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Research Article

Occurrence of Extended Spectrum Beta Lactamase (ESBL) Producers in Post Operative Wound Infection

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Abstract: Postoperative wound infections have been an important cause of morbidity and cost burden for the patients and also the determination of antimicrobial resistant strains makes the clinicians a hectic role in management. The main objective of this investigation is to find out the ESBL producing gram negative bacilli in postoperative wound infections in various specialities. The methodology comprised the incorporation of the pus swabs and aspirates from 108 postoperative wound infection cases. The bacterial etiology was identified and antimicrobial susceptibility test was done. All gram negative bacilli resistant to 3rd generation cephalosporins were subjected to ESBL detection by double disk synergy test, E test and ESBL screening agar test. Of the 108 patients, various bacterial pathogens were recovered from 94 patients (87%). Monomicrobial and polymicrobial infection was observed in 62.8% and 37.2% patients respectively. A total of 134 isolates were recovered from 94 patients with postoperative wound infections (38.8% gram negative and 61.2% gram positive). The antibiotic sensitivity test was performed using a battery of antibiotics and ESBL production among 44.2% isolates was observed. ESBL production was detected in 47.4% of *Escherichia coli* and 50% of *Klebsiella pneumoniae*. Further, managing the infection with precise empirical treatment, policy of reviewing prescription patterns and continuous monitoring of resistance patterns of the etiological agents can help the prevention of post operative infections.
Keywords: Postoperative wound infection, Bacteria, ESBL production.

INTRODUCTION

All the wounds are contaminated by pathogens and body commensals ranging from bacteria to other infectious entities. Surgical wound infection is clinically defined as purulent discharge from the surgical wound or the insertion wound of the drain, or spreading cellulitis from the wound [1]. This type of wound is characterized by inflammation in periwound area. These are the second most common cause of nosocomial infections [2,3]. The high rate of these types of infections is associated with higher morbidity, mortality and increased health care expenses [3, 4]. In spite of the availability of newer antibiotics, surgical wound infection still ruins a menace due to secondary bacterial contamination and pervasive use of prophylactic antibiotics that lead to the emergence of multi-drug resistant bacteria [3]. These wounds are expected to heal within an expected period, depending on the category of the wound and degree of surgery.

The prime closure of a clean, surgical wound would be expected to require superficial intervention to enable healing to progress naturally and quickly without difficulties in the surgical wound [1]. This may however be complicated by infection or existing co-morbidity. The management of wound infection has become more challenging due to wide spread bacterial resistance to antimicrobials. Thus, the knowledge of the aetiological agent of wound infection has been proven to be helpful in the selection of the empirical antimicrobials for the treatment of the infection. Some significant factors influencing the incidence of subsequent infection include skin preparations, surgical techniques, timing and method of wound closure and antibiotic prophylaxis after certain types of surgery. The major classification of operative wounds infections is done on the basis of the degree of microbial contamination (clean wound, clean contaminated wound, contaminated wound and dirty or infected wound). The most common isolates in all types are

Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Klebsiella spp, *Enterococcus* spp, etc [5].

Beta-lactamases are bacterial enzymes that inactivate betalactam antibiotics [6]. The beta-lactamases inactivating all the penicillins and cephalosporins including the extended spectrum cephalosporins are called as Extended Spectrum Beta-Lactamases (ESBLs). There are almost 500 different ESBLs described, that are mutations of the classical broad-spectrum beta lactamase enzymes, initially named TEM and SHV (TEM-1, TEM-2, SHV-1). Treatment is complicated by the presence of ESBL-producing Enterobacteriaceae, because they are often multidrug resistant. Thus, infections caused by ESBL-producing Enterobacteriaceae are of serious concerns. Many ESBLs are frequently expressed in gram-negative bacteria. They confer resistance to ampicillin, amoxicillin, and other penicillin derivatives, as well as to early but not later-generation cephalosporins [7]. Advances in control of infections have not eliminated the risk of post-operative wound infections due to the emergence and spread of resistant microbes.

The condition is particularly serious in developing countries where irrational prescribing of antimicrobial agents is common. Measures including new antimicrobial production, better infection control program and rational use of existing antimicrobial agents have been suggested to reduce the problem [8-10]. This study was carried out as part of the hospital infection surveillance and control. It seeks to study the prevalence of ESBL producing Gram negative bacilli in postoperative wound infections.

