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ORIGINAL ARTICLE

Frequency of ESBL in Surgical Site Infection at a Tertiary Care Hospital

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Abstract

Background: Infection caused by ESBL in the surgical site infection is very alarming. **Objective:** The purpose of the present study was to see the status of ESBL bacteria isolated from surgical site infection with their antimicrobial sensitivity pattern. **Methodology:** This cross sectional study was conducted in the Department of Microbiology at Dhaka Medical College, Dhaka from January, 2005 to December, 2005 for a period of one (1) year. All the patients presented with surgical site infections at any age with both sexes were included a study population. Detection of extended spectrum beta lactamase producing Gram negative bacteria was done by using disc diffusion method and was confirmed by E- test ESBL method. Sensitivity pattern of ESBL producers were observed against quinolone and fluoroquinolones. ESBLs are the enzymes capable of hydrolyzing all penicillin, monobactam and cephalosporins except cephamycin, but inactive against imipenem. **Result:** A total number of 92 surgical wound samples were collected of which 68(73.9%) samples were culture positive. Interestingly, most of the *E. coli* was ESBL positive (55.0%). *Klebsiella* species was 33.1% ESBL positive. ESBL positivity of *Proteus* and *Pseudomonas* species were low (11.1%). Among the isolated *Pseudomonas* species, 1(6.67%) of the 15 strains isolated from wound swab was ESBL producers. ESBL positivity was significantly found in surgically wound samples (p=0.0001). Among the ESBL producers, all the *E. coli*, *Klebsiella* species, *Proteus* species and *Pseudomonas* species were resistant to amoxicillin, cephradine, ceftriaxone, aztreonam, ceftazidime and cefotaxime. All the Gram negative bacteria were sensitive to imipenam. **Conclusion:** A considerable numbers of ESBL producing bacteria were detected from surgical wound.

Keywords: ESBL, surgical site infection, antibiotic resistant bacteria

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Introduction

Surgical site infection has played a great role in the morbidity and mortality of the patients[1]. Many bacteria are responsible for this infection. These bacteria are gradually developing resistance to beta-Lactam antibiotics by producing beta-Lactamase[2]. 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[3].

Extended spectrum beta-Lactamase (ESBL) producing strains are steadily increasing in incidence over the past few years resulting in limitation of their therapeutic options[4]. Hospital outbreak of multidrug resistance which are now being frequently caused by ESBL producers[1]. Prevalence of ESBLs among the clinical isolates varies in different countries and even in different institutions of the same country[5]. Resistance to beta-lactam drugs are susceptible to beta-Lactamase inhibitors like clavulanic acid, sulbactam, tazobactam containing antibiotics are considered in favour of ESBL[5]. This study has been designed to isolate ESBL producing organisms from surgical site infection.

Methodology

This was a cross sectional study conducted in the Department of Microbiology at Dhaka Medical College, Dhaka from 1st January, 2005 to 31st December, 2005 for a period of 1(one) year. Samples were collected from surgical wound by sterile swab stick. Samples were collected from inpatients and outpatients of various departments of Dhaka Medical College Hospital (DMCH) from both sexes of different age groups. Patients with infected wound receiving antibiotic treatment especially 3rd generation cephalosporins for at least 5-7 days without any improvement were included in this study. Patients receiving antibiotics <5 days were excluded from this study. Isolation, identification and antibiotic susceptibility of different organisms were done following NCCLS guidelines[6]. All wound swabs were stained by Gram stain as per standard method and were examined under microscope for the presence of bacteria[7]. All wound swabs were inoculated in blood agar and MacConkey agar media and incubated at 37^0C aerobically for 18-24 hours, plates were taken out and were examined for the presence of colonies of bacteria. All the organisms were identified by their colony morphology, staining character, pigments production, haemolysis, motility and other relevant biochemical tests as per standard methods[7-8]. Antimicrobial susceptibility test was performed[4]. Antibiogram for all bacterial isolates were done by disc diffusion method of modified Kirby-Bauer technique using Mueller Hinton agar plates and commercially available antimicrobial disc (Oxoid Ltd. UK). For *E. coli, KIebsiella* species, *Proteus* species and other enterobacteriaece, the discs that were used were amoxicillin (Amx), co-trimoxazole (SXT), gentamicin (CN) amikacin(AK) nalidexic acid (Na), nitrofurantoin (Nf.), netilmycin (NET) ciprofloxacin (CIP), pivmecillinum (Mel), cephradine (CL), ceftriaxone (CRO), ceftazidime (CAZ), imipenem (I), aztreonam (ATM). azithromycin (Az). For *Pseudomonas* species gentamycin (CN), ciprofloxacin (CIP), aztreonam (ATM), ceftazidime (CAZ), ceftriaxone (CRO), netilmycin (Net), amikacin (AK), cefoxitine (Cef), imipenern (I) were used. ESBL was detected by phenotypic method named as double disc diffusion test[10] and by E test[3]. *E. coli* ATCC 25922 as negative control and *K. pneumonia* ATCC 700603 as positive controlled were used.

