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Research Article

Detection of Extended Spectrum Beta Lactamases among Gram Negative Bacilli Isolated From Surgical Site Infection in a Tertiary Care Hospital

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ABSTRACT
Background: Surgical site infection (SSI) is a common complication that contribute to significant morbidity and mortality. The common organisms encountered in SSI are Staphylococcus aureus, Enterococci, E.Coli, Klebsiella spp and Pseudomonas. Knowledge of antibiotic susceptibility and drug resistant pattern will be of great help to treating physician and patient. This study is carried out to determine the prevalence of ESBL producing gram negative bacteria associated with SSI. **Material & Methods**: This is a prospective study carried out in a tertiary care hospital for a period of 2.5 years. 248 cases were diagnosed as having surgical site infection. Pus samples were processed for culture and antibiotic sensitivity. ESBL detection among gram negative bacteria was done by Predictor disk method and AmpC detection was done by AmpC disk test **Result**: Out of the 248 pus samples 221 were culture positive. Gram negative isolates171 (69.35%) were predominantly isolated as compared to gram positive86 (34.68%). Among the gram negative isolates 133(77.78%) were ESBL producer of which 33.33% were pure ESBL producer, 27.48% isolates shown both ESBL & AmpC production while 16.95% isolates were pure AmpC producer. These isolates were sensitivity to Imipenem & Amikacin. **Conclusion**: Present study indicated high prevance of ESBL producing bacteria associated with SSI. To prevent such type of infections change of antibiotic prophylaxis, screening for ESBL colonization, better infection control practices should be considered.

INTRODUCTION

Surgical site infection is a common healthcare associated infection. Even with advancement of infection prevention and control practices, better surgical techniques, antimicrobial prophylaxis, better wound care, surgical site infection still account for a major cause of prolonged hospital stays, morbidity and mortality Surgical site infections are infections at the site of surgery within 30 or 90 days of the operation, depending on the procedure. Procedures warranting a 90-day surveillance period for the development of a surgical site infection include breast surgery, cardiac surgery, coronary artery bypass graft with both chest and donor site incisions, coronary artery bypass graft with chest incision only, craniotomy, spinal fusion, open reduction of fracture, herniorrhaphy, hip prosthesis, knee prosthesis, pacemaker surgery, peripheral vascular bypass surgery, and ventricular shunt placement.[1]

Source of SSIs include the patient‘s own normal flora or organisms present in the hospital environment that are introduced into the patient by medical procedures, specific underlying disease, trauma or burns which may cause a mucosal or skin surface interruption. Factors which promote SSIs include length of hospital stay, obesity, Diabetes mellitus, smoking etc. The development of a surgical site infection depends on the complex interplay of many factors. Most postoperative wounds are endogenous.[2]

Common organisms associated with SSI are mostly gram positive bacteria such as Staphylococcus aureus, coagulase negative Staphylococcus, Enterococcus species[3,4] Many reports are associated with rising trend of gram negative bacilli mainly enterobacteriaceae.[5]

Isolation of gram negative bacteria may be associated with intestinal perforation, prolonged hospitalization. Many of these gram negative bacteria are ESBL producing. Risk factor for the development of such type of infections are prolonged hospital stay, inadvert

antibiotic use, previous colonization with such organism[6]

The beta lactam antibiotics are the commonly prescribed antibiotics especially given as an empirical therapy in intensive care units. Most of these beta lactam antibiotics had developed resistance by producing beta lactamase and it's a major concern in these days. The Extended spectrum beta lactamase (ESBL) are plasmid mediated enzymes that hydrolyze the oxyimino beta-lactam (3rd generation cephalosporins) and monobactams (aztreonam) but has no effect on cephamycins (cefoxitin and cefotetan).[7] Infections caused by them are multidrug resistant and are very difficult to treat and led to use of an expensive broad spectrum antibiotics. Identification of the causative organism and its antibiotic sensitivity pattern along with ESBL detection can help in timely management of surgical site infections.

Aims & Objectives

1. To isolate and identify gram negative bacteria from Surgical site infection
2. To detect the ESBL production by phenotypic methods.

MATERIAL AND METHODS

The present study was a prospective study carried out in the Department of Microbiology in a tertiary care hospital. The patients who developed surgical site infection were selected from General surgery, Obstetrics & Gynaecology and Orthopaedic wards

The sample size was calculated using the formula[8]

$$N = \frac{Z^2 (1-\alpha/2) p (1-P)}{D^2}$$

P = with anticipated population proportion-10%

D = Allowed error 5%.

Confidence level 95%

Patients developing stitch abscess, episiotomy wound infections were excluded from study.