MATERIALS AND METHODS

This is an analytical prospective study. Pus swab, aspirate from postoperative wound infections involving the skin and subcutaneous tissue is included; wound swab from trauma, burns, stitch abscess, episiotomy wounds, circumcision site and post operative infections of deeper tissue are excluded.

Study area and design

The study was conducted in tertiary care superspeciality teaching hospital, Puducherry. The duration of the study was one year and three months (from April 2010 to June 2011). A total of 108 postoperative patients was included in this study. Two swabs or aspirates were obtained from the patient (one for Gram's staining and another for bacteriological culture.

Sample processing

All the samples were subjected for culturing in Blood agar, MacConkey agar, Chocolate agar and Thioglycollate broth. After 24 to 48 hours, the isolates were identified by colony morphology, Gram's staining and biochemical tests. The antibiotic sensitivity test by disc diffusion (Kirby Bauer) method was performed according to the Clinical and Laboratory Standard Institute (CLSI) guidelines for all isolates [11].

Primary ESBL screening (phenotype)

Screening of ESBL procedures was done by using 3rd generation cephalosporin by disc diffusion method and also in ESBL screen agar. Confirmation of ESBL was done by double disc method, combined disc and E test [12]. Isolates showing minimum zone of inhibition ≤22mm for ceftazidime, ≤27mm for cefotaxime, ≤25mm for ceftriaxone, ≤17mm for cefpodoxime and ≤27mm for Aztreonam were identified as ESBL producers were selected for checking the ESBL production as recommended by CLSI [13]. Every isolate that showed resistance to at least one of the screening agents was tested for ESBL production [12]. The use of more than one of these agents for screening improves the detection of sensitivity. Then were tested further by ESBL screen agar where *E. coli* produced pink to burgundy, translucent colonies and *Klebsiella* produced Green to blue colonies.

Phenotypic confirmatory test by ESBL E-test

The ESBL E strips were obtained from the supplier that contains ceftazidime (TZ) gradient on one end and ceftazidime + clavulanic acid (TZL) gradient on the opposite end. The MIC range of TZ and TZL are 0.5 to 32 µg/ml and 0.064 to 4 µg/ml + 4 µg/ml respectively. After overnight growth, the organism was emulsified in saline solution to a turbidity of 0.5 McFarland standard. The suspension was spread on a Muller Hinton agar plate with a sterile cotton swab. After the plates were dried for 15 min, the E - Test strips were placed on them, after incubation at 35ºC for 18 hrs. The MIC was interpreted as the point of intersection of the inhibition ellipse with the edge of the test strip. After overnight growth, the organism was emulsified in saline solution to a turbidity of 0.5 McFarland standard. One percent (1%) v/v solution of sulphuric acid solution was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water. A quantity of 1% w/v of barium chloride solution was prepared by dissolving 0.5g of dehydrate barium chloride in 50ml of distilled water. 0.06ml of barium chloride solution was added to 99.4ml of sulphuric acid solution and mixed. Ten tubes of the turbid solution of different concentration were prepared, same type as used for preparing the test and control inoculum.

The suspension was spread on a Muller Hinton agar plate with a cotton swab. After the plates were dried for 15min, the E-Test strips were placed on them after incubation at 35ºC for 18 hrs. The MIC was interpreted as the point of intersection of the inhibition ellipse with the edge of the test. ESBL positive: a. If TZ ≥1 and TZ/TZL ≥8. b. The presence of a phantom zone or ellipse deformation; ESBL negative: If TZ <1 or TZ/TZL <8; Non determinable (ND): TZ >32 and TZL

>4; Strains showing non determinable (ND) results with TZ/TZL strips [14].

RESULTS AND DISCUSSION

During this period of study (March 2010 to July 2011), about 108 patients suspected to have postoperative wound infections were prospectively evaluated, where the pus culture yielded no growth for 14 patients. Further the potential pathogens were isolated possibly from 94 patients (87%). The majority of the patients included in this study had undergone gastrointestinal surgery (27.8%) followed by orthopedic surgery (24.1%). Out of 134 isolates, 52 (38.8%) were gram negative and 82 (61.2%) were gram positive. *Staphylococcus aureus* was found to be predominant isolate (38%) followed by *Escherichia coli* and *Klebsiella pneumoniae.* Polymicrobial isolation was possible among 35 patients (37.2%). The majority of the *E. coli* and *K. pneumoniae* strains were susceptible to Imipenem, meropenem, piperacillin/ tazobactum and amikacin. The initial confirmation of ESBL producers was positive for 9 isolates of *E. coli* and 8 isolates of *K. pneumoniae* (Table 1).