Results

A total number of 92 surgical wound samples were collected of which 68(73.9%) samples were culture positive (Table 1).

Table 1: Distribution of Surgical wound Samples according to Culture Result (n=92)

Culture	Frequency	Percentage
Growth Positive	68	73.9
Growth Positive	24	26.1
Total	**92**	**100.0**

Out of 68 isolated organisms, majority were *E. coli* (29.4%) followed by *Pseudomonas* species (22.1%), *Staphylococcus aureus* (22.1%), *Klebsiella* species (13.2%) and *Proteus* species (13.2%). Interestingly, most of the *E. coli* was ESBL positive (55.0%). Klebsiella species was 33.1% ESBL positive. ESBL positivity of *Proteus* and *Pseudomonas* species were low (11.1%) (Table 2).

Among different samples, 16(30.19%) ESBL positive strains were isolated from surgical/traumatic wound. Among the isolated *E. coli*, 11(55%) of the 20 strains isolated from wound swabs were ESBL producers. Among the isolated *Klebsiella species* three (33.33%) of the nine strains isolated from wound swab were ESBL producers. Among the isolated *Pseudomonas* species, 1(6.67%) of the 15 strains isolated from wound swab was ESBL producers. Among the isolated *Proteus* species, 1(11.11%) of the nine strains isolated from wound swab was ESBL producers. ESBL positivity was significantly found in surgically wound samples (p=0.0001) (Table 2). Among the ESBL producers, all the *E. coli*, *Klebsiella* species, *Proteus* species and *Pseudomonas* species were resistant to amoxicillin, cephradine, ceftriaxone, aztreonam, ceftazidime and cefotaxime. All the Gram negative bacteria were sensitive to imipenam (Table 3).

Table 2: Distribution of Samples according to Isolated Bacteria (n=68)

Isolated Bacteria	ESBL		Total	P value
	Positive	Negative		
E. coli	11(55.0)	9(45.0)	20(100.0)	0.6282*
Klebsiella spp.	3(33.3)	6(66.7)	9(100.0)	0.4927**
Proteus spp.	1(11.1)	8(88.9)	9(100.0)	0.0331**
Pseudomonas spp.	1(6.7)	14(93.3)	15(100.0)	0.0005**
Staph. aureus	0(0.0)	15(100.0)	15(100.0)	-
Total	**16(30.2)**	**52(69.8)**	**68(100.0)**	0.0001*

*Figures in parentheses represent percentage; spp.=species; *chi-square test has been performed;
**chi-square test with Fisher's exact test has been performed; p value <0.05 is statistically significant

Discussion

ESBLs are the enzymes produced by a variety of organisms like enterobacteriacace and *Pseudomonas aeruginosa*[4]. Failure to detect these enzymes has contributed to their uncontrolled spread and therapeutic failure[11]. A total number of 92 surgical wound samples were collected of which 73.9% samples were culture positive; however, majority were *E. coli* (29.4%) followed by *Pseudomonas* species (22.1%), *Staphylococcus aureus* (22.1%), *Klebsiella* species (13.2%) and *Proteus* species (13.2%). Interestingly, most of the *E. coli* was ESBL positive (55.0%). Klebsiella species was 33.1% ESBL positive. ESBL positivity of *Proteus* and *Pseudomonas* species was low (11.1%).

A study in Bangabandhu Sheik Mujib Medical University by Mostaqim[12] found 69.4% bacteria from various samples and among them 90% was Gram negative and 10% were Gram positive bacteria. Among the Gram negative bacteria, 30.9% were ESBL producers. Among the isolated bacteria, 40.6% *E. coli*, 18.44% *Proteus* species, 12.80% *Pseudomonas* species and 7.19% were *Klebsiella* species, which correlated with the findings of the present study. In contrast to the findings of the present study, over all isolation rate of bacteria were more in the study of Mostaquim[12]. This might be due to the fact that most of the patients of the present study were hospitalized patients, who were taking antibiotics for at least five days.

