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Full Length Research Paper

A study on isolation and detection of drug resistance gram negative bacilli with special importance to post operative wound infection

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Isolation and identification of the drug resistant gram negative bacilli bacteria were carried out from post operative wound infections. Out of 214 samples collected, 167 showed bacterial growth and gram negative bacilli (GNB) growth was observed in 121 samples. In the present study *E. coli* was found to be the predominant agent isolated from wound infections (37.3%), followed by *Pseudomonas* (20.9%), *Klebsiella* (17.2%), *Acinetobacter* (14.2%) and other agents were less common. It was found that out of 121 gram negative bacilli isolates, 28 were found to be extended spectrum beta lactamase (ESBL) producers and 16 were mettallo beta lactamase (MBL) producers. Out of 52 isolates tested for minimum inhibitory concentration (MIC), 7 isolates showed MIC of 8 µg/ml, 42 isolates showed MIC of 0.5 µg/ml and other isolates showed intermediate concentration. All the ESBL and MBL positive isolates showed phantom zone in E-test.

Key words: Antibiotic resistance, post operative wound infection, beta lactamase, minimum inhibitory concentration.

INTRODUCTION

Wound infections are recognized as potentially serious complications in patients subjected to surgical operations. Outbreaks of post-operative sepsis are common and such infection significantly leads to the severity of the patient's illness.

Accurate information of the incidence, etiology and source of infections acquired within the hospital is essential for prevention (David et al., 2009).

The development of a post operative wound infection depends on the complex interplay of many factors. When there is a decrease in integrity and protective function of the skin, large number of different pathogens will enter into the wound and initiate an inflammatory response characterized by the classic signs of redness, pain, swelling, raised temperature and fever. This process ultimately aims to restore homeostasis. Most post-operative wounds are endogenous and are acquired from the skin, mucous membranes or gastrointestinal tract of the patient (Green et al., 1976). Exogenous infections are mainly acquired from the nose or skin flora of the operating team and transmitted on the hands of the surgeon or through the air directly or indirectly from instruments. Infections are mainly caused by *Staphylococcus aureus*, coagulase negative *Staphylococci* or occasionally by other organisms (gram negative Bacilli) and in clean wounds the infection rate is usually low (1 to 3%) (Kamat et al., 2008).

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of post operative wound infections. However, over the years, *via* several constantly changing mechanisms, many bacterial species have acquired resistance to the most common classes of antibiotics. Resistance of bacteria to an antibiotic can either be natural or acquired (Jacoby and Medeiros, 1991). The main challenges are to limit the spread of existing resistance and to detect novel resistance early enough to try and prevent the spread of new resistant genes.

The most widely used antibiotics for treatment of post operative wounds are -lactams due to their broad spectrum of activity, safety profile and proven clinical efficacy (Ayyagari and Bhargava, 2001). Three principal mechanisms cause resistance to -lactams: 1) a

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reduction in the affinity of the drug targets (penicillin-binding proteins) via amino-acid substitution, a phenomenon occurring in both gram positive and gram-negative bacteria; 2) Gram negative species, alteration in outer-membrane permeability that denies passage to the -lactams and 3) in both Gram-positive and Gram-negative bacteria, the production of -lactamases that inactivate the drug through hydrolysis of the -lactam ring (Johnnie et al., 2009).

Their widespread use, however, has resulted in the emergence and rapid spread of resistance. The past decade has seen an increase in the frequency of resistance to modern antibiotics, including the "third-generation" cephalosporins due to overuse or misuse of antibiotics (Chaudhary and Aggarwal, 2004). Bacterial resistance has greatly hampered effective treatment of patients in clinical settings. Many works have been carried out on detection of drug resistance of bacterial species (Chaudhary and Aggarwal, 2004, Xiao et al., 2008). In this study we have attempted to isolate and identify the drug resistant gram negative bacilli and to determine the rate of post operative wound infection.

