Prevalence of Extended Spectrum β-Lactamases (ESBL) Producers Among Gram-Negative Bacilli in Wound Infection

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Abstract
Background: Extended Spectrum β-Lactames Producing Organisms (ESBLs) are increasing in number and causing more severe infections because of their continuous mutation and multidrug resistance property with limited therapeutic option. Aims and Objectives: Present study was undertaken to detect the prevalence of the ESBLs producing bacteria in wound infection, so as to provide a base line data in treating them & prevent unnecessary use of antibiotics 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: 105 (One hundred five) (91.30%) bacterial strains were isolated from 115 samples of wound swab & pus from different patients were studied of which 84(80.00%) were Gram-negative and 21(20.00%) were Gram-positive. Among the isolated Gram-negative bacteria 79(94.05%) were found suspected ESBLs producers of which 54(68.35%) were found as confirmed ESBL producers. The prevalence of ESBLs producing organisms in the present study were found to be 64.29% 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 : Gram-negative bacilli; ESBL; Klebsiella Species; Infection.

INTRODUCTION
Antimicrobial resistance is emerging as one of the major public health threats at the beginning of the 21st century worldwide including Bangladesh. The wide spread use and in some cases, misuse of antimicrobials in all health care settings over the past several decades has been cited as a contributing factor in the development of drug resistance in virtually all bacterial species. The accelerated emergence of antibiotic resistance among the prevalent pathogens is the most serious threat to the management of infectious diseases1. Infections by extended spectrum beta lactamases (ESBLs) producing organisms are causing significant diagnostic and therapeutic problems in affected patient’s2. ESBLs are mutant forms of β-lactamases enzymes coded by genes located on transferable plasmids, which can easily spread form one organism to another. The ESBL producing organisms are often multi drug resistant, as the plasmids producing ESBLs can carry resistance to other antibiotics3. Extended spectrum β-lactamases producing bacteria produce Extended Spectrum β-Lactamases (ESBLs) enzymes that mediate resistance to extended spectrum (Third generation) cephalosporins and monobactams but do not affect cephamycins (e.g. Cefoxitin and cefotatan) or carbapenems and are inhibited by β-lactamase inhibitors such as clavulanate, sulbactam and tazobactam1,4,5.
The ESBL producing bacteria are increasingly 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 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. The widespread use of the third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in these enzymes\(^7\). Drug resistance of this form is often difficult to recognize 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\(^8,9\). This study was designed to investigate the prevalence of ESBLs producing organisms among wound infections 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. Samples were collected after taking informed written consent from patients admitted in different wards of Chittagong Medical College Hospital, Chittagong.

**Inclusion criteria:** The following categories of patients were included in this study:

i. Patients with infected wound.

ii. Infected burn patients.

**Exclusion criteria:** Newly admitted burn/surgical cases.

A sterile technique was applied to aspirate or collect pus or wound swab from abcess or wound infection, either by disposable syringe or by sterile swab stick and inoculated in Blood agar and Mac Conkey agar media. After overnight incubation at 37\(^\circ\)C, plates were checked for presence of any suspected pathogens. Identification of organisms were done as per standard laboratory methods of identification and antimicrobial sensitivity of the isolates were tested against different antibiotics.

**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 Minimum Inhibitory Concentration [(MIC)] of third generation cephalosporins, namely 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\(^10\).

**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 (10µg) 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\(^10\).

**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 115 samples of wound swab & pus from different patients were studied, of which 105 (91.30%) bacterial strains were isolated & 10(08.70%) found no growth (Figure 1).

![Figure 1: Frequencies of isolates from wound infection.](image)

Among the 105 bacterial isolates 84(80.00%) were Gram-negative and 21(20.00%) were Gram-positive, of which majority were Klebsiella spp 26(24.76%) followed by *E. Coli* 25(23.81%) *Pseudomonas* spp 23(21.90%) *Staphylococcus aureus* 18(17.14) *Proteus* spp 10(09.53%) & Coagulase negative *Staphylococci* 03(2.86). (Table 1).
Table 1: Distribution of bacterial species among the isolates (n = 105).

<table>
<thead>
<tr>
<th>Name of bacterial species</th>
<th>Number(n=105)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-negative organism</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella species</td>
<td>26</td>
<td>24.76</td>
</tr>
<tr>
<td>E. Coli</td>
<td>25</td>
<td>23.81</td>
</tr>
<tr>
<td>Pseudomonas species</td>
<td>23</td>
<td>21.90</td>
</tr>
<tr>
<td>Proteus species</td>
<td>10</td>
<td>09.53</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>84</td>
<td>80.00</td>
</tr>
<tr>
<td><strong>Gram-positive organism</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>18</td>
<td>17.14</td>
</tr>
<tr>
<td>Coagulase negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococci</td>
<td>03</td>
<td>02.86</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>21</td>
<td>20.00</td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>105</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

It appears from Figure 2 that total 84 isolated gram-negative bacteria were screened for suspected ESBLs producers on the basis of MIC ESBL breakpoints by agar dilution method, out of which 79(94.05%) were found suspected ESBLs producers & 05(05.95%) gave negative result.

