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Detection of Extended Spectrum Beta Lactamases (ESBLs) in Surgical Wound and Burn Infections

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Extended spectrum beta lactometers are the enzymes, which were mainly occurred in hospital acquired infections (nosocomial infections) due to unhygienic conditions of the hospital environment. ESBL production is usually plasmid mediated, it is possible for one specimen to contain both ESBL producing and non ESBL producing cells of the same species. This suggests that for optimal detection, several colonies must be tested from a primary culture plate. In the process of detection of these enzymes, first we isolate the organism from wound based on the culture characters and biochemical characters and proceed for antibiotic sensitivity. If the organism shows resistant, then we have proceed for test for Beta lactamases, and confirmatory tests for ESBLs.The prevalence of ESBLs in government general hospitalk kakinada and surroundings places in kakinada district is 9.33%. It is much lower than other reports from India and abrod. In our study surprisingly out of 21% of esbl positive cases, 16% of the isolates were *Proteus* species. All the isolates were found sensitive to the Carbapenem antibiotics.

Key words: ESBLs - Extended Spectrum Beta-Lactamases, 3GC-third generation cephalosporins, TEM-Temonera (gene), SHV-sulphydril variable (gene) enzymes, CONS-Coagulase negative *Staphylococci*.

Extended spectrum beta lactamases (ESBLs),as the name suggests, are beta lactamases that are capable of hydrolyzing broad or extended spectrum beta lactams.[1]Beta-Lactamases are a type of enzymes produced by some bacteria that are responsible for their resistance to Beta-Lactam antibiotics. These antibiotics have a common element in their molecular structure; a four atom ring known as a beta-lactam. A Beta-Lactam ring (β-Lactam) or penem is a lactam with a heteroatomic ring structure, consisting of these carbon atoms and one nitrogen atom. The Beta-Lactam ring is part of the structure of several antibiotic families, principally the *Penicillins, Cephalosporins, Carbapenams* and *Monobactams* which are therefore also called Beta-Lactam antibiotics[4].

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ESBLs have serine at their active site and attack the amide bond in the lactam ring of antibiotics causing their hydrolysis and consequently abolishing their antimicrobial activity. In the past it was believed that cephalosporins were relatively immune to attack by b-lactamases. It was surprising to find cephalosporin resistant *Klebsiella* spp. among the clinical isolates. The mechanism of this resistance was production of extended spectrum b-lactamases (ESBLs).[5]

Genes responsible for Beta lactamase enzyme production

Bacteria under normal conditions, Repressor gene (Amp D) keeps the inducer gene (Amp R) in check and allows the beta lactamase production in controlled quantities. When an antibiotic come in contact with a bacterium with penicillin binding proteins (PBP),Amp E (which code for PBP) converts to ampE' and inactivates the repressor gene.. By this inactivation of repressor gene the inducer gene is de-repressed

and RNA-polymerase is activated. Consequences are hyper production of beta lactometers and finally resistant against that particular antibiotic.The ESBL's are frequently plasmid encoded. Plasmid responsible for ESBL Frequently carry genes encoding resistance to other drug classes (for example, amino glycosides). Members of the family *Enterobacteriaceae* commonly express plasmid-encoded Beta-Lactamases.[3] Therefore the purpose of the present study was to detect ESbL producing strains from different wounded patients and analyse the drugs of choice for this ESBL producing strains and analyse the prevelance rate of the ESbL producing strains and multi drug resistant organisms in surgical ward of Government General Hospital, kakinada and surrounding areas of kakinada.

MATERIAL AND METHODS

Medias

Nutrient agar, Blood agar, Muller Hinton agar, IMViC media and reagents, Triple sugar iron agar, Phenyl pyruvic acid media(PPA).

Antimicrobial agents (antibiotic discs)

The following antimicrobial agents were tested: amikacin (30 mcg), amoxicillin/clavulanic acid (20/10 mcg), ampicillin (10 mcg),amoxycillin(30mcg) cefepime (30 mcg), cefotaxime (30 mcg), ceftazidime (30 mcg), ceftriaxone (30 mcg) gentamicin (10 mcg), imipenem (10 mcg), erythromycin (15 mcg), netillin (30 mcg), penicillin(100units), tetracycline(75 mcg), vancomycin(30 mcg).

Methods of detection

Specimens (pus samples) were collected from ulcers (wounds) of different pars of the body by touching the infected area with a sterile swab. Microorganism was identified based on the results of the cultural characteristics and biochemical tests and followed by Antimicrobial susceptibility testing

Antimicrobial susceptibility testing

Disk-diffusion tests were carried out with antibiotic-containing disks on Mueller-Hinton agar plate (Hi-Media).[1] The results were expressed as susceptible or resistant according to the criteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS)[3]. Isolated organism shows resistant to that particular antibiotic the fallowing reasons are predicted [4]. 1,Antibiotic modifying enzymes, 2,Alteration of targets 3,Active transport of antimicrobial agents out of the bacterial cell 4,Lack of penicillin receptors 5,Modifications of target enzymes 6, β-lactamase inhibitors. Despite of these conditions how can we say that isolate produce beta lactamases and for this we have to go for test for beta lactamases.[6]

Tests for beta-lactamases

Isolated organism was resistant to the particular beta – lactam antibiotics by producing beta-Lactamase enzymes.[1] Penicilloic acid is produced when beta-lactamases hydrolyze benzyl penicillin. It can be detected by

Nitrocefin test

Using a single disc dispenser, dispense the required number of discs from the cartridge into an empty Petri dish or onto a microscopic slide.