MATERIALS AND METHODS

Source of samples

Patients admitted to J.S.S. Hospital, Mysore, India, who had undergone surgical procedures and were clinically suspected as cases of post operative wound infections formed the source of samples for this study. The study was conducted over a period of 3 months and 214 pus samples were obtained from different departments of JSS hospital. These pus samples were used to culture the pathogens and the pathogenesis was studied. All the personnel and clinical data including the antibiotics administered were recorded before taking the sample. The details about the patients (name, age, sex, ward and wound site) were recorded along with the history of infection. 129 samples received in the study were from (cases of wound infection) general surgery ward, 31 samples were from orthopedic ward and remaining 52 were from other departments.

Collection of samples

The wounds were examined for suggestive signs/symptoms of infection in the post operative period, during wound dressing or when the dressings were soaked. When infection was clinically suspected, the area around the surgical wound was cleaned with 70% ethyl alcohol. The exudates (pus sample/aspirate) were collected from the depth of the wound using two sterile cotton swabs. All the samples collected were immediately transferred to the laboratory for further processing and the isolates were preserved under refrigerated conditions.

Culture methods for identification

Collected samples were subjected to direct microbial observation using Gram staining method. All the samples were inoculated on a sterile Mackonkey agar and Blood agar plates and the plates were incubated at 37°C for 18 to 24 h. Based on growth in MacConkey agar isolates were identified as Lactose Fermenting (LF - pink colonies) and non lactose fermenting (NLF - colorless colonies) gram negative bacilli. For further isolation following standard identification tests were used. The growth on blood agar was observed for colour and the type of haemolysis seen around the colony. Biochemical tests such as indole test, urease production, citrate utilization, triple sugar iron tests were carried out identification of gram negative Bacilli.

Antibiotic susceptibility test

Antibiotic susceptibility tests were performed as per Murray et al. (1995) method. This was further confirmed by determining the minimum inhibitory concentration (MIC) of imipenem by agar dilution using concentrations ranging from 0.5 to 8 g/ml in Mueller-Hinton agar. *E. coli* obtained from American Type Culture Collection (ATCC 25922) was used as control. All the isolates were subjected for antibiotic sensitivity testing by disk diffusion method. Using ampicillin, gentamicin, amikacin, cefotaxime, ceftriaxone, piperacillin, imipenem, meropenem, piperacillin, piperacillin+tazobactum, ceftazidime, imepenem disks.

A fresh subculture of each isolate was suspended in a sterile bottle containing 5 ml peptone water, and incubated overnight at 37°C. The overnight broth cultures were diluted with sterile saline to 106 colony forming unit/ml by adjusting the turbidity of the inoculum to 0.5 McFarland turbidity standards. A sterile cotton-tipped applicator was introduced into standardized inoculum and used to inoculate Mueller-Hinton agar plates. Sterile antibiotic discs were placed 15 mm apart on each plate and incubated for 24 h aerobically at 37°C. Zone diameter of inhibition of each isolate to the disc was read with a calibrated ruler and compared with zone diameter interpretive standard of the National Committee for Clinical Laboratory Standards (NCCLS) to determine sensitivity or resistance. The NCCLS values were cross-checked for correctness with the new guidelines for antibiotic disc susceptibility test of the Clinical and Laboratory Standards Institute.

Detection of drug resistance mechanism

Detection of ESBL isolates

In this test disc of third generation Cephalosporins and Augmentin are kept 30 mm apart from center on inoculated Mueller-Hinton Agar (MHA). A clear extension of the edge of the inhibition zone of cephalosporin towards augmentin disc is interpreted as positive for ESBL production (Chaudhary and Aggarwal, 2004). This test provides the advantage of simultaneous determination of antibiotic susceptibility and -lactamase substrate profile. Inoculum produced in this method contains between 109 and 1010 CFU/ml of cells that actively produce -lactamase. Two types of inocula are prepared one disc diffusion test inoculum (optical density equal to that of 0.5 McFarland standard) and a three dimensional inoculum (contain between 109 and 1010 CFU of cells). Plate is inoculated by disc diffusion procedure. A circular slit is cut on the agar 4 mm inside the position at which the antibiotic discs are placed. Conventional (two dimensional) disc diffusion susceptibility test results are measured according to the recommendations of NCCLS. Distortion or discontinuity in the circular inhibition zone is interpreted as positive for ESBL production.

