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Research Paper

Isolation of Extended Spectrum Beta Lactamase Producing Enterobacteriaceae from Wound Infections in a Tertiary Care Hospital.

Medical Science

KEYWORDS : ESBL, enterobacteriaceae, wound infection

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ABSTRACT

Wounds can be broadly categorized as having either an acute or chronic etiology. A total of 140 patients with wound infection attending Surgical, Orthopaedic and Plastic Surgery Departments in a Tertiary Care Hospital,Chennai were studied from June 2007-May 2008.Specimens obtained from patients with acute and chronic wound infection, were analysed for bacteriological profile and antimicrobial susceptibility pattern. Enterobacteriaceae isolates obtained were analysed for ESBL production. Out of the 140 specimens collected 149 aerobic isolates and 50 anaerobic isolates were obtained. Of the 149 aerobic isolates 74 were identified as enterobacteriaceae. 30 (40.54%) of the 74 enterobacteriaceae isolates were found to be ESBL producers. 30% and 66.6% of the ESBL producers were sensitive to ciprofloxacin and amikacin respectively. All the ESBL producers (100%) were sensitive to imipenem. This study emphasizes the need for routine evaluation of ESBL producers among clinical isolates which is essential in formulating treatment guidelines and infection control measures.

Introduction

Infection in a wound delays healing and may cause wound breakdown, herniation of the wound and complete wound dehiscence[1]. The patient suffers increased trauma, treatment costs rise and general wound management practices become more resource demanding. The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by extended spectrum beta lactamase (ESBL) producing strains, polymicrobial flora and by fungi.

ESBLs are now found in a significant percentage of Escherichia coli and Klebsiella pneumoniae strains. They have also been found in Pseudomonas aeruginosa and other enterobacteriacae strains like Enterobacter, Citrobacter, Proteus, Morganello morganii, Serratia marsescens, Shigella dysenteriae.[18] ESBL (Amber class A penicillinases) that hydrolyse and cause resistance to oxyamino cephalosporins(extended spectrum cephalosporins) and aztreonam.Production of these enzymes is either chromosomally mediated or plasmid mediated. Point amino acid substitution of the classical plasmid mediated beta lactamases like TEM-1 TEM-2 and SHV-1 increases the spectrum of activity from earlier generation beta lactams to 3rd generation cephalosporins and monobactams. However, they have no detectable activity against cephamycins and carbapenems and are inhibited to an extent by beta lactamase inhibitors (clavulanic acid, sulbactam and tazo bactam). Today over 575 different ESBLs have been described.

Materials and Methods

Clinical samples like pus, tissue material and discharge from the incised lesions or ulcers were collected from the patients using sterile syringe and needle, sterile punch biopsy forceps and sterile swabs respectively for aerobic and anaerobic culture. A Gram stained direct smear of the specimen was examined. The specimens were cultured on Blood agar plate and MacConkey agar plate for aerobic culture and on thioglycollate broth,Robertson cooked meat broth(RCM),Blood agar(BA), Neomycin blood agar(NBA) and Bacteroides bile esculin agar plates(BBEA) for anaerobic culture. The aerobic organisms cultured from specimens were identified based on colony morphology, Grams staining, motility and standard biochemical reactions[4]. The anaerobic organisms were presumptively identified based on aerotolerence, Gram stain and colonial morphology-(Level I identification)[20]. The antimicrobial susceptibility testing was done for aerobic isolates according to Kirby Bauer method. [5] in Mueller Hinton agar medium.

ESBL Detection

Enterobacteriaceae isolates with zone inhibition diameter ≤27mm for cefotaxime and ≤22mm for ceftazidime were further tested for ESBL production by following methods.

Double Disc Diffusion Synergy Test (DDST)[9]

In the DDST synergy was determined between a disk of augmentin (20µg amoxycillin and 10µg clavulanic acid)and a 30µg disk of third generation cephalosporin(cefotaxime/ceftazidime) placed at a distance of 15mm apart (center to center) on a lawn culture of the resistant isolate under test on Mueller Hinton agar plate. A clear extension of the edge of the third generation cephalosporin inhibition zone toward the disk containing clavunate was interpreted as synergy indicating the presence of an ESBL.

Phenotypic Confirmatory Test (PCT) :[8]

Mueller Hinton Agar plate was inoculated with a standardised inoculum (0.5 Mc Farland) [14] of test strain to form a lawn culture .The antibiotic disks used were cefotaxime and ceftazidime each 30µg alone and in combination with clavulanic acid 10 µg. The test organism were confirmed ESBL producers if the zone size around the third generation cephalosporin plus clavulanic acid increased more than 5mm in comparison to the third generation cephalosporin disk alone.