After obtaining verbal consent, pus samples were collected from 248 patients suffering from surgical site infection. Swabs were collected from deeper aspect of wound and transported immediately to the laboratory. Samples were processed for microscopy and cultured on Blood agar & MacConkeys agar. Isolates were identified based on the colony characteristic, biochemical reactions as per standard microbiological protocols[9,10]

antibiotic susceptibility testing was done on Muller Hinton agar as per CLSI guidelines.[11.12] ESBL detection was done by predictor disk method.[13] Antibiotic disk were placed as shown in figure.

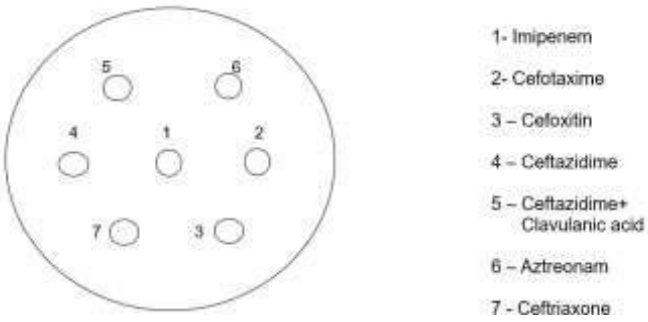

Figure 1

ESBL producers showed resistance to cefotaxime, ceftazidime, ceftriaxone and susceptible to cefoxitin. Increase in zone diameter by 5 mm with addition of inhibitor. Zone of Ceftazidime (30µm) and clavulanic acid (10 µm) > zone of Ceftazidime by 5mm.

Screening of Amp C was done if isolate is showing blunting of zone of any cephalosporin towards Imipenem and no increase in zone with addition of inhibitor. Confirmation of AmpC is done with AmpC disk test[14] the combination with clavulanic acid bringing the susceptibility back confirms the ESBL production. A standard ESBL producing organism is usually susceptible to cefoxitin. If there is an improvement with clavulanic acid, but not to the completely susceptible range, it would suggest either a depressed AmpC + ESBL or could also suggest the presence of an ESBL with several other (non-AmpC) enzymes. High level AmpC production has minimal effect on activity of cefepime making this drug more reliable for ESBL detection in presence of AmpC. Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603 for ESBL, Staphylococcus aureus ATCC 25923, were used as control strains.

RESULT

248 cases were diagnosed as having surgical site infection. Among them79 (31.85%) were male and 169(68.15%) were female. Predominantly cases were from Obstetrics & gynecology ward. Common surgery infected was LSCS

Common age group affected was 21-30 yrs. gram negative isolates were predominant as shown in the graph Staphylococcus aureus was the predominant organism isolated. Other organisms isolated as shown in chart

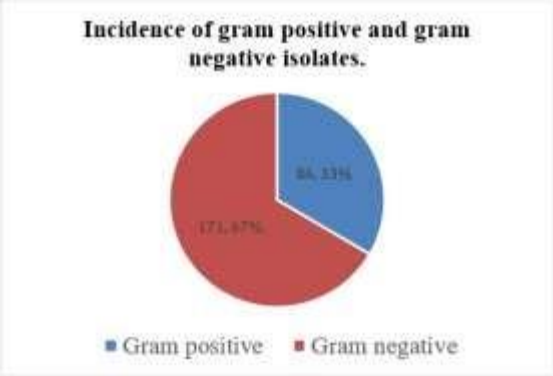

Figure 2

Figure 3

All the gram-negative isolates in the study exhibited maximum sensitivity to Carbapenems Imipenem,(93%) Amikacin(71%), Gentamicin(66%) and lesser sensitivity to 3rd generation cephalosporin's(14.15/%)

ESBL detection were observed as shown in the following table

Table no. 1 Incidence of ESBL, AmpC, production in Gram negative Isolates (n= 171)

Organisms	ESBL	ESBL + AmpC	AmpC
E.coli N=50	18 (36%)	13 (26%)	
Acinetobacter baumannii N=46	6 (13.4%)	21 (45.65%)	5 (10.86%)
Klebsiella spp. N=42	19(45.23%)	9 (21.42%)	7 (16.67%)
Citrobacter spp. N=12	5 (41.67%)	3 (25%)	3 (25%)
Pseudomonas aeruginosa N=9	4 (44.44%)	0 (0)	0 (0)
Proteus spp. N=8	5 (62.50%)	0 (0)	3 (37.5%)
Enterobacter spp.	0 (0)	1 (25%)	3 (75%)

N=4			
Total N=171	57(33.33%)	47(27.48%)	29(16.95%)

