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Status of Extended Spectrum Beta-Lactamase (ESBL) Producing Bacteria Isolated from Surgical and Burn Wound at Tertiary Care Hospital in Dhaka City

Mostaqimur Rahman[1], Hafiza Sultana[2], Md. Abdullahil Mosawuir[3], Latifa Akhter[4], Md. Abdullah Yusuf[5]

[1]Assistant Professor, Depart of Microbiology, Rangpur Medical College, Rangpur, Bangladesh; [2]Assistant Professor, Depart of Microbiology, Rangpur Medical College, Rangpur, Bangladesh; [3]Associate Professor, Department of Physiology, Rangpur Medical College, Rangpur, Bangladesh; [4]Assistant Professor, Department of Skin &VD, Rangpur Medical College, Rangpur, Bangladesh; [5]Assistant Professor, Department of Microbiology, National Institute of Neuroscience & Hospital, Dhaka, Bangladesh

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Abstracts

Background: Surgical and burn wound infection are the most common infection in the hospital settings. **Objective:** The aim of the present study was to see the status of extended spectrum beta-lactamase (ESBL) producing bacteria isolated from patients presented with surgical and burn wound infection. **Methodology:** This cross-sectional study was conducted in the Department of Microbiology, BSMMU from January to December 2006, at a period of one year. This study was carried out to detect extended spectrum β-lactamase producing Gram negative bacteria rapidly by using a kit containing chromogenic cephalosporin directly from primary culture by comparison with phenotypic confirmatory method. **Result:** Total 181 samples were collected from patients with wound infections of which 170(93.9%) bacteria were isolated. Among individual samples ESBLs positive strains were highest in surgical wound which was 22(31.42%) and 24(28.24%) isolates respectively. From surgical wound swab ESBL was found 3(42.9%) isolates from *Klebsiella* species. ESBL producing *E. coli* was found in 12(35.3%) isolates. *Pseudomonas* species showed in 2(22.2%) isolates and 1(33.3%) isolate of *Acinetobactor* species. ESBL positive *E. coli* was found in 5(45.45%) isolates from burn wound. ESBL positive *Proteus* species was detected in 11(28.94%) isolates from burn wound. **Conclusion:** Most common bacteria isolated from the infected surgical and burn wound are *E. coli* and *Proteus* species, though *Klebsiella* species is the most common ESBL producing bacteria isolated from both infected surgical and burn wound. [*Bangladesh Journal of Infectious Diseases 2018;5(1):21-26*]

Keywords: ESBL; burn wound; surgical wound; infection

Correspondence: Dr. Md. Mostaqimur Rahman, Assistant Professor, Depart of Microbiology, Rangpur Medical College, Rangpur, Bangladesh; Email: rupom68@yahoo.com
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Introduction

Many genera of Gram negative bacteria possess a naturally occurring, chromosomally mediated β-lactamase[1]. Being plasmid and transposon mediated has facilitated the spread of these enzymes to other species of bacteria. Within few years after its first isolation, the extended spectrum β-lactamase spread worldwide and is now found in many different species of members of the family Enterobacteriaceae, *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Neisseria gonorrhoeae*[2]. β-lactamases producing bacteria are increasing in number and causing more severe infections, because of their continuous mutation[3]. Extended mutation has led to the emergence of extended spectrum β-lactamases enzymes, the incidence and types of which vary with geographical location and time. The functional and molecular classifications are complex for the bacteria producing these enzymes. Awareness and detection of these enzymes are necessary for optimal patient care[3].

Burn patients are infected by hospital-acquired bacteria by various invasive and noninvasive procedures[4]. Early diagnosis of microbial infections and screening for drug resistance is aimed to institute the appropriate antibacterial therapy and to avoid further complications. Now-a-days, majority of the bacteria that cause burn infection in hospitals are resistant to at least one of commonly used drugs[5]. Among the Gram-positive cocci, methicillin-resistant Staphylococcus aureus (MRSA) is the most important nosocomial pathogen. Sensitivity of MRSA to only a few antibacterial agents limits therapeutic options and poses a threat to the patient life[6]. Extended-spectrum b-lactamases (ESBLs) and metallo-beta-lactamases (MBLs)-producing organisms pose a major problem for treating burn victims[7]. ESBLs are beta-lactamases capable of conferring bacterial resistance to penicillins, first, second, and third-generation cephalosporins, and aztreonams, but not to cephamycins or carbapenems. MbL is a group of carbapenem-hydrolyzing b-lactamase but not aztreonams and resists currently available beta-lactamase inhibitors, but are inhibited by chelating agents such as ethylenediamine tetra-acetic acid (EDTA)[8].