Double disc synergy test (DDST) / Disc approximation method:

In DDST, synergy was determined between a disc of -lactamase inhibitor (Ac) and a 30 mg disc of each of third generation cephalosporin's (Ca and Ce) test antibiotic placed at a distance of 30 mm apart on lawn culture of the resistant isolates on

Muller- Hinton Agar. The test organism was considered to produce ESBL, if the zone size around the antibiotic disc increased towards the - lactamase inhibitor disc. This increase occurs because the clavulanic acid inactivates the ESBL produced by the test organism resulting in the formation of extended inhibitory zone (Hirakata et al., 2006*)*.

Detection of MBL isolates

Imipenem resistant strains were checked for production of metallo – -lactamase (MBL) by the modified Hodge test and EDTA disc synergy test. Modified Hodge test was carried out using Mueller-Hinton agar medium. The plate was inoculated using a cotton swab dipped in an overnight culture suspension (after adjusting the turbidity of 0.5 McFarland turbidity standards) . 10 g imipenem disc was placed at the center of the plate and test strains were streaked from the edge of the disc to the periphery of the plate in four different directions. After overnight incubation the plates were observed for the presence of a 'cloverleaf shaped' zone of inhibition. The plates with such zones were interpreted as Modified Hodge test positive / MBL producers.

For the EDTA-disk diffusion synergy test an overnight broth culture of the test strain, (turbidity adjusted to 0.5 McFarland turbidity standards) was used to inoculate a plate of Mueller- Hinton agar. A 10 g imipenem disc and a blank filter paper disk (6 mm in diameter, Whatmann filter paper no. 2) were placed next to each other and 10 l of 0.5 M sterile EDTA solution was applied to the blank disc. The previously prepared EDTA-Imepenem disc and the Imepenem disc were also placed on the test culture, 30 mm distance apart. After overnight incubation, the presence of an enlarged zone of inhibition around the ready EDTA-Imipenem discs was interpreted as EDTA synergy positive (Yan et al., 2001).

Detection of minimum inhibitory concentration (MIC)

The isolates which were found to be MBL / ESBL producers, MIC for imipenum were detected by agar dilution method and E-test.

Agar dilution method

The lowest concentration of the antimicrobial agent that inhibited the visible growth of microorganisms was taken as MIC. Sterilized Muller Hinton agar was allowed to cool to 50°C, a dilution series of antimicrobial agent (Imipenem) was prepared in sterilized test tubes and a drug-free control was included in the experiment. About 19 ml of molten agar was added to each test tubes and mixed thoroughly. The agar was poured into the pre-labelled sterile Petri dishes and was allowed to set at room temperature.

The density of inoculums was standardized to give 100 colony-forming units (CFU) per spot on the agar. The inoculum was prepared by diluting a broth culture. A 0.5 McFarland standard was used for visual comparison to adjust the suspension to a density equivalent to approximately 108 CFU/ ml. The suspensions of organisms were diluted in 0.85% saline or broth to give 107 CFU/ ml. Plates were inoculated within 30 min after standardizing the inoculum, to avoid changes in inoculums density. Inoculum was transfer to the series of agar plates, including a control plate (without antimicrobial agent). The plates were incubated at 37°C for 18 h.

E-test

Confirmation of ESBL was done by E-test ESBL strips based on the MIC values. Double-ended strips containing gradient of cefotaxime (CT) or ceftazidime (TZ) at one end and cefotaxime or ceftazidime plus clavulanic acid (CTL and TZL) at the other end were tested in parallel. The presence of ESBL was confirmed by the appearance of phantom zone below CT or deformation of TZ inhibition ellipse or when clavulanate caused a more than or equal to three doubling concentration decrease (ratio of 8) in the MIC values of cefotaxime and ceftazidime. Confirmation of MBL was also done by E-test ESBL strips based on the MIC values. Double-ended strips containing gradient of Imepenem (IP) at one end and Imepenem overlaid with EDTA (IPI) at the other end were tested in parallel. The presence of MBL was confirmed by the appearance of phantom zone below IP or a reduction in the Imipenem MIC in the presence of EDTA of greater than or equal to eight-fold (IP/IPI >= 8) was interpreted as indicating MBL activity.

