

PREVALENCE OF EXTENDED SPECTRUM β -LACTAMASES (ESBL) PRODUCERS AMONG GRAM-NEGATIVE BACILLI IN CHITTAGONG MEDICAL COLLEGE HOSPITAL, CHITTAGONG

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Summary

The emergence and spread of antibiotic resistance in microorganisms 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. The present study was undertaken to detect the prevalence of the ESBLs producing bacteria in patients attending Chittagong Medical College Hospital, so as to provide a guideline in treating them & prevent unnecessary use of antibiotics. All the isolates were identified by standard procedure & isolated gram-negative bacteria initially screened by Minimum Inhibitory Concentration (MIC) ESBLs breakpoints. Then confirmed by Phenotypic Confirmatory Test (PCT). In the present study, 176(74.89%) bacterial strains were isolated from 235 samples of wound swab, pus and urine. Among the isolates, 150(85.23%) were gram-negative and 26(14.77%) were gram-positive bacteria. Isolated gram-negative bacteria were screened for suspected ESBLs producers where 142(94.67%) were found suspected ESBLs producers, of which 89(62.68%) confirmed as

ESBL producers. Among the gram negative isolates, ESBLs producers was found to be 59.33%, where Klebsiella species (67.50%) was the leading ESBLs producers. It is essential to report ESBL production along with routine sensitivity reporting, which will help the clinician in prescribing the proper antibiotics.

Key words: Gram Negative Bacilli; Extended Spectrum β -Lactamases (ESBL); Klebsiella Species.

Introduction

The accelerated emergence of antibiotic resistance among the prevalent pathogens is the most serious threat to the management of infectious diseases [1]. β -lactam antibiotics are commonly used antimicrobials to treat bacterial infections. The most important mechanisms of resistance to β -lactam antibiotics among gram-negative bacilli involve the production of β -lactamases which destroy the β -lactam ring of β -lactam antibiotics [2]. The extended-spectrum β -lactam antibiotics became widely used in the treatment of serious infections caused by gram-negative bacteria in the 1980's. Resistance to these newer β -lactams due to β -lactamases emerged quickly. The first report of plasmid encoded β -Lactamases capable of hydrolyzing the extended spectrum cephalosporins was published in 1983 [3,4].

Extended Spectrum β -Lactamases (ESBLs) producing bacteria produce extended spectrum β -lactamase enzymes that mediate resistance to extended spectrum (Third generation) cephalosporins (eg. Ceftazidime, cefotaxime, ceftriaxone etc) and monobactams (eg. Aztreonam) but do not affect cephamycins (eg. Cefoxitin & cefotetan) or carbapenems (eg. Meropenem or imipenem) and are inhibited by β -lactamase inhibitors such as clavulanate, sulbactam and tazobactam [1,4,5,6].

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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].

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 [10]. Another study carried out in Dhaka Medical College Hospital (DMCH) & BSMMU, Dhaka showed 30.90% ESBLs producers & the other study in BSMMU found, 80% ESBLs producers [11,12].

Currently, ESBLs are becoming a major threat for patients in the hospital, long-term care facilities and community. Inappropriate antibiotic selection in infections caused by these organisms is associated with treatment failures, poor clinical outcomes, increased mortality and longer hospital stays [7]. Many clinical laboratories are not fully aware of the importance of ESBLs and a serious challenge facing clinical laboratories is that clinically relevant ESBL mediated resistance is not always detectable in routine susceptibility tests. The inability of the clinical laboratory to accurately detect and report ESBLs has resulted in avoidable therapeutic failures in patients, and outbreaks of multi-drug resistant gram-negative bacterial pathogens.

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

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: The following categories of patients were included in this study: 1. Patients with infected wound 2. Infected burn patients 3. Patients with clinical signs/symptoms of urinary tract infection.

Exclusion Criteria: Pus cell <10/HPF in a centrifuged urine sample [13].

Laboratory Procedure

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). Identification of organisms was done as per standard laboratory methods of identification.

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 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 [14].

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 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 [14].

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

Table II, Among the 176 bacterial isolates 150(85.23%) were gram-negative, of which majority were *E. coli* 70(39.77%), followed by *Klebsiella* species 40(22.73%), *Pseudomonas* species 25(14.21%), *Proteus* species 12(6.82%) & *Acinetobacter* species 03(1.70%) and 26(14.77%) were gram-positive bacteria, of which *Staphylococcus aureus* 18(10.23%), *Enterococci* species 05(2.84%) & *Coagulase negative staphylococci* 03(1.70%).

It appears total 150 isolated gram-negative bacteria were screened for suspected ESBLs producers on the basis of MIC ESBL screening breakpoints, out of which 142(94.67%) were found suspected ESBLs producers & 08(5.33%) gave negative result (Fig 1).