Again the surgical site infection is also very common. It has played a great role in the morbidity and mortality of the patients[9]. Many bacteria are responsible for this infection. These bacteria are gradually developing resistance to beta-Lactam antibiotics by producing beta-Lactamase[10]. ESBL producing organisms can cause both community and hospital acquired surgical site infections which can be very difficult to treat with common drugs. Isolates may be susceptible to 3rd generation cephalosporin in vitro; however, it results in clinical failure when used in vivo[11]. In this context this present study was undertaken to see the status of extended spectrum beta-lactamase (ESBL) producing bacteria isolated from patients presented with surgical and burn wound infection.

Methodology

This cross-sectional study was carried out in the Department of Microbiology and Immunology at Banglabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from January 2006 to December 2006 for a period of one (01) year. Samples were collected from in-patient and out-patient department of Dhaka Medical College Hospital, Dhaka and BSMMU, Dhaka after getting informed verbal consent from the patients or from the attendants. Laboratory work was performed in department of Microbiology & Immunology, BSMMU, Dhaka. *K. pneumoniae* ATCC 700603 (positive control) and *E. coli* ATCC 25922 (negative control) were used for quality control of ESBL tests (NCCLS, 1999).

Samples were inoculated on appropriate culture media and plates were incubated at 37° C aerobically for 24 to 48 hours. Plates were checked for presence of suspected pathogens. All the organisms were identified by their colony morphology, staining characters, pigment production, motility and other relevant biochemical tests as per standard methods[8]. Phenotypic confirmation of ESBLs producing isolates were done by inhibitor potentiated disc diffusion test according to CLSI recommendation.

Third generation cephalosporin i.e. cefotaxime (30µg) and ceftazidime (30 µg) disc alone and in combination with clavulanic acid (10 µg) were placed on inoculated plate. Mueller Hinton plates were inoculated with test bacteria. Ceftazidime, cefotaxime disc without clavulanic acid was placed on one side of inoculated plate and ceftazidime, cefotaxime disc combined with clavulanic acid was placed on other side of plate. Then the plates were incubated at 35°C overnight. After overnight incubation inhibition zone diameter was measured with scale.

It was observed whether there was an increase in zone diameter for cefotaxime and ceftazidime in

combination with clavulanic acid compared to its zone diameter for cefotaxime and ceftazidime tested alone; thus, ≥ 5 mm increase in a zone diameter for cefotaxime and ceftazidime in combination with clavulanic acid then the zone diameter of cefotaxime and ceftazidime when tested alone, was confirmed an ESBL producing organism[9]. Genotypic detection of ESBL was performed by plasmid extraction.

Results

Total 181 samples were collected from patients with wound infections from BSMMU and DMCH of which 87 were surgical wound samples and 94 were burn samples. Total 170(93.9%) bacteria were isolated from these 181 samples. In infected wound and burn swab majority was yielded culture positive result which were 80(91.9%) and 90(95.7%) isolates respectively (Table 1).

Table 1: Rate of Isolation of Bacteria from Surgical and Burn Wound (N=181)

Types of Sample	Culture		Total	P value
	Positive	Negative		
Surgical Wound	80(91.9%)	7(8.1%)	87(100.0%)	0.000
Burn wound	90(95.7%)	4(4.3%)	94(100.0%)	
Total	**170(93.9%)**	**11(6.1%)**	**181(100.0%)**	

Among 80 culture positive surgical wound swabs *E. coli* was the most common isolated bacteria from surgical wound which was 34(42.5%) isolates followed by *Proteus* species, *Pseudomonas* species, *Staph. aureus*, *Klebsiella* species and *Acinetobactor* species which were 17(21.2%) isolates, 9(11.3%) isolates, 9(11.3%) isolates, 7(8.7%) isolates and 3(3.7%) isolates respectively. However, out of 90 culture positive burn swabs majority bacteria was *Proteus* species which was 38(42.3%) isolates followed by *Pseudomonas* species, *E. coli*, *Klebsiella* species, *Staph. aureus* and *Acinetobactor* species which were 27(30.0%) isolates, 11(12.2%) isolates, 5(5.6%) isolates, 4(4.4%) isolates and 3(3.3%) isolates respectively (Table 2).