RESULTS AND DISCUSSION

Distribution of bacterial growth

Out of 214 samples 167 showed bacterial growth and 47 remained sterile even after 48 h of incubation. High rate of bacterial growth was seen in the samples collected from surgery wards (106/214), followed by other departments (35/214), orthopedic ward (26/214). Among the 167 positives cultures, Gram negative bacilli (GNB) growth was observed in 121 samples, mixed growth of GNB and Gram positive cocci (GPC) was seen in 18 samples and only GPC growth was observed in 28 samples. Out of 214 samples, 134 Gram negative bacilli isolates were obtained in the present study. In this study *E. coli* was found to be the predominant agent isolated from wound infections (37.3%), followed by *Pseudomonas* (20.9%), *Klebsiella* (17.2%), *Acinetobacter* (14.2%) and other agents were less common. Table 1 shows the isolated gram negative bacilli species from pus samples.

Antibiotic susceptibility tests

Antibiotic sensitivity was observed after incubation for 24 h at 37°C as described in Materials and Methods. Table 2 shows antibiotic susceptibility pattern of Gram negative bacilli isolates**.** Majority of the *E. coli* isolates showed resistance to Cephotaxime, followed by Ciprofloxacin, Ceftriaxone. Minimum resistance was seen to Imipenem, Amikacin, Chloramphenicol and Meropenem (Table 3). The *Klebsiella* showed highest susceptibility against Piperacillin, Meropenem and Imipenem, showed maximum resistance to Ceftazidime, Ciprofloxacin, Amoxycillin and Gentamicin (Table 4). However *Pseudomonas* and *Acinetobacter* showed resistance to all the antibiotics tested (Tables 5 and 6).

Detection of drug resistance mechanism

Detection of ESBL production

Extended spectrum-lactamases (ESBLs) are plasmid

Table 1. Number of gram negative bacilli isolated.

Genus	Number	Percentage (%)
E. coli	50	37.3
Klebsiella	23	17.2
Pseudomonas	28	20.9
Acinetobacter	19	14.2
Citrobacter	3	2.2
Enterobacter	2	1.5
Proteus	8	6
Providencia	1	0.7
Total	134	

Table 2. Antibiotic susceptibility pattern of gram negative bacilli isolates.

Antibiotics	A		B		C		D		E		F		G	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Amoxyclav	23	27	13	10	8	20	14	5	3	0	1	7	0	2
Cefuroxime	8	42	2	21	1	27	3	16	2	1	2	6	0	2
Cefoxitin	17	32	8	15	0	27	2	17	0	3	0	8	0	2
Ceftriaxone	32	18	20	3	6	22	16	3	2	1	4	4	0	2
Cefotaxime	13	37	5	18	3	25	5	14	3	0	2	6	1	1
Ceftazadime	27	23	15	8	18	10	7	12	2	1	4	4	0	2
Cefipime	30	20	10	13	15	13	12	7	2	1	1	7	0	2
Amikacin	1	49	7	16	6	22	6	13	1	2	1	7	2	0
Gentamicin	13	37	12	11	16	12	10	9	3	0	3	5	0	2
Piperacillin	31	19	14	9	17	11	14	5	2	1	1	7	0	2
Pipe+ Tazo	15	35	12	11	10	18	14	5	1	2	2	6	0	2
Ciproflox	35	15	20	3	18	10	11	8	3	0	2	6	1	1
Meropenem	10	40	1	22	3	25	3	16	0	3	2	6	0	2
Imipenem	1	49	7	16	6	22	6	13	1	2	1	7	2	0
Aztreonam	25	25	17	6	10	18	8	11	3	0	3	5	0	2