Minimum inhibitory concentration (MIC) determination

This was done by agar dilution method as per standard technique. ESBL isolates were tested for various concentration of cefotaxime (0.25µg to 2048µg/ml of agar) and ceftazidime (0.25µg to 2048µg/ml of agar) and for various concentration of cefotaxime and ceftazidime combined with 2µg/ml of clavulanic acid and the MIC determined.

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Quality control strains -Non ESBL producing organism (Escherichia coli ATCC 25922) and an ESBL producing organism (Klebsiella pneumoniae ATCC 700603) were used as controls. Statistical analysis was done using microiso analysis.

Results

The total number of patients were 140 of which 92 were males and 48 were females.(Males:Females=1:0.52).The age ranged from 18 to 70 years of age and maximum cases were recorded in the age group between 41-50years.The types of wounds were surgical site infection(18%),cutaneous abscess(18%),traumatic wound(18%), cellulitis(10%), infected chronic leg ulcer(18%) and diabetic foot ulcer(18%).

The organisms isolated from 140 wound specimens are given in **Table-1.**The p value for the same is 0.00000 which is significant.Aerobic culture yielded 149 aerobes and anaerobic culture yielded 50 anaerobes.

ESBL producers among enterobacteriaceae isolates are shown in **Table-2.**

74 enterobacteriaceae isolates were obtained and 30 (40.54%) were found to be ESBL producers,the commonest ESBL producers were Klebsiella pneumoniae(50%) and Escherichia coli(47.82%).

Among the different clinical samples ESBL producers were found most commonly in cases of Diabetic foot ulcer(45.83%), Chronic leg ulcer(44.4%) and Surgical site infection(42.85%).**-Table-3.**The p value for the same is 0.00000 which is significant. The antimicrobial susceptibility patterns of the ESBL producers are shown in **Table-4.** 30% and 66.6% of the ESBL producers were sensitive to ciprofloxacin and amikacin respectively. All the ESBL producers (100%) were sensitive to imipenem.

In the present study by agar dilution method, MIC for cefotaxime for 30 ESBL producers varied from 32 to 2048 µg/ml. When cefotaxime was combined with 2 µg/ml of clavulanic acid the MIC was reduced to 0.25µg-128µg/ml. MIC for ceftazidime for 30 ESBL producers was between 32 µg/ml -2048µg/ml. MIC was reduced to 0.25 to 8µg/ml when ceftazidime was combined with 2 µg/ml of clavulanic acid.

ESBL producers were demonstrated by different methods(DDST, PCT,MIC) and found to be positive by all three methods.(100%).

Discussion

The prevalence of ESBLs among clinical isolates varies greatly world wide and in geographic areas and is rapidly changing over time.In India the prevalence rate varies in different institutions from 28% to 84% whereas in the US it varies from 0 to 25%.

In the present study the occurence of ESBL producing enterobacteriaceae in wound infections was 40.54%. The occurence of ESBL producing Escherichia coli in the study was (11/23)47.82% and that of Klebsiella pneumoniae (9/18) was 50%. In a study by Aswhin [2] 58.06% and 43.75% of ESBL positive Escherichia coli and Klebsiella pneumoniae respectively were obtained.The prevalence of Escherichia coli ESBL producers varies in different studies ie in Leblebicioglu H [13] study (20.9%) and in Ozgunes et al [15] study (12%). ESBL's among Klebsiella pneumoniae of this study correlates with Leblebicioglu H [13](50%) and Ozgunes[15]study (47%). In a study by Shukla et al [17] 36.1% and 27.7% by Bithika et al [6] of ESBL positive Klebsiella pneumoniae were obtained.

In the present study 27.27% Klebsiella oxytoca,36.36% Proteus mirabilis, 33.33% of Proteus vulgaris and Citrobacter fruendii each were obtained as ESBL producers.36.36% of ESBL producing Proteus mirabilis obtained in this study is higher than Laura Pagani [12] study (24.82%), Khan M K R [10] study (27.77%) and Chanal C [7] study (14.2%.).33.33% of ESBL producing Citrobacter freundii obtained in this study is higher than Kumar M.S.[11] study (38/336)11.3%. This difference may be because of the less number of isolates obtained in this study.

In post operative wound infection the occurence of ESBL producers in the study were 6/22(27.27%) which correlates with Ashwin's [2] study 11/49(22.4%). In diabetic wound infection ESBL Escherichia coli 3/5 (60%) were obtained and ESBL Klebsiella pneumoniae 2/4 (50%) were obtained. This correlates with the study of Ravisekhar et al [16] which reported 54.5% Escherichia coli isolates to be ESBL producers and of Varaiya A[19] study which reported 46.51% Escherichia coli ESBL producers and 43.44%Klebsiella pneumoniae ESBL producers.