Table 1: Distribution of ESBL producing Enterobacteriaceae members among post operative wound cases

Organism	No. of ESBL producers	Percentage
Escherichia coli (n=19)	9	47.4
Klebsiella pneumoniae (n=16)	8	50
Citrobacter spp. (n=4)	2	50

The description of the ESBL producers of E. coli isolates 9 (47.4%) yielded growth and 10 (52.6%) yielded no growth on CHROMagar. Among the K. pneumonia, 50% showed positive growth and supported ESBL formation. In comparison with E test, sensitivity of CHROM agar was 100%, while specificity was found as 87.5% and 80.8% among *E. coli* and *K. pneumoniae* respectively (Table 2).

Table 2: Identification of ESBL producer and non ESBL producer among the isolates on CHROMagar

Results of growth	CHROMagar			
	Escherichia coli		*Klebsiella pneumoniae*	
	No. of isolates	Percentage	No. of isolates	Percentage
Growth	9	47.4	8	50
No growth	10	52.6	8	50
Specificity	87.5%		80.8%	
Sensitivity	100%		100%	

The colony morphology of ESBL screen agar where *E. coli* produced pink to burgundy, translucent colonies and *K. pneumoniae* produced Green to blue colonies showed 100% sensitivity. Regarding the data results of E strip test, the plates after 15min of inoculation, the E-Test strips were placed on them and incubated at 35ºC for 18 hrs. The MIC was interpreted as the point of intersection of the inhibition ellipse with the edge of the test. All the test isolates were shown positive to ESBL production by observing TZ ≥1 and TZ/TZL ≥8 and the presence of a phantom zone or ellipse deformation confirmed the same.

The alarm of emerging antibiotic resistance among pathogenic bacteria has become apparent in most of our hospital environments and it is a matter of great concern leads to major hospital acquired infections. This may result due to misuse of antibiotic by the clinicians, incomplete antibiotic course completion by the patients and counter sales which result in the production of β-lactamase enzymes. Out of one hundred and thirty four isolates identified in this study only 19 isolates among gram negative bacterial groups were considered and eligible for the detection of ESBL formation by using the antibiotics tested (TZ/TZL).

The bacterial isolates including *Staphylococcus aureus*, *Coagulase negative S. aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *MRSA*, *Citrobacter spp*, *Pseudomonas aeruginosa*, *Proteus mirabilis* are the highest isolate in this study; which is already identified and documented in variation in the percentages [15,16].

Extended spectrum β lactamases (ESBLs) producing Gram negative bacteria are large, rapidly evolving group of plasmid mediated enzymes that is referred as emerging pathogens and may signaling a threat alarm among hospital acquired infections. Clinicians, microbiologists, infection control practitioners, policy makers and hospital epidemiologists are concerned about ESBL producing bacteria because of the increasing incidence of such infections [17]. In the present study, out of 19 and 16 *E. coli* and *K. pneumoniae* strains 9 (47.4%) and 8 (50%) showed positive to ESBL production respectively. ESBL producers were detected by E strip test (golden

method for confirmation of ESBL according to CLSI [13]. Other studies reported that 57.4% supported to *E. coli* [18, 19].

The cause of the upsurge in community acquired infections with ESBL-producing organisms is not yet clear, but associations with foodstuffs, animal consumption of antibiotics, and frequent patient contact with health care facilities need to be explored [20]. Methods to detect ESBL producing organisms from clinical specimens should have high sensitivity and high specificity combined with a short time to the reporting of results. In order to identify ESBL producing gram-negative bacilli from clinical samples, it is an easy and reliable method of using selective media and it should be an ideally achieving identification method for detecting ESBL strains in one step. At the least, it should decrease the workload and reduce the need of unnecessary confirmations [21]. Some studies highlighted that the ESBL producers among *Klebsiella pneumoniae* and *Serratia* species that had transferable plasmids encoding a mutated enzyme that made the bacteria resistant to ceftazidime. Since then, these enzymes have been described in isolates of *Escherichia coli* recently [22, 23].

CONCLUSION

Preoperative antimicrobial prophylaxis in the appropriate dose, time and duration, selected on the basis of the antimicrobial susceptibility pattern of the most common isolate in the hospital, would ensure a decreased rate of postoperative wound infections. Therefore, there is a need for continuous monitoring to determine the sensitivity pattern of the common isolates which are found in the hospital environment.

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