study, 94.67% were found as suspected ESBLs producers, of which 62.68% confirmed as ESBL producers. The prevalence of ESBLs producers was found to be 59.33%, where Klebsiella species (67.50%) was the leading ESBLs producers. Among them 6.74% were imipenem resistant ESBLs producers. **Conclusion:** It is essential to report ESBL production along with routine antimicrobial sensitivity testing time to time for the selection of antibiotics for empirical treatment.

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Introduction

The emergence and spread of antibiotic resistance in micro organisms have rendered the management of infectious diseases difficult. 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[1]. Moreover, imipenem resistant ESBLs bacteria will create a real challenge for the physician.

Extended-Spectrum β-lactamases (ESBLs) producing bacteria produce Extended-Spectrum β-lactamase (ESBL) enzymes that mediate resistance to extended spectrum (Third generation) cephalosporins and monobactams (eg. aztreonam) but do not affect cephamycins (eg. cefoxitin and cefotatan) or carbapenems (eg. Meropenem or imipenem) and are inhibited by β-lactamase inhibitors such as clavulanate, sulbactam and tazobactam[2,3,4].

ESBLs have been found in a wide range of gram-negative rods. Klebsiella pneumoniae seems to remain the major ESBLs producer. Another very important organism is Escherichia coli[1]. Other organisms reported to harbour ESBLs include Enterobacter species, Salmonella species, Morganella morganii, Proteus mirabilis, Serratia marcescens and Pseudomonas aeruginosa[5,6].

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. The prevalence of ESBLs among clinical isolates varies greatly worldwide, from country to country and from institution to institution, and is rapidly changing over time. In

the United States, occurrence of ESBL production in Enterobacteriaceae ranges from 0 to 25%, depending on the institution. In India, the prevalence rate varies in different institution from 6.6 to 91%[7,8]. In Bangladesh, one study in Bangabandhu Sheikh Mujib Medical University (BSMMU) Dhaka showed, 23.19% ESBLs producing organisms among the isolated gram-negative bacteria[9]. Another study carried out in Dhaka Medical College Hospital (DMCH) & BSMMU, Dhaka, showed 30.90% ESBLs producers & the other study in BSMMU found, 80% was ESBLs producers[10,11].

Carbapenems (Imipenem, Meropenem, Ertapenem and Doripenem) are the latest developed β-lactam antibiotics that possess exceptionally broad spectrum of activity involving coverage of Gram-positive and Gram negative aerobes and anaerobes. These agents are primarily used in hospitals as an empiric therapy for the treatment of life threatening infections. However, clinical use of carbapenem drugs were recently increased following the emergence and dissemination of ESBLs producers, which were capable to hydrolyze all β-lactams except carbapenems[12,13]. Carbapenems are often one of the few therapeutic options available for the treatment of multi-drug resistant Gram-negative bacterial infections. Never the less, in the recent past, reports were accumulating on the emergence of carbapenem resistance all over the world limiting its usage[14].

Materials and 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. Total 235 samples (Wound swab, pus & urine) 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.

Inclusion Criteria:

i) Patients with infected wound

ii) Infected burn patients

iii) Patients with clinical signs/symptoms of urinary tract infection.

Exclusion Criteria:

i) Pus cell <10/HPF in a centrifuged urine sample[15].

After collecting samples under all aseptic precautions, wound swabs & pus were inoculated in Blood agar and MacConkey agar media and urine samples were inoculated in Cystine Lactose Electrolyte Deficient (CLED) agar media by calibrated wire loop (0.01ml). Colony counts exceeding 105 CFU/ml were taken as significant bacteriuria[16,17]. Identification of organisms were done as per standard laboratory methods of identification & antimicrobial sensitivity by disc diffusion method using the Kirby-Bauer technique and as per recommendations of CLSI[18,19].

Agar Dilution Method: The screening for ESBL producers was done by agar dilution method as was recommended by Clinical Laboratories Standard Institute (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 ≥2μg/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[19].

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 ie. Ceftazidime (30 μg) and cefotaxime (30 μg) disc alone and in combination with clavulanic acid (10 μg) were used. Ceftazidime, cefotaxime discs 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℃, 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[19].

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

Table I : Distribution of isolated bacteria from different samples (n = 235)

Samples	Number of samples studied	Number of isolated bacteria	Percentage (%)
Wound Swab & Pus	115	105	91.30
Urine	120	71	59.17
Total	235	176	74.89

Table II : Distribution of bacterial isolates (n = 176)