Table 2: Distribution of different bacterial species among Surgical and Burn Wound (n=170)

Name of Bacteria	Wound Swab		Total	P value
	Surgical	Burn		
Proteus spp.	17(21.2%)	38(42.3%)	55(32.4%)	0.000
Escherichia coli	34(42.5%)	11(12.2%)	45(26.5%)	
Pseudomonas spp.	9(11.3%)	27(30.0%)	36(21.2%)	
Staph. aureus	9(11.3%)	4(4.4%)	13(7.6%)	
Klebsiella spp.	7(8.7%)	5(5.6%)	12(7.1%)	
Acinetobactor species	3(3.7%)	3(3.3%)	6(3.5%)	
Enterococci species	1(1.3%)	1(1.1%)	2(1.2%)	
Enterobactor species	0 (0.0%)	1(1.1%)	1 (0.6%)	
Total	**80(100.0%)**	**90(100.0%)**	**170(100.0%)**	

Among individual samples ESBLs positive strains were highest in surgical wound which was 22(31.42%) isolates and from burn wound 24(28.24%) isolates. Among isolated ESBL producing bacteria *Klebsiella* species was highest in all types of sample. From culture positive surgical wound swab ESBL was found 3(42.9%) isolates from *Klebsiella* species. ESBL producing *E. coli* was found in 12(35.3%) isolates. ESBL producing *Proteus* species was reported in 4(23.5%) isolates. *Pseudomonas* species showed in 2(22.2%) isolates and 1(33.3%) isolate of *Acinetobactor* species (Table 3).

Table 3: ESBLs Producer among Different Species of Bacteria from Culture Positive Surgical Wound Swab (n=70)

Name of Bacteria	ESBLs		Total	P value
	Positive	Negative		
Escherichia coli	12(35.3%)	22(64.7%)	34(100.0%)	0.000
Klebsiella species	3(42.9%)	4(57.1%)	7(100.0%)	
Proteus species	4(23.5%)	13(76.5%)	17(100.0%)	
Pseudomonas species	2(22.2%)	7(77.8%)	9(100.0%)	
Acinetobactor species	1(33.3%)	2(66.7%)	3(100.0%)	
Total	**22(31.4%)**	**48(68.6%)**	**70(100.0%)**	

Klebsiella species were 3(60%) in infected burn wound. ESBL positive *E. coli* was found in 5(45.45%) isolates from burn wound. ESBL positive *Proteus* species was detected in 11(28.94%) isolates from burn wound. ESBL positive *Pseudomonas* species was detected in 4(14.81%) isolates from infected burn wound. ESBL positive *Acinetobactor* species was found in only 1(33.33%) isolates (Table 4).

Table 4: ESBLs Producer among Isolated Bacterial Species from Culture Positive Burn Wound Swab (n=85)

Name of Bacteria	ESBLs		Total	P value
	Positive	Negative		
Escherichia coli	5(45.5%)	6(54.5%)	11(100.0%)	0.000
Klebsiella spp.	3(60.0%)	2(40.0%)	5(100.0%)	
Proteus spp.	11(28.9%)	27(71.1%)	38(100.0%)	
Pseudomonas spp.	4(14.8%)	23(85.2%)	27(100.0%)	
Enterobactor spp.	0(0.0%)	1(100.0%)	1(100.0%)	
Acinetobactor spp.	1(33.3%)	2(66.7%)	3(100.0%)	
Total	**24(28.2%)**	**61(71.8%)**	**85(100.0%)**	

Discussion

Bacterial antibiotic resistance has become a major clinical concern worldwide including Bangladesh[12]. Failure to detect these enzymes- ESBLs, AmpC β-lactamases, Metallo- β-lactamases has contributed to their uncontrolled spread and therapeutic failure[13].

Total 181 samples were collected from patients with wound infections from BSMMU and DMCH of which 87 were surgical wound samples and 94 were burn samples. Total 170(93.9%) bacteria were isolated from these 181 samples. In infected wound and burn swab majority was yielded culture positive result which were 80(91.9%) and 90(95.7%) isolates respectively. Among 80 culture positive surgical wound swabs *E. coli* was the most common isolated bacteria from surgical wound which was 34(42.5%) isolates followed by *Proteus* species, *Pseudomonas* species, *Staph. aureus, Klebsiella* species and *Acinatobactor* species which were 17(21.2%) isolates, 9(11.3%) isolates, 9(11.3%) isolates, 7(8.7%) isolates and 3(3.7%) isolates respectively. However, out of 90 culture positive burn swabs majority bacteria was *Proteus* species which was 38(42.3%) isolates followed by *Pseudomonas* species, *E. coli, Klebsiella* species, *Staph. aureus* and *Acinatobactor* species which were 27(30.0%) isolates, 11(12.2%) isolates, 5(5.6%) isolates, 4(4.4%) isolates and 3(3.3%) isolates respectively. Similar to the present study Rahman et al[14] in Bangladesh found 33% *E.coli*, 27% *Klebsiella* species in an urban hospital.