Moisten each disc with a drop of water

With a sterilized loop or applicator stick remove several well isolated colonies and smear onto a disc surface. Detection of beta-Lactamases depends on the color change from yellow to red produced on hydrolysis of the beta-lactam ring of the chromogenic cephalosporins with in 5 mins.[6] If the test result was positive we have to proceed for the confirmatory tests for ESBL's.

Confirmatory tests for ESBLS

Double disc synergy test

In this test discs of 3rd Generation Cephalosporins (3GC) and augmentin (3GC with beta lactamases inhibitor) are kept 30 mm apart from center to center on inoculated Mueller-Hinton Agar (MHA). A clear extension of the edge of the inhibition zone of cephalosporin towards augmentin disc. Distortion of the zone size in synergistic fashion indicates that isolate was positive for ESBL production. [1]

RESULTS AND DISCUSSION

Total of 75 patients admitted in the surgical ward of Government General Hospital, Kakinada were studied for the detection of Extended spectrum Beta lactamases among wound infections & burn infections during the period of May and June 2007.

The total number of 75 isolates 73 cases were culture positive, Remaining 2 cases were negative cases. The study of etiological causes in surgical wound infections and prevalence of beta lactamases shows the following from results.

In our study it was observed that more number of males were affected than females with surgical wounds and the most common isolate in these cases is *Pseudomonas* sp. Regarding the age wise distribution of surgical wound cases, maximum number of cases seen at the age of 30-45 years. It was observed that *Pseudomonas* species are the predominant organism with a positive of 22 cases, causing wound infections inpatients . The next common isolate in surgical ward in our study is *Staphylococcus* species, those are *S. aureus* and Coagulase Negative *Staphylococcus* with positive culture of 25 samples. The other bacteria that were isolated were, *E.coli. Proteus* species *Acinetobacter, Klebsiella* species were isolated from 9 cases.

It was observed that 95% of the isolates were multidrug resistant. 56% of the isolates showed resistance or decreased susceptibility to at least one of the three 3GC used for the study. 21% of the isolates should resistant to all the three 3GC antibiotics (Cephalosporins, Cefotaxime, Ceftriaxone) and this resistant to all the three 3GC was found to coexist with resistant to other antibiotics. Reports of ESBL in *proteus* spp is relatively rare because of low frequency of plasmid conjugation[1,2]. Surprisingly out of 21%, 16% of the isolates were *Proteus* species. All the isolates were found sensitive to the Carbapenem antibiotics.

In addition resistance to 3GC, 70% of the isolates showed resistance to Amoxycillin, 69% to Netilmicin, and 64% to Gentamicin. In the study resistance to 3GC was found to coexist with resistance to other antibiotics, since all the isolates showed multidrug resistance, the therapeutic use of all 3GC should be against isolates that appear resistant to any such compound. Since all the isolates were sensitive to Imipenam. It might serve as the drug of choice for the treatment of ESBL producing strains. Our study highlights the emergence of ESBL producing strains endowed with extremely wide spectrum of antibiotic resistance, including resistance to Sulfonamides, Streptomycin, Gentamicin and Amikacin. the prevalance rate of this ESBL and multidrug resistant strains is 9.33%.

Table 1. Distribution of cases according to their age group and gender: total number of cases tested:175

S.No	Patients different age groups	Male	Female	Total	Percentage (%)
1	< 5 years	11	3	14	8%
2	5-15 years	28	7	35	20%
3	15-30 years	30	8	38	21.7%
4	30-45 years	35	10	45	25.71%
5	45 > years	31	9	40	22.85%
6	0-45> years	135	37	172	98.2885%

Table 2. Distribution of cases according to clinical diagnosis: total number of cases tested: 175

S. No.	Diagnosis type	Number of cases	Percentage (%)
1	Surgical wounds	46	26.28%
2	Cellulites	28	16%
3	Post Operative wounds	37	21.14%
4	Ulcers	21	12%
5	Electrical burns	16	9.14%
6	Miscellaneous a) Snake bite cellulites b) Amputation c) Etc.	24	13.71%

CONCLUSION

ESBL mediated resistance to 3GC was found in 9.33% of isolates. This prevalence rate is much lower than other placesin India and abroad. The ESBL-producing isolates in the United States has been reported to be 5%. In France and England 14 to 16% ESBL producers among clinical

Table 3. Distribution of cases according to beta lactamase and ESBL producers

S. No.	Category of Organism	No. of Cases	Percentage (%)	No. of Cases shows resistant to all Antibiotics	No of Cases produce B-lactamases	No. of Cases produce ESBLs	% of ESBL producers
1.	*Pseudomonas*	52	29.71	15	11	7	17.94
2.	*E. coli*	10	5.71	3	3	3	7.69
3.	*Klebsiella*	21	12	9	8	4	10.25
4.	*S. aureus*	32	18.28	5	5	-	-
5.	CONS	28	16	3	2	-	-
6.	*Proteus vulgaris*	14	8	10	10	6	15.38
7.	*P. mirabilis*	8	4	0	0	-	-
8.	*Acinetobacter*	7	4	0	0	-	-

isolates has been reported. In particular regions or hospitals, the incidence can reach 25 to 40%.In a previous study in central India, 76.5% of isolates resistant to 3GC antibiotics was found to produce ESBLs[1]. Propably due to indisremenate use of 3GC. During the past decade, ESBL producing strains have emerged as one of the major multidrug resistant organisms. Avoid over use or miss use of antibiotics. Unnecessary drugs may reverse the undesired effects of multidrug resistant and ESBL producing strains. In our study pre-reccurence rate is much lower because medical team conducted an educational programmes for medical staff on increase awareness on the discreminate use of 3GC.

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