Name of bacterial species	Wound swab & pus (n = 105)	Urine (n = 71)	Total number of bacteria (n = 176)
E. coli	25 (23.81)	45 (63.38)	70 (39.77)
Klebsiella species	26 (24.76)	14 (19.72)	40 (22.73)
Pseudomonas species	23 (21.90)	02 (02.82)	25 (14.21)
Proteus species	10 (09.52)	02 (02.82)	12 (06.82)
Acinetobacter species	00 (00.00)	03 (4.22)	03 (01.70)
Total gram-negative bacteria	**84 (80.00)**	**66 (92.96)**	**150 (85.23)**
Staphylococcus aureus	18 (17.14)	00 (00.00)	18 (10.23)
Enterococci species	00 (00.00)	05 (7.04)	05 (02.84)
Coagulase negative Staphylococci	03 (2.86)	00 (00.00)	03 (01.70)
Total gram positive bacteria	**21 (20.00)**	**05 (7.04)**	**26 (14.77)**

● Figures within parentheses indicate percentages

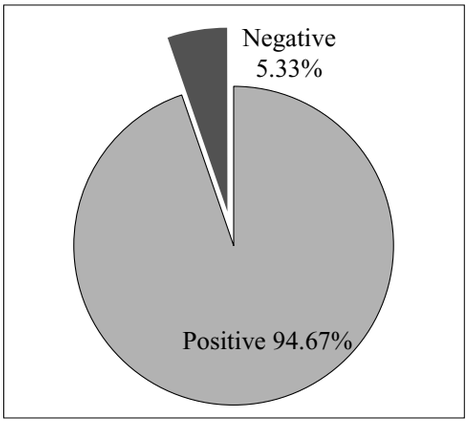

Fig 1 : Detection of ESBL producing bacteria on the basis of MIC (Screening test)

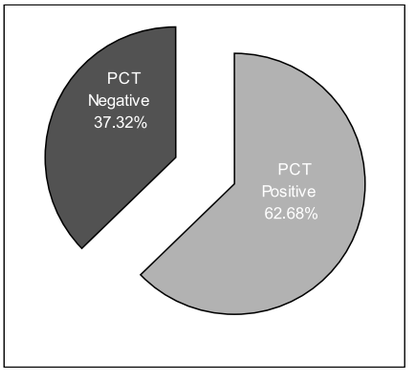

Fig : Detection of ESBL producing bacteria by Phenotypic Confirmatory Test (PCT)

Table III : Distribution of ESBLs producers among gram-negative bacteria (n=150)

Name of bacteria	Total no. of gram-negative bacteria	Number of ESBL producers(%)
E. coli	70	41(58.57)
Klebsiella species	40	27(67.50)
Pseudomonas species	25	13(52.00)
Proteus species	12	07(58.33)
Acinetobacter species	03	01(33.33)
Total	150	89(59.33)

● Figures within parentheses indicate percentages

Table IV : Resistant pattern of ESBL strains against imipenem (n=89)

Name of the bacteria	Number ESBL positive strains	Number of resistance strains
E. coli	41	01(02.44)
Klebsiella species	27	01(03.70)
Pseudomonas species	13	03(16.07)
Proteus species	07	00(00.00)
Acinetobacter species	01	01 (100.00)
Total	89	06 (06.74)

● Figures within parentheses indicate percentages

A total 235 samples were studied, of which 115 were wound swab & pus, and 120 were urine samples. Of these samples, 176 (74.89%) bacterial strains were isolated, of which 105(91.30%) from wound swab & pus and 71(59.17%) from urine samples (Table I).

Among the 176 isolates 150(85.23%) were gram-negative bacteria, of which majority were E. coli 70(39.77%) followed by Klebsiella spp. 40(22.73%)

Pseudomonas spp. 25(14.21%) Proteus spp. 12(06.82%) & Acinetobacter spp. 03(1.70%) and 26(14.77%) were gram-positive bacteria, of which Staphylococcus aureus 18(10.23%) Enterococci spp. 05(2.84%) & Coagulase negative staphylocci were 03(1.70%) (Table II).

Isolated 150 gram-negative bacteria were screened for suspected ESBLs producers on the basis of MIC ESBL breakpoints, where 142(94.67%) found as suspected ESBLs producers (Fig 1).

Suspected 142 ESBLs producing bacteria were further tested by phenotypic confirmatory test where 89(62.68%) confirmed as ESBL producers & 53(37.32%) gave negative result (Fig 2).

Out of 150 gram-negative bacteria 89(59.33%) were found to ESBLs producer. Higher rate of ESBLs was observed among Klebsiella spp. 27(67.50%) out of 40, followed by E. coli 41(58.57%) out of 70, Proteus spp. 07(58.33%) out of 12, Pseudomonas spp. 13(52.00%) out of 25 & Acinetobacter spp. 01(33.33%) out of 03 (Table-III).

Table IV shows resistance pattern of ESBL strains against imipenem. Out of 89 ESBL isolates 06(6.74%) were found resistant to imipenem, among which, 1(2.44%) from E. coli, 1(3.70%) from Klebsiella spp, 3(23.07%) from Pseudomonas spp & 1(100%) from Acinetobacter spp.