Among different samples, isolation rate of *E.coli* was highest 34(42.5%) in surgical wound and 11(12.22%) in burn wound. *Klebsiella* spp. was

found 7(8.75%) isolates in surgical wound and 5 (5.55%) in burn wound. *Proteus* spp. was highest in burn wound (42.22%) followed by surgical wound (21.25%). *Pseudomonas* species was highest in burn wound (30.0%) followed by surgical wound (11.25%). *Staph aureus* was found highest in surgical wound 9(11.2%) followed by burn wound 4(4.4%).

Isolation rate of different strains among surgical wound sample showed highest rate in *E. coli* (42.5%) followed by *Proteus* species (21.25%), *Pseudomonas* species (11.25%), *Staph. aureus* (11.25%), *Klebsiella* species (8.75%). In a study of pus samples Rahman et al. (2004) found *Staph. aureus* (36%), *E. coli* (32%), *Klebsiella* species (24%). Isolation rate of different strains among burn wound sample showed highest rate in *Proteus* spp. 38(42.22%), followed by *Pseudomonas* spp. 27(30%), *E. coli* 11(12.22%), *Klebsiella* spp. 5(5.55%), *Staph. aureus* 4 (4.44%). In a study *Pseudomonas aeruginosa* was found as the highest isolated bacteria from burn patients (12.5%) followed by *Enterobacter* species (2.6%), *E coli* (1.4%), *Klebsiella* species (0.8%) and *Proteus* species (0.2%)[15]. In another study at BSMMU, ESBL was detected in 23.19% Gram negative bacteria, among them *Klebsiella* species was highest(40.90%) followed by *Proteus* spp. (40.62%), *E.coli* (26.92%) and less in *Pseudomonas* spp. (4.87%)[16]. In another study at urban hospital in Dhaka showed (43.21%) *E. coli* and (39.5%) *Klebsiella* species as ESBL producers[14].

ESBL producing strains were isolated from surgical wound and burn wound. Highest rate of ESBLs (32.33%) was found in surgical wound (31.42%) and in burn wound (28.24%). Among isolated ESBL producing bacteria *Klebsiella* species was highest in all types of sample. Among surgical wound strains *E. coli* (35.3%), *Klebsiella* species (42.9%), *Proteus* species (23.5%), *Pseudomonas* species (22.2%), *Acinetobactor* species (33.3%) were ESBLs producers. Among burn wound strains *E. coli* (45.45%), *Klebsiella* species (60.0%), *Proteus* species (28.9%) and *Pseudomonas* species (14.81%) and *Acinetobactor* species (33.3%) were ESBLs producers. Higher rate was also found in surgical wound (31.43%) followed by burn wound (28.24%). This may be due to most of the patients were post-operative with improper handling of wound, overcrowding, understaffing or nursing workload with cross-transmission of ESBL producing *Enterobacteriaceae*[15].

The isolation rate of ESBL producing *Klebsiella* spp. was highest among burn wound (60%), followed by surgical wound (42.86%). Similar higher rate ESBL producing strain s of *Klebsiella* spp. (44%) also observed in Singapore hospital[16]. In the study by Rahman et al[14] ESBL producer *Klebsiella pneumoniae* was highest in pus (54.5%). *Klebsiella* spp. has the ability to spread rapidly in hospital environment and tends to cause nosocomial outbreak[17].

ESBL producing *Proteus* spp. was observed in 16 (27.11%) out of total 59 samples of which highest rate was observed in burn wound 28.94%, probably due to high rate of isolation from burn unite. Multi drug resistant Pseudomonas spp. also found in burn unite. Increase number of ESBLs producer is probably due to previously treated with β-lactam drugs, extreme ages, bed retention, immune suppuration, association with other diseases, temporary or permanent urinary catheter[18]. In a study in BSMMU it was also found lower rate of *Pseudomonas* species (4.9%) ESBLs producer[12]. Lower rate of ESBL producing *Pseudomonas* is due to *Pseudomonas* spp. exhibits multiple mechanism of drug resistance simultaneously other than ESBL[19], such as AmpC β-lactamase enzymes, and Metallo β-lactamase. These enzymes are resistant to clavulanic acid that is used to detect ESBL producing bacteria in double disc and phenotypic method[20].

Conclusion

In conclusion most common bacteria isolated from the infected surgical and burn wound are *E. coli* and *Proteus* species. However, the most common ESBL producing bacteria is *Klebsiella* species isolated from both infected surgical and burn wound. Proper detection of ESBL producing bacteria should be performed from the infected surgical and burn wound.

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