^{S} Susceptible, ^{R} Resistant, Numbers - total number of isolates. A- *E. coli, B- Klebsiella, C- Pseudomonous, D- Acinitobacer, E- Citrobacter, F- Proteus, G- Enterobacter.*

Table 3. Antibiotic sensitivity of *E. coli.*

Antibiotics	Concentration (µg)	Zone diameter (mm)	Pattern
Amikacin (Ak)	30	18	S
Chloramphenicol (C)	30	18	S
Cephotaxime (Ce)	30	3	R
Ceftriaxone (Ci)	30	8	R
Ciprofloxacin (Cf)	5	4	R
Imipenem (I)	10	18	S
Meropenem (Mr)	10	14	I

^{S}Susceptible, ^{R} Resistant, ^{I} Intermediate.

mediated enzymes, most commonly found in *Klebsiella* species, and followed by *E. coli*. These enzymes are capable of hydrolyzing broad spectrum cephalosporins and monobactams but inactive against cephamycins and imipenem. In addition, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics resulting in limitation of therapeutic option. ESBLs have serine at their active site and attack the amide bond in

Table 4. Antibiotic sensitivity of *Klebsiella*.

Antibiotics	Concentration (µg)	Zone diameter (mm)	Pattern
Ampicillin (A)	30	7	R
Amoxycillin (Ac)	10	6	R
Ceftazidime (Ca)	30	2	R
Ciprofloxacin (Cf)	5	3	R
Gentamicin (G)	10	6	R
Imipenem (I)	10	18	S
Meropenem (Mr)	10	19	S
Piperacillin (Pt)	100	22	S

[S] Susceptible, [R] Resistant.

Table 5. Antibiotic sensitivity of *Pseudomonas*.

Antibiotics	Concentration (µg)	Zone diameter (mm)	Pattern
Amikacin (Ak)	30	8	R
Ceftazidime (Ca)	30	3	R
Ciprofloxacin (Cf)	5	3	R
Cefepime (Cpm)	30	3	R
Imipenem (I)	10	4	R
Piperacillin (Pc)	100	4	R
Piperacillin / Tazobactum (Pt)	100	4	R
Ticarcillin (Ti)	75	3	R

[R] Resistant.

Table 6. Antibiotic sensitivity of *Acinetobacter* (P 286).

Antibiotics	Concentration (µg)	Zone diameter (mm)	Pattern
Amoxycillin (Ac)	30	5	R
Aztreonam (Ao)	30	5	R
Ciprofloxacin (Cf)	5	3	R
Ceftriaxone (Ci)	30	3	R
Gentamicin (G)	10	3	R
Imipenem (I)	10	5	R
Piperacillin (Pc)	100	4	R
Piperacillin / Tazobactum (Pt)	100	4	R

[R] Resistant.

the lactam ring of antibiotics causing their hydrolysis. Because of inocolum effect and substrate specificity their detection is a major challenge. Two indicators of ESBLs are eight fold reductions in MIC and inhibition zone of third generation cephalosporin in the presence of clavulanic acid (Chaudhary and Aggarwal, 2004). Table 7 shows Drug Resistance pattern for ESBL positive isolates. Out of the 121 samples 18 isolates showed ESBL positive. Due to the variable affinity of these enzymes for different substrates and inoculum effect, some ESBL isolates may appear susceptible to a third generation cephalosporin *in vitro*. Cefpodoxime and Ceftazidime have been proposed as indicators of ESBL production as compared to Cefotaxime and Ceftriaxone (Nathisuwan et al., 2001). For ESBL producing bacteria there is a dramatic rise of MIC for extended spectrum cephalosporins as the inoculum is increased beyond that used in routine susceptibility (Nordmann, 1998).

Sensitivity breakpoints of MIC as designated in the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for *Klebsiella* and *E. coli* against cefotaxime, ceftriaxone and ceftazidime are less than or equal to 8 mg/mL. Some ESBL producing organisms may have increased MIC against these agents compared

Table 7. Drug resistance pattern of ESBL positive Gram negative bacilli isolates.

