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Prevalence of various β-Lactamases production among gram negative isolates in burn care unit in a tertiary care hospital

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Abstract
Background: The worldwide emergence of antimicrobial resistance among a wide variety of human bacterial burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections.
Aim: study was aimed to detect the prevalence of various types of β-Lactamases present among the gram negative bacilli isolated from burn wound infection (BWI).
Materials and Methods: Present study was carried out in the Department of Microbiology Dr. V M Government medical college, Solapur Maharashtra from December 2012 to July 2014.Wound swabs collected from 50 patients having total body surface area (TBSA) of burn in between 20-40% on 4th, 10th and 16th day. A total of 202 wound swabs were collected aseptically and cultured for the growth of bacteria.158 gram negative bacilli were isolated and screened for the presence of Extended Spectrum β-Lactamases (ESBL), AmpC β-Lactamases, Metallo β-Lactamases (MBL) and confirmed by the standard confirmatory tests.
Results: 27.21% of all Gram negative isolates (158) produced extended spectrum β-lactamases, AmpC β-lactamases produced by 14.55%, 21.51% showed co-production of ESBL and AmpC and 4.43% strains produced metallo β-lactamases. *Klebsiella pneumoniae* was the predominant bacteria producing ESBL, AmpC and co-production of ESBL and AmpC mediated resistance, whereas *Pseudomonas aeruginosa* was the predominant MBL producer.
Conclusion: The emerging antimicrobial resistance in burn wound pathogens poses serious therapeutic challenge. Thus proper antibiotic policy and measures to restrict the indiscriminate use of cephalosporins and carbapenems should be taken to minimize the emergence of this multiple β-Lactamases producing pathogen.

Keywords: AmpC β-Lactamases, Burn wound infection (BWI), Extended spectrum β-Lactamases (ESBL), Gram negative bacilli, Metallo β-Lactamases (MBL).

Introduction
Approximately 75% of the mortality following burn injuries is related to infections. Despite advances in antimicrobial therapies, bacterial infections remain a significant problem in the management of burn victims. Therefore, knowledge of the responsible bacterial flora in burn wounds and its prevalence and resistance is crucial in making fast and reliable therapeutic decisions [1].
Due to extensive use of β-lactam antibiotics in clinical practice over the past several decades, various β-lactamases have emerged. One of the most important mechanisms of microbial resistance to β-lactam antibiotics is hydrolysis by β-lactamases [2]. Extended Spectrum β-Lactamases (ESBL), Amp C β-lactamase and Metallo β-lactamase (MBL) producing organisms pose a major problem for treating burn victims [3]. ESBLs are typically inhibitor susceptible β-lactamases that hydrolyze penicillins, cephalosporins and aztreonam and are encoded by mobile genes. Amp C β-lactamases preferentially hydrolyze cephalosporins and cephamycins and resist inhibition by clavulanate, sulbactam and tazobactam. MBLs hydrolyze carbapenems and other β-lactams. Resistance to carbapenems is of great concern as these are considered to be antibiotics of last resort to combat infections by multidrug-resistant bacteria, especially in intensive care units and burn wards [4]. Bacteria that produce carbapenemases are often referred as "superbugs".

There is paucity of information regarding the prevalence of β-lactamases mediated resistance among gram negative bacteria in burn infection from the Indian subcontinent. The aim of the present study is to find the prevalence of β-lactamases mediated resistance among gram negative bacteria in burn infection.