Out of 84 gram-negative bacteria 54(64.29%) were found to ESBLs producer. Higher rate of ESBLs was observed in Klebsiella spp 19 (73.03%) out of 26, followed by E. Coli 17(68.00%) out of 25, Pseudomonas spp 13(56.52%) out of 23 & Proteus spp 05(50.00%) out of 10 (Table 2).

Table 2: Distribution of ESBLs producers among gram-negative bacteria (n=84).

<table>
<thead>
<tr>
<th>Name of bacteria</th>
<th>Total no. of gram-negative bacteria</th>
<th>Number of ESBL producers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella species</td>
<td>26</td>
<td>19 (73.03)</td>
</tr>
<tr>
<td>E. Coli</td>
<td>25</td>
<td>17 (68.00)</td>
</tr>
<tr>
<td>Pseudomonas species</td>
<td>23</td>
<td>13 (56.52)</td>
</tr>
<tr>
<td>Proteus species</td>
<td>10</td>
<td>05 (50.00)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>84</strong></td>
<td><strong>54 (64.29)</strong></td>
</tr>
</tbody>
</table>

Figures within parentheses indicate percentages

DISCUSSION

In the present study, 105(91.30%) bacteria were isolated from 115 wound swab & pus samples from different patients (Figure 1). This result is closely related to that of Rahman in Bangabandhu Sheikh Mujib Medical University (BSMMU) Dhaka, who found 93.92% bacterial isolates from wound swab & pus samples. Among the bacterial isolates, 84(80.00%) were gram-negative and 21(20.00%) were gram-positive in our study (Table 1). Similar to present study, Rahman found 90% gram-negative & 10% gram-positive isolates. Amongst the isolates, Klebsiella spp (24.76%) was the predominant organism, followed by E. Coli (23.81%) Pseudomonas spp (21.90%) Staphylococcus aureus (17.14%) Proteus spp (9.53%) and Coagulase-negative staphylococci (2.86%). Sule et al. in Nigeria also found Klebsiella spp (25.3%) as the most common bacterial isolates from wound swab. In contrast to present study Rahman et al. of Dhaka, found Staphylococcus aureus (39.44%) was the principal organism, followed by Klebsiella spp (21.13%) E. Coli (11.27%) Proteus spp (08.45%) and Pseudomonas spp (05.63%) in surgical wound. These different findings may be due to that etiological agents may however, vary from country to country, from hospital to hospital and from one community to another.

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 79(94.05%) suspected ESBLs producers from 84 gram-negative isolates, based on Minimum Inhibitory Concentration (MIC) ESBLs screening breakpoints (Figure 2). As using more than one antibiotic increase the sensitivity, we used three third generation cephalosporins (Ceftriaxone, ceftazidime & cefotaxime) for the screening.
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. When these 79 screening positive isolates were subjected to the confirmatory tests, 54 (68.35%) were confirmed as ESBL producers by phenotypic confirmatory test (Figure 3).

The prevalence of ESBLs producing organisms in the present study were found (Table II) to be 64.29%, 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%15, 11, 14, 17. Ullah et al. in Pakistan found 58.7% ESBL producers18. 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 institution73.

The variation on ESBL positivity might be due to the number of isolates studied, variation in institution to institution & geographic location54. 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 profuse7. The uncontrolled use of 3rd generation cephalosporins at the hospital could be a leading contributory factor to the high ESBLs prevalence19.

ESBLs are most commonly recognized in Klebsiella spp and E. Coli and most prevalent in Klebsiella pneumoniae20,21. We also found Klebsiella spp (73.03%) as the leading ESBLs producers followed by E. Coli (68.00%) Pseudomonas spp (50.00%) and Proteus spp (50.00%) in our study, which correlates with those of Rahman & Alim both in BSMMU, Yasmin of Mymensingh who also found Klebsiella spp as the most common ESBL producers11,15,17.

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 & multidrug resistance efflux pump, easy spreading nature, pathogenic and efficient at acquiring and disseminating resistance plasmid and production of endotoxin, carbapenemases, which make it more resistant22.

CONCLUSION
Existing of extended spectrum β-lactameses in bacteria and their potential multidrug resistance will create serious problem 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.
REFERENCES