When 142 suspected ESBLs producing bacteria were further tested by phenotypic confirmatory test where 89(62.68%) found as confirmed ESBL producers & 53(37.32%) showed 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 1 : 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)
<i>E. coli</i>	25 (23.81)	45 (63.38)	70 (39.77)
<i>Klebsiella</i> species	26 (24.76)	14 (19.72)	40 (22.73)
<i>Pseudomonas</i> species	23 (21.90)	02 (02.82)	25 (14.21)
<i>Proteus</i> species	10 (09.52)	02 (02.82)	12 (06.82)
<i>Acinetobacter</i> species	00 (00.00)	03 (4.22)	03 (01.70)
Total gram-negative bacteria	84 (80.00)	66 (92.96)	150 (85.23)
<i>Staphylococcus aureus</i>	18 (17.14)	00 (00.00)	18 (10.23)
<i>Enterococci</i> species	00 (00.00)	05 (7.04)	05 (02.84)
<i>Coagulase negative staphylococci</i>	03 (2.86)	00 (00.00)	03 (01.70)
Total Gram positive bacteria	21 (20.00)	05 (7.04)	26 (14.77)

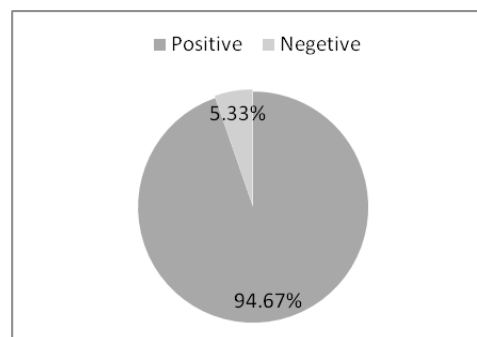


Fig 1 : Suspected ESBL producers on the basis of MIC ESBL screening breakpoints(n = 150)

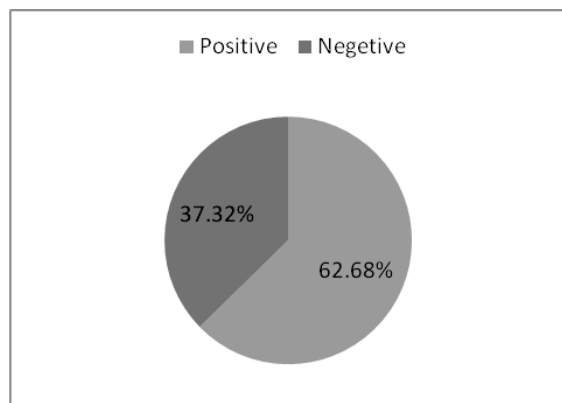


Fig 2 : ESBL producers on the basis of phenotypic confirmatory test (n = 142)

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

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 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% organisms were from wound swab & pus, and 53.57% from urine samples[11].

Among the bacterial isolates, 150(85.23%) were gram-negative and 26(14.77%) were gram-positive in our study (Table II). 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 [10,11]. 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. coli (40.63%) & Proteus spp. (18.44%) and Alim revealed E. coli (42.39%) & Pseudomonas spp (22.28%) as the prevalent isolates in their study [10,11]. These sorts of variation are not unexpected, because it depends upon some external factors like socioeconomic conditions, hygienic status, environmental factors, level of education, and genetic factors [15].

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 142(94.67%) suspected ESBLs producers from 150 gram-negative isolates, based on Minimum Inhibitory Concentration (MIC) ESBLs screening breakpoints (Fig 1). As using more than one antibiotic increase the sensitivity, we used three third generation cephalosporins (Ceftriaxone, ceftazidime & cefotaxime) for the screening [14]. 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) (Fig 2). Closely similar to our study, Giriyaapur et al. of Karnataka and Dalela of Rajasthan, both in India detected 63.89% and 61.6% ESBL producers respectively [8,16].

The prevalence of ESBLs producing organisms in the present study were found (Table III) 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% [10,11,12,17]. 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]. The prevalence of ESBL production is high in the referral centers and the intensive care units 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 [18].

ESBLs are most commonly recognized in *Klebsiella* spp and *E. coli* and most prevalent in *Klebsiella pneumoniae* [19,20]. 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 of Mymensingh, Metri et al. & Giriyaapur et al., both in India who also found *Klebsiella* spp as the most common ESBL producers [10,11,17,7,8].

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 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. It has some virulence factor like hyper-viscosity, polysaccharide capsule and production of endotoxin, carbapenemases, which make it more resistant [21,22].

Conclusion

Existing of extended spectrum β -lactamases in bacteria and their potential multidrug resistance 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. Indiscriminate use of antibiotics especially 3rd generation cephalosporins and monobactams should be avoided. The regular detection of ESBLs producing organisms by conventional methods should be carried out in every laboratory where molecular methods cannot be performed.

Disclosure

All the authors declared no competing interest.

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