Material and method: Present study was carried out in the Department of microbiology Dr. V M Government medical college, Solapur, Maharashtra from December 2012 to December 2014.Total 202 wound swab collected from 50 adult patients on 4th,10th and 16th day of admission in burn ward of Shri Chatrapati Shivaji Maharaj General Hospital Solapur, Maharashtra.158 gram negative bacteria were isolated and identified by standard laboratory techniques [5, 6] Antimicrobial sensitivity testing was performed on Mueller-Hinton agar plates with commercially available discs (Hi-Media, Mumbai) by the Kirby-Bauer disc diffusion method. The results were recorded and interpreted as per CLSI recommendations [7] ESBL detection was done in those gram negative isolates which showed resistance/decrease susceptibility to 3rd generation Cephalosporin i.e. Ceftazidime (30ug) or Cefotaxime (30ug) or both according to CLSI guideline. (Screening test) [7] Confirmation of ESBL production done by Predictor disc approximation method according to Rodrigues C *et al* [8] Same test was used for screening of AmpC β-lactamases production. The combination of 3rd generation Cephalosporin 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.

Confirmation of AmpC production was done in those isolates which showed resistance to cefoxitin in screening test by Amp C Disk test according to Singhal S *et al*. [9] Production MBL was detected in those isolates which showed resistance to imipenem by Imipenem- EDTA combined disk test according to Behera B *et al*. [10].

Result

Percentage of resistance exhibited by the 158 Gram negative bacilli isolates to various antimicrobial agents is shown in table no -1. High level of drug resistance was observed for Ampicillin, cefotaxime and Trimethoprim-sulfamethoxazole, while Imipenem, Amikacin and Ciprofloxacin were found to be most effective among gram negative isolates.

β-lactamases production was demonstrated in 107 out of 158 (67.72%) of Gram negative isolates. Among these 43 (27.21%) isolates were pure ESBL producer, 34 (21.51%) isolates were both ESBL and AmpC producer, 23 (14.55%) isolates were pure Amp C producer and 7 (4.43%) isolates were MBL producer.

ESBL, coproduction of ESBL with Amp C and Amp C production was seen maximum in *Klebsiella pneumoniae* followed by *P. aeruginosa*. MBL production was demonstrated maximum in *P. aeruginosa*.

Table 1: Antibiotic Resistance pattern of gram negative isolates from burn wound

Organisms/ Antibiotics	Cefotaxime (30 µg)	Gentamicin (10 µg)	Amikacin (30 µg)	Ciprofloxacin (5 µg)	Trimethoprim – sulfamethoxazole (1.25/23.75 µg)	Tetracycline ((30 µg)	Piperacillin (100 µg)	Ampicillin (10 µg)	Ampcillin-Sulbactam (10/10 µg)	Imipenem (10 µg)
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Klebsiella pneumoniae (75)	51(68)	36(48)	12(16)	31(41.33)	43(57.33)	53(70.66)	--	75(100)	--	1 (0.01)
P.aeruginosa (52)	35(67.30)	22(42.30)	16(30.76)	24(46.16)	--	--	18(34.61)	--	--	3(5.76)
E. coli (16)	11(68.75)	7(43.75)	5(31.25)	7(43.75)	9(56.25)	8(50)	--	14(87.50)	--	1(6.25)
Acinetobacter spp. (6)	4(66.66)	3(50)	2(33.33)	3(50)	3(50)	4(66.66)	--	--	4(66.66)	1(16.66)
Proteus mirabilis(6)	5(83.33)	3(50)	3(50)	3(50)	4(66.66)	6(100)	--	6(100)	--	1(16.66)
Citrobacter spp. (3)	1(33.33)	2(66.66)	0(0)	2(66.66)	2(66.66)	3(100)	--	2(66.66)	--	0(0)

Table 2: Incidence of ESBL, Amp C and MBL production in Gram negative bacilli isolated (n= 158)

Enzyme type	Number	Percentage
ESBL	43	27.21%
ESBL + Amp C	34	21.51%
Amp C	23	14.55%
MBL	7	4.43%
Non-β-Lactamases producer	51	32.28%