Table 3: Antimicrobial drug resistance among the ESBL producing organisms

Antibiotics	*E. coli* (n=11)	*Klebsiella* (n=3)	*Proteus* (n=1)	*Pseudomonas* (n=1)
Amoxicillin	11(100)	3(100.0)	1(100)	1(100)
Cotrimoxazole	9(81.8)	3(100.0)	0(0.0)	-
Gentamycin	7(63.6)	2(66.7)	0(0.0)	0(0.0)
Ciprofloxacin	4(36.4)	1(9.1)	0(0.0)	0(0.0)
Cephradine	11(100)	3(100)	1(100)	1(100)
Aztreonam	11(100)	3(100)	1(100)	1(100)
Amikacin	10(90.9)	2(66.7)	1(100)	0(0.0)
Netilmycin	8(72.7)	2(66.7)	0(0.0)	0(0.0)
Piperacillin	-	-	-	0(0.0)
Carbenicillin	-	-	-	1(100)
Ceftriaxone	11(100)	3(100.0)	1(100)	1(100)
Ceftazidime	11(100)	3(100.0)	1(100)	1(100)
Cefotaxime	11(100)	3(100.0)	1(100)	1(100)
Imipenem	0(0)	0(0)	0(0)	0(0)

*Figures in parentheses represent percentage

From wound samples, 29.41% *E. coli* and 13.24% *Klebsiella spp.* were isolated, of which 55% and 33.33% were ESBL producer respectively. Alim[13] found 16.7% *E. coli* and 18.2% *Klebsiella* species and among them 65.4% and 37.5% were ESBL producers respectively. This finding coincides with the findings of the present study. A total of 15(22.06%) *Pseudomonas* species were isolated from surgical wound of which 6.7% were ESBL producer. Alim[13] found 6.7% *Pseudomonas* species in wound swab and none of them was ESBL producer. Among the Gram negative bacteria, the percentage of ESBL production is lowest in case of *Pseudomonas* strains, although *Pseudomonas* species shows more resistance. This may be due to *Pseudomonas* species has many determinants of pathogenicity other than ESBL that mediate resistance against antibiotics. Among 29 the isolated *Proteus* species 13.2% was isolated from wound swab and among them, 11.1% were ESBL producer. These findings agree with the finding of Alim[13] who found 18.8% *Proteus* species in wound swab and among them, 16.7% were ESBL producers. Alim[13] found 22.28% *Pseudomonas* species of which 4.9% were ESBL producers.

Lower rate of ESBL producer among *Pseudomonas* species might be due to the fact that they exhibit multiple mechanism of drug resistance simultaneously other than ESBL such as, efflux pump, AmpC enzyme mutation of porin proteins, Metallo β-lactamases etc (Virginia and Quinin, 2004). Increased production of AmpC enzyme (β-lactamases) leads to resistance during course of treatment; therefore, these enzymes are resistant to clavulanic acid that is used to detect ESBL producing bacteria in double disc diffusion and phenotypic confirmatory method[14]. Among 68 isolated ESBL producing strains, 100% were resistant to 3rd generation cephalosporins, aztreonam and amoxicillin. Against ciprofloxacin *E. coli* showed 39.5%, *Klebsiella spp.* showed 42.9% *Proteus* species showed 28.6%., and *Pseudomonas* species showed. 22.2% resistance.

Higher resistance to other antibiotics like cephradine, cotrimoxazole, gentamycin, amikacin against ESBL producer were observed in this study which indicates that ESBL produing organisms are multidrug resistant and genes that code for ESBL are linked to other resistance genes[5]. In this present

study, sensitivity of ESBL strains to cephamycin were 92.10%, 85.71%, 100% and 66.7% for *E. coli, Klebsiella, Proteus* and *Pseudomonas* species respectively. ESBL strains were 100% sensitive to imipenem. According to CDC, ESBL are defined as enzymes which hydrolyze 3rd generation cephalosporin and aztreonam; however, sensitive to cephamycin and imipenem[15].

When ESBL producing organisms are confirmed by NCCLS guidelines, results should be reported as resistance to all penicillin, aztreonam and cephalosporin excluding cephamycin[16]. Treatment of ESBL producing organisms can be done by imipenem or cephamycin. Imipenem is costly and not within the reach of the peoples of developing country like Bangladesh. Cephamycin is also costly and multi-dose drug. Therefore, early correct detection of ESBL producing organisms by E test ESBL method and rational use of quinolone and fluoroquinolones can limit the spread of multidrug resistant pathogens[17].

Conclusion

The present study showed a considerable number of ESBL producing organisms among the Gram negative bacteria, isolated from surgical wound samples. Sensitivity were higher in case of imipenem and cephamycin.

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