Sample number	A	Ac	Ao	C	Ca	Ce	Cf	Ci	Cpm	G	I	Mr	Pc	Pt	Ti
P-165							R	R	R						
P-207					R		R					R	R		
P-208							R				R		R	R	
P-218			R		R		R								
P-240	R	R			R		R			R					
P-241	R	R				R	R	R							
P-249	R	R			R		R	R							
P-260	R	R				R	R	R		R					
P-283					R	R	R			R					R
P-289		R	R		R		R	R		R			R		
P-305		R			R		R		R	R		R			
P-315		R	R				R	R	R				R		
P-321			R		R			R					R	R	
P-339								R	R						
P-344					R					R		R		R	
P-345					R		R	R				R		R	
P-347	R	R	R	R	R	R		R	R						
P-368			R		R			R	R					R	

Ampicillin (A), Amoxycillin (Ac), Aztreonam (Ao), Chloramphenicol (C), Ceftazidime (Ca), Cephotaxime (Ce), Ciprofloxacin (Cf), Ceftriaxone (Ci), Cefepime (Cpm), Gentamicin (G), Imipenem (I), Meropenem (Mr), Piperacillin (Pc), Piperacillin / Tazobactum (Pt), Ticarcillin (Ti).

with those of non-ESBL isolates. However, the increased MIC is still lower than the cut off value for susceptibility.

In double disc diffusion technique after 24 h of incubation of the cultures with -lactam antibiotics in the presence of - lactamase inhibitor, the formation of extended zone was observed between the two Cephalosporin discs and the Amoxicillin disc then those isolates were interpreted as ESBL producers. It was found that out of 121 Gram negative bacilli isolates 28 were found to be ESBL producers.

Detection of MBL production

In Modified Hodge test after 24 h of incubation of cultures with Imepenem disk at 37°C, the formation of clover leaf shaped zone of inhibition was observed then those isolates were interpreted as MBL producers (Hirakata et al., 1998). Table 8 shows the some of the MBL positive isolates. In EDTA double disc synergy test after 24 h of incubation of isolates inoculated onto the Muller Hilton agar medium with Imipenem disk and a blank Whatmann filter paper disk applied with EDTA, formation of enlarged zone of inhibition was observed in all the MBL positive cultures which were interpreted as EDTA synergy positive. It was found that out of 121 Gram negative bacilli isolates 16 were MBL producers (Table 9).

MIC detection by agar dilution

The MIC values were detected for all the MBL positive and ESBL positive isolates after spot inoculation of all the isolates onto Muller Hilton agar medium plates containing different concentrations of Imipenem and incubating them for 24 h (Table 10).

Based on the MIC detection it was found that all *E.coli* isolated were susceptible to Imipenem. Two *Klebsiella* species showed MIC of 8 µg/ml. These were identified as intermediate in susceptibility. Similarly *Psuedomonas aeruginosa* had a MIC of 8 µg/ml, one isolate of *Acinetobacter* showed MIC of 8 µg/ml. Two isolates of *Proteus* showed MIC 2 µg/ml and one isolate showed MIC of 4 µg/ml. Out of 52 isolates tested for MIC 7 isolates showed 8 $\mu g/ml$ and 42 isolates had MIC of 0.5 µg/ml and other isolates showed intermediate concentration.

E-test

The above results were interpreted as positive if the phantom zone was observed below Clavulanate or when Clavulanate caused a more than or equal to three doubling concentration decrease (ratio of >=8) in the MIC values of Cefotaxime in E-test. All the ESBL and MBL positive isolates showed phantom zone in E-test (NCCLS, 2000).

DISCUSSION

Fifty years ago the antibiotic era began with the discovery of penicillin. With in a few years of introduction of

Table 8. Drug resistance pattern of MBL positive gram negative bacilli isolates.