In the present study 66.6% of ESBL producer were sensitive to amikacin and 30% were sensitive to ciproflaxacin. In the study by Baby Padmini S.[3] senstivity of ESBL producers to amikacin was 82.6% and to ciprofloxacin 17.4%.All the ESBL's in the study were sensitive to imipenem (100%) which correlated with the study by Baby Padmini S. [3] From a study by Bithika et al,[6] MIC for 3rd generation cephalosporins was between 2-1024µg/ml and it was reduced to 0.25-128µg/ml when clavulanic acid was added at a concentration of 2µg/ml. So the present study correlates well with the study of Bithika et al.[6]

Conclusion:By employing proper microbiological techniques causative agents can be isolated from cases of wound infection and antimicrobial sensitivity can be assessed. This helps in the proper treatment thereby reducing the morbidity and also prevents ultimate complications like amputation as in diabetic patients.

The high percentage of ESBL producing enterobacteriaceae may be due to selective pressure imposed by extensive use of antimicrobials. Routine detection of ESBL producing microorganisms is required by reliable laboratory methods and since most of these are multi drug resistant, the therapeutic strategies to control infections in the hospital set up have to be carefully formulated. Screening for ESBL as per CLSI guidelines by disk diffusion method and confirming it by Phenotypic confirmatory test is economical, less time consuming and less skill demanding procedure that should be included in the microbiology laboratories as a routine test.

Essential infection control practices should include hand washing by hospital personnel, basic cleaning of all surface levels (hand touch sites),increased barrier precautions and isolation of patients colonized or infected with ESBL producers.A multidisciplinary approach, coordinated participation of microbiologists, clinicians, nursing personnel, hospital infection control team is necessary for management of wound infection and ESBL producing infection.

TABLE-1
Organisms isolated from 140 wound specimen.

ORGANISMS	ACUTE WOUNDS				CHRONIC WOUNDS		TOTAL n=140
	SSI n=25	Abscess n=25	Traumatic wound n=25	Cellulitis n=15	Chronic leg ulcer n=25	Diabetic foot ulcer n=25	
Escherichia coli	5	3	5	2	3	5	23 (16.4%)
Citrobacter freundii					1	2	3 (2.1%)
Citrobacter koseri						2	2 (1.4%)
Klebsiella pneumoniae	5	2	4	1	2	4	18 (12.9%)
Klebsiella oxytoca	2	2	1	1	1	4	11 (7.9%)
Proteus mirabilis	1	2	3		1	4	11 (7.9%)
Proteus vulgaris	1			1	1	3	6 (4.3%)
Pseudomonas aeruginosa	1	1	6		5	4	17 (12.1%)

Acinetobacter species			1				1 (0.7%)
Staphylococcus aureus	5	9	4	3	6	6	33 (23.6%)
Coagulase negative staphylococcus	1	2	1	2	2	2	10 (7.1%)
Beta hemolytic streptococci	1	2	2	1	1	1	8 (5.7%)
Enterococcus species					2	4	6 (4.3%)
Candida species					1		1 (0.7%)
Peptostrepto coccus species	4	7	6	2	6	6	31 (22.14%)
Bacteroides fragilis	4	2	4	1	3	2	16 (11.4%)
Clostridium species			2		1		3 (2.1%)

TABLE-2
ESBL producers among Enterobacteriaceae

Organisms	Total	ESBL	Percentage
Escherichia coli	23	11	47.82%
Citrobacter freundii	3	1	33.33%
Citrobacter koseri	2	0	0.00%
Klebsiella pneumoniae	18	9	50%
Klebsiella oxytoca	11	3	27.27%
Proteus mirabilis	11	4	36.36%
Proteus vulgaris	6	2	33.33%
Total	74	30	40.54%

TABLE-3
ESBL producing Enterobacteriaceae obtained from various wound specimens

Type of wound	Total enterobacteriaceae isolates	ESBL producers	Percentage
Surgical site infection	14	6	42.85%
Abscess	19	2	22.22%
Traumatic wound	13	5	38.46%
Cellulitis	5	2	40%
Chronic leg ulcer	9	4	44.44%
Diabetic foot ulcer	24	11	45.83%
Total	74	30	40.54%

TABLE - 4
Antibiotic sensitivity pattern of ESBL producing enterobacteriaceae.

Antibiotics	ESBL producers n=30	Percentage
Ampicillin	0	-
Cotrimoxazole	0	-
Gentamicin	0	-
Amikacin	20	66.6%
Ciprofloxacin	9	30%
Cefotaxime	0	-
Ceftazidime	0	-
Imipenem	30	100%

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