ESBL producing isolates n= 57 shown maximum susceptibility to Imipenem (100%) followed by Amikacin (75.95%), Gentamicin (47.36%) Ciprofloxacin (45.61%), tetracyclin(24.56%), Trimethoprim sulphamethaxazole(24.56%) Pure AmpC producing isolates n= 29 shown maximum susceptibility to Imipenem (100%) followed by Amikacin (57.60%), Gentamicin (34.62%) Ciprofloxacin (26.92%), Tetracyclin(15.38%), Trimethoprim sulphamethaxazole(3.85%) Both ESBL and AmpC producing isolates n= 47 shown maximum susceptibility to Imipenem (100%) followed by Amikacin (59.57%), Gentamicin (29.79%) Ciprofloxacin (8.51%), Trimethoprim sulphamethaxazole(6.38%) tetracyclin(4.25%)

DISCUSSION

Surgical site infection represents a substantial burden of diseases for patients & health services. Although the total elimination of wound infection is not possible, a reduction in the infection rate to a minimum level & spread of resistant pathogens could have significant benefits in terms of both patients comfort & resources used.

The incidence of post-operative wound infection in India ranges from 4.04- 30%.[15,16,17] In the present study pus samples were collected from the total 248 cases of post operative wound infection. Maximum pus samples were collected from the Obstetrics & Gynaecology ward (59.27%) followed by General Surgery ward (29.03%) and Orthopaedics ward (11.69%). Predominant number of cases were operated for LSCS (30.24%). LSCS is commonly associated with the development of wound infections as various obstetric variables like prolong rupture of membrane, chorioamnionitis, and meconium increases the chances of infection.[18] In this study more number of cases were from the age group of 21- 40 yrs (56.26 %) followed by 41-60 yrs (27.82%). It indicate working age group is affected. Many prospective studies on post operative wound infection pointed out that there is no evidence of relation of sex with infection of operative wound[19,20]. In this study higher incidence of infection was found in females as compared to males. This is due to the fact that more no. of cases occurred in Obstetrics & Gynaecology ward. Mawalla et al[21] also showed female preponderance. Gram negative isolates (69.35%) were predominant as compared to Gram positive isolates (34.68%). This finding is in consistence with Sonawane J et al (2008)[22] & Mohanty S et al (2004)[23] The most common bacterial isolate in our study was Staphylococcus aureus. Among the gram negative isolates E.coli were predominant followed by Acinetobacter baumannii, klebsiella spp. Our observations are comparable to Wassef MA et al[24] and Shahane V et al[25]

Acinetobacter spp is an emerging pathogen. Recently many workers have reported it's association with wound infection and Health Care Associated Infection's. We observed higher rate of Acinetobacter spp. association with wound infection. The pattern of organisms isolated in wound types in the present study suggest the skin colonizers to be the main source of infection in clean procedures. The higher incidence of Gram negative bacilli in the clean contaminated wounds demonstrates the profound influence of endogenous contamination from the bowel and hollow muscular organs. Higher incidence of Acinetobacter spp may suggest hospital acquired infection Present study demonstrated highest number of beta lactamases producing isolates the third generation Cephalosporins are routinely used in inpatient & outpatient setting. Injudicious use result in selective pressure & emergence of drug resistance mechanism in organisms Plane ESBL production was associated with Proteus spp (62.50%), Klebsiella spp(45.23 %), Pseudomonas aeruginosa(44.44%), E.coli(36%). Our result are in comparable with other studies done with Wassef at al[24] & Shriyan et al.

Some workers had demonstrated ESBL production in the organisms belonging to enterobacteriaceae. There are many studies which have demonstrated ESBL production in Acinetobacter spp & Pseudomonas spp[26,27] ESBL producing isolates were found to be susceptible to Imipenem & Amikacin which is in consistent with the observation of Sonawane J et al[22]. AmpC production was associated maximum in Acinetobacter baumannii, E.coli, Klebsiella spp. These isolates were found to be susceptible to Imipenem(100%) and to some extent to Amikacin (57.60%). Sarma et al[28] reported 14% AmpC producing E.coli from the

surgical site infection. Presence of AmpC alters the therapeutic regimen. Higher proportion of AmpC detected in our study suggest that there is a need for the continuous monitoring of detection and control of spread of these organisms. Coexistence of ESBL and AmpC production seen predominantly in Acinetobacter baumannii (45.65 %), E.coli (26%). These isolates were susceptible to Imipenem (100%) and Amikacin (57.57%). Association of AmpC with ESBL is associated with resistance to betalactam- betalactam inhibitor combination and increased amount of resistance to other classes of antibiotics.

CONCLUSION

In present study, majority of isolates from surgical site infections were Staphylococcus aureus 62(24.12%) followed by E.coli (19.34%), Acinetobacter baumannii 46(17.90%), Klebsiella spp 42(16.34%), Coagulase negative staphylococcus16 (6.2%). Significant number of gram negative bacteria were identified to be ESBL Producers. ESBL production limits therapeutic option so detection of resistance pattern is important to suggest proper treatment and also help in preventing the spreads of antimicrobial resistance. Strict infection control policies should be made to prevent the spread of these resistant bacteria.

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