Sample number	A	Ac	Ao	C	Ca	Ce	Cf	Ci	Cpm	G	I	Mr	Pc	Pt	Ti
P-229							R		R	R	R	R			R
P-238	R	R						R		R				R	
P-242	R	R	R			R	R	R		R	R			R	
P-255	R	R					R	R	R	R	R		R		
P-270				R	R		R			R	R	R			R
P-271	R				R	R	R			R		R	R		
P-286		R	R	R			R	R		R	R			R	
P-326					R		R	R	R			R	R	R	R
P-340				R			R	R	R						
P-350			R		R			R							

Ampicillin (A), Amoxycillin (Ac), Aztreonam (Ao), Chloramphenicol (C), Ceftazidime (Ca), Cephotaxime (Ce), Ciprofloxacin (Cf), Ceftriaxone (Ci), Cefepime (Cpm), Gentamicin (G), Imipenem (I), Meropenem (Mr), Piperacillin (Pc), Piperacillin / Tazobactum (Pt), Ticarcillin (Ti).

Table 9. -lactamase production by gram negative bacilli.

Organism	Total number of isolates	No. of GNB isolates that exhibited growth at different concentrations of Imipenem (µg/ml)					
		0.25	0.5	1	2	4	8
E.coli	12	12	6	1	0	0	0
Klebsiella	11	11	10	4	4	2	2
Pseudomonas	14	14	13	13	13	11	4
Acinetobacter	9	9	7	3	3	2	1
Proteus	6	6	6	3	2	1	0
Total	52	52	42	24	22	16	7

Table 10. Minimum inhibitory concentration of gram negative bacilli isolates.

Type of Beta-lactamase	A	B	C	D	E	F	G	H	Total
ESBL	12	8	2	1	2	0	3	0	28
MBL	2	1	5	8	0	0	0	0	16
Total	14	9	7	9	2	0	3	0	44

(A- *E. coli*, B- *Klebsiella,* C- *Pseudomonas* , D- *Acinetobacter* , E- *Citrobacter* , F- *Proteus*, G- *Enterobacter*, H= -*Providencia*).

penicillin into clinical use, penicillinase producing *Staphylococcus aureus* started to proliferate in hospitals. To overcome this problem, penicillinase resistant penicillins came into picture. Shortly afterward, the broad spectrum penicillins and first generation cephalosporins were introduced. They remained a first line of defense against microbes for over 20 years, before resistance due to -lactamases produced by gram negative bacilli became a serious problem (Medeiros, 1997). To counter this threat, the pharmaceutical industry marketed six novel classes of -lactam antibiotics (cephamycins, oxyimino cephalosporins, carbapenems, monobactams and clavam and penicillianic acid sulfone inhibitors) within a relatively short span of 7-8 years (Chaudhary and Aggarwal, 2004). Although, novel - lactamases had emerged gradually after the introduction of new -lactam agents, their number and variety accelerated at an alarming rate. -lactamase production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to penicillins and cephalosporins (Robert et al., 2009). The resistant organisms can be found in a variety of *Entrerobacteriaceae* species, however, the majority of ESBL producing strains are *K. pneumoniae*, *K. oxytoca* and *E. coli.* Other organisms reported to harbor ESBLs include *Enterobacter*, *Salmonella, Morganella morganii, Proteus mirabilis, Serratia marcescens* and *Pseudomonas aeroginosa.* However, the frequency of

ESBL production in these organisms is low (Nathisuwan et al., 2001). The emergence of gram-negative bacterial species with acquired resistance to various broad-spectrum -lactams is becoming a worldwide clinical problem. Strains producing TEM or SHV-derived ESBLs usually demonstrate high-level resistance to broad-spectrum oxyimino -lactams such as CAZ and cefotaxime (Medeiros, 1997). Moreover, several *K. pneumoniae* strains that showed resistance to cephamycins as well as oxyimino cephalosporins were also found to produce AmpC-type -lactamases such as MOX -1 (Jacoby and Medeiros, 1991). In this regard this case study is an initiative to isolation and distinguishing of Gram negative bacilli producing ESBL and MBL at JSS Medical College and Hospital, Mysore, India. Study on isolation of different antibiotic resistance bacterial species from post operative wound infection showed the extent of development of antibiotic resistant Gram negative bacilli among the post operative wound infections.

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