Table 3: Organism wise distribution of ESBL, Amp C and MBL producers

Organisms	ESBL (n=43)	ESBL + Amp C (n=34)	Amp C (n=23)	MBL (n=7)
Klebsiella spp(75)	22(51.16%)	13(38.23%)	15(65.21%)	1(14.28%)
P. aeruginosa (52)	15(34.88%)	12(35.29%)	5(21.73%)	3(42.84%)
E.coli (16)	5(11.62%)	3(8.82%)	2(8.69%)	1(14.28%)
Acinetobacter Spp(6)	0	3(8.82%)	0	1(14.28%)
Proteus spp.(6)	1(2.32%)	2(5.88%)	1(4.34%)	1(14.28%)
Citrobacter spp.(3)	0	1(2.94%)	0	0

Discussion: The resistance to β-lactam antibiotics is an increasing problem worldwide and β-lactamases production is the most common mechanism of drug resistance. Both global and Indian figures showed a marked increase in the number of β-lactamases producing organisms [11] Numerous β-lactamases are encoded either by chromosomal genes or transferable genes located on plasmids or transposons.[12] Based on amino acid and nucleotide sequence studies, four distinct classes of β-lactamases have been defined. Class A (Extended spectrum β-lactamases), class B (Metallo β-lactamases), class C (Amp C β-lactamases) and Class D (Cloxacillin hydrolysing β-lactamases) [13] Extended spectrum β-lactamases (ESBLs) are plasmid mediated, TEM and SHV derived enzymes, first isolated in Western Europe in mid 1980s. Initially theses enzymes were commonly found in Klebsiella species and E.coli, but now these enzymes are produced by all the members of Enterobacteriaceae and few other gram negative bacilli. These enzymes are capable of hydrolyzing broad spectrum cephalosporins and monobactams but inactive against cephamycins and imipenem [3]. In addition, ESBL producing organisms exhibit coresistance to many other classes of antibiotics resulting in limitation of therapeutic option [14].

In the present study 27.21% of gram negative bacteria were ESBL producers. Similar result were recorded in Studies by Bandekar *et al.* [3] and Ananthakrishanan *et al.* [15] in burn infection from India. *Klebsiella pneumoniae* was the predominant ESBL producer followed by *Pseudomonas aeruginosa, E.coli* and *Proteus species*. Major risk factors for colonization or infection with ESBL producing organisms are long term antibiotic exposure, prolonged hospital stay, high rates of the third generation cephalosporin use and invasive procedures [16]. Similar risk factors were observed in our study also.

AmpC β-lactamases are presumed to be chromosomally or plasmid mediated. They have been described in pathogens e.g., *Klebsiella pneumoniae*, *E.coli*, *Salmonella spp*., *Proteus mirabilis, Citrobacter freundii*, *Acinetobacter*, *Enterobacter* spp. and *Pseudomonas aeruginosa* [9] In the present study 14.55 % were Amp C producers and *Klebsiella pneumonia* (65.21%) was the predominant Amp C producer, which is in contrast to studies of Kumar V *et al* [4] and Shahid M *et al* [17] who recorded Amp C production predominant in *Pseudomonas species*.

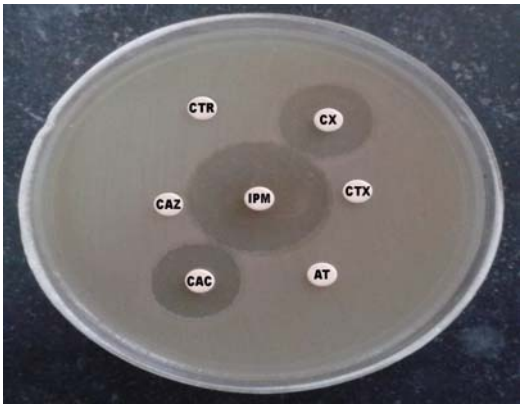
It was also observed that 21.51% of gram negative bacteria were showed co-production of ESBL and AmpC β-lactamases. There are no similar studies to compare the incidence of co-production of ESBL and AmpC mediated resistance among burn infection in India and abroad. Predominant co-production of ESBL and AmpC was noted in *Klebsiella pneumonia* (38.23%).

Amp C β-lactamases producing isolates. Reason behind it may be that third generation Cephalosporins are routinely used in inpatient & outpatient setting. Regular use result in selective pressure & emergence of drug resistance mechanism in organisms.