Discussion

The prevalence of ESBLs producing organisms is increasing worldwide. Serious infections with these organisms are associated with high mortality rate as therapeutic options are limited. The emergence of carbapenem resistance (Imipenem) ESBLs create a real challenge for both clinical microbiology laboratories and clinicians because of their dynamic evolution and epidemiology, wide substrate specificity with its therapeutic implications, their significant diagnostic challenges and infection control issues[1].

In the present study, a total of 235 samples were collected and of which 115 were wound swab & pus, and 120 were urine samples. From these samples, culture positive bacterial isolates were 176(74.89%) and among which 105(91.30%) from wound swab & pus, and 71(59.17%) from urine samples. This result is closely related to that of Rahman in BSMMU, Dhaka, who found 69.41% culture positive isolates and isolated 93.92% bacteria were from wound swab & pus, and 53.57% from urine samples[10].

Among the bacterial isolates, 150(85.23%) were gram-negative and 26(14.77%) were gram-positive in our study. Similar to present study Alim and Rahman of BSMMU, Dhaka found 90.21% gram-negative & 9.79% gram-positive and 90% gram-negative & 10% gram-positive isolates respectively[9,10]. Amongst the isolates in our study, the majority were E. coli 70(39.77%) followed by Klebsiella spp. 40(22.73%) Pseudomonas spp. 25(14.21%) Staphylococcus aureus 18(10.23%) Proteus spp. 12(06.82%) Enterococci spp. 5(2.84%) Acinetobacter spp. 3(1.70%) and Coagulase-negative staphylococci 3(1.70%). In contrast to our findings, Rahman revealed E. coil (40.63%) & Proteus spp. (18.44%) and Alim revealed E. coli (42.39%) & Pseudomonas spp. (22.28%) as the prevalent isolates in their study[9,10]. These sorts of variations are not unexpected, because it depends upon some external factors like socioeconomic conditions, hygienic status, environmental factors, level of education, and genetic factors[20].

In the present study, we found 142(94.67%) suspected ESBLs producers from 150 gram-negative isolates, based on Minimum Inhibitory Concentration (MIC) ESBLs screening breakpoints. As using more than one antibiotic increase the sensitivity, we used three third generation cephalosporins (Ceftriaxone, ceftazidime & cefotaxime) for the screening[19]. 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 142 screening positive isolates were subjected to the confirmatory tests, 89(62.68%) were confirmed as ESBL producers by Phenotypic Confirmatory Test (PCT). Closely similar to our study, Giriyapur et al of Karnataka and Dalela of Rajasthan both in India detected 63.89% and 61.6% ESBL producers respectively[8,21].

The prevalence of ESBLs producing organisms in the present study were found to be 59.33%, 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%[9,10,11,22]. 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[7,3]. 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].

ESBLs are most commonly recognized in Klebsiella spp. & E. coli and most prevalent in Klebsiella pneumoniae[23,24]. We also found Klebsiella spp. (67.50%) as the leading ESBLs producers followed by E. coli (58.57%) Proteus (58.33%) Pseudomonas (52%) and Acinetobacter spp. (33.33%) in our study, which correlates with those of Alim & Rahman, both in BSMMU, Yasmin at Mymensingh, Metri et al & Giriyapur et al both in India who also found Klebsiella spp. as the most common ESBL producers[9,10,22,7,8]. 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 some virulence factor like hyper-viscosity, polysaccharide capsule, multidrug resistance efflux pump, acquiring & disseminating resistance plasmid. and production of endotoxin, carbapenemases, which make it more resistant[25,26].

Of all available anti-microbial agents, carbapenems are the most sensitive and reliable treatment options for infections caused by ESBLs producing bacteria[4]. We found 6(6.74%) ESBLs producers were resistant to imipenem, among which, 1(2.44%) E. coli, 1(3.70%) Klebsiella spp, 3(23.07%) Pseudomonas spp & 1(100%) Acinetobacter spp (Table-IV). Similar to ours, Metri et al and Gupta et al both in India, found 7.4% and 17.32% imipenem resistant ESBLs producers respectively in their studies[7,27]. Thokar, Ahmed and Ahmed, found 25% imipenem resistant ESBLs producing E. coli[28]. The emergence of resistance to carbapenem (Imipenem) is an important growing threat to public health, since it is the final therapeutic option currently available for the treatment of life threatening infections caused by them. Notoriously, the genes responsible for this resistance were located in plasmids-facilitate the rapid spread of resistance and worsen the scenario and requires efforts toward detection and infection control strategies[14].

Conclusion

Existing of Extended Spectrum β-lactameses (ESBLs) producing bacteria and their potential multidrug resistance, especially imipenem, will create serious problem in the future as their continuous mutation and limited therapeutic option. Coordinated participation of microbiologists, clinicians, nursing personnel, hospital infection control team is essential to enforce strict infection control measures, to decrease horizontally transferable resistance. However, we need to keep in mind that the carbapenem must be kept in reserve for life-threatening infections.

Disclosure

All the authors declared no competing interest.

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