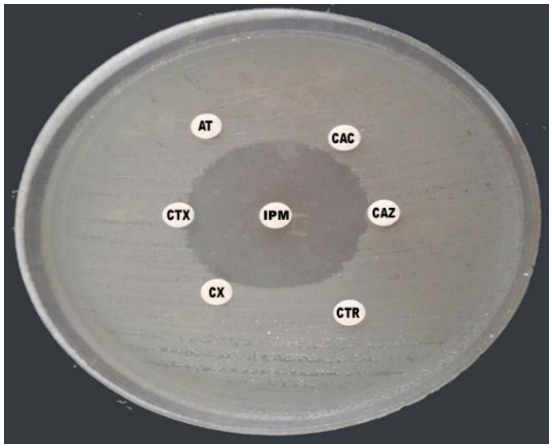
Metallo β-lactamase (MBL) is a group of carbapenem hydrolysing β-lactamase. The MBLs are inhibited in-vitro by CuCl3, FeCl3, EDTA and thiol compounds like 2 mercaptopropionic acid but not by β-lactamase inhibitors like Clavulanic acid, sulbactam or tazobactam [18] Detection of MBL from burn infection has tremendous therapeutic consequences, as the treatment option for such isolates are limited [3].

Not all gram negative bacteria were tested for MBL production in this study. Only those gram negative bacilli resistant to imipenem were screened for MBL production. 4.43% of gram negative bacteria were MBL producers. In contrast to this, studies by Bandaker N *et al.* [3] in burn infection recorded as high as 29.5% to be MBL production. Pseudomonas aeruginosa (42.84%) was the predominant MBL producers in present study while *Proteus mirabilis* was common in study by Bandaker N *et al.* [3]. The low prevalence of MBL in present study may be due to less use of carbapenems in burn ward at government hospital due to their high cost.

Conclusion: Infection is an important cause of morbidity and mortality in burn patients. Early appropriate antimicrobial therapy may improve outcome in burn wound infection. But emerging antimicrobial resistance in burn wound pathogens poses serious therapeutic challenge. Thus proper antibiotic policy and measures to restrict the indiscriminate use of cephalosporins and carbapenems should be taken to minimize the emergence of multiple β-lactamases producing pathogen. screening techniques to detect various β-lactamases should be perform routinely in microbiology laboratories so that the suitable antimicrobial therapy can be instituted and the dissemination of these isolates may be prevented by employing appropriate infection control measures. (i.e., physical isolation in a private room, use of gowns and gloves during patient contact and hand washing before and after each patient visit)

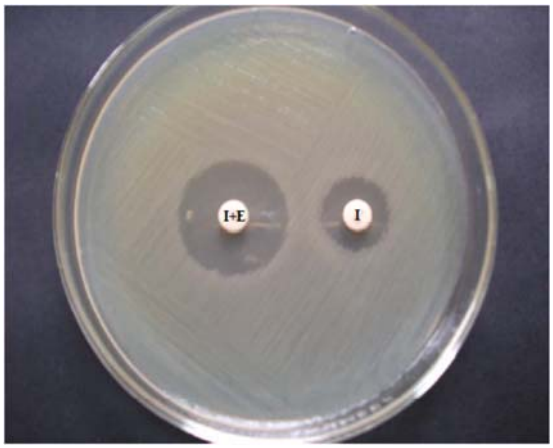


Photograph 1: Showing ESBL producer



Photograph 2: Showing AmpC Screening positive (CAZ- Ceftazidime, CAC- Ceftazidime+ clavulanic acid, CTX- Cefotaxime, CTR- Ceftriaxone, Cx- Cefoxitin, AT- Aztreonam, IPM- Imipenem)

Photograph 3: Showing Amp C Producer (Cx – Cefoxitin)



Photograph 4: Showing MBL producer (I - Imipenem, I+E – Imipenem + EDTA)

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