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Original Article

Beta lactamases mediated resistance amongst gram negative bacilli in Burn infection

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ABSTRACT

Introduction: Burn is one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality. Significant thermal injuries induce a state of Immuno- suppression, which predisposes infectious complications in burn patients. In burn patient infections arise from multiple sources and infect burn wounds by a variety of micro-organisms. Gram negative bacterial infection results from translocation from colon, further more burn patients are infected by Hospital acquired bacteria by various invasive and non invasive procedures Early diagnosis of microbial infections and screening for mechanism of drug resistance is aimed to institute the appropriate antibacterial therapy and to avoid further complications. Objectives: Beta lactamases are enzymes responsible for the resistance to beta lactam antibiotics. This study is aimed at the detection of various types of beta lactamases present among the gram negative bacilli isolated from burn infection. Methods: patients admitted to burn intensive care unit were included in the study.83 gram negative bacilli were isolated and screened for the presence of extended spectrum beta lactamase, AmpC lactamase, Metallo beta lactamase and confirmed by the respective confirmatory tests. Results: 39.8% produced extended spectrum beta lactamases, 22.9% AmpC beta lactamase and 15.7% strains produced metallo beta lactamases. Pseudomonas aeruginosa was the predominant bacteria producing ESBL and AmpC mediated resistance, whereas Acinetobacter baumannii was the predominant MBL producer. Conclusion: It is important to monitor the bacteriology in burn patients at all time, and understand the changing pattern of bacterial flora, antibiotic susceptibility and bacterial strains spreading in burn ward. Extended-spectrum beta-lactamases is the cause of resistance. After sulbactam added to the third generation of cephalosporins, the beta-lactamases were inhibited, but it lead to increase use of carbapenems leading to emergence of metallo beta lactamase mediated resistance. Hence, screening techniques should be performed routinely to detect these β-lactamase producers so that suitable antimicrobial therapy can be instituted.

1. Introduction

Burns provide a suitable site for bacterial multiplication and are more persistent richer sources of infection than surgical wounds, mainly because of the larger area involved and longer duration of patient stay in the hospital [1]. Infection is a major cause of morbidity and mortality in hospitalized burn patients[2]. It is now estimated that about 75% of the mortality following burn injuries is related to infections rather than osmotic shock and hypovolemia [3]. The pattern of infection differs from hospital to hospital; the varied bacterial flora of infected wound may change considerably during the healing periodIn the past 65 years, antibiotics have been critical in the fight against infectious disease caused by the

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bacteria and other microbes. Antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the Twentieth Century. Despite the advances in patient care and the use of a large number of antimicrobial agents, infections which complicate the clinical course of patients who had sustained severe thermal injures continue to be a major unsolved problem.

Nowadays, majority of the bacteria that cause burn infection in hospitals are resistant to at least one of the drugs most commonly used for treatment [4]. Extended Spectrum β-Lactamases (ESBL), AmpC β-lactamase and Metallo β-lactamase producing organisms pose a major problem for treating burn victims [5,6]. The routine susceptibility tests done by clinical laboratories fail to detect β-lactamases positive strains and can erroneously detect isolates sometimes to be sensitive to any of the broad-spectrum cephalosporin like cefotaxime, ceftazidime, ceftriaxone and for imipenem or meropenem [7-9]. Hence, it is necessary to know the prevalence of β-lactamase positive strains in a burn unit so as to formulate a policy of empirical therapy in high risk units where infections due to resistant organisms are much higher. Equally, important is the information on an isolate from a patient to avoid misuse of extended spectrum cephalosporins, and carbapenems which still remain an important component of antimicrobial therapy in burn wards [10,11]. There is not enough information from the Indian subcontinent regarding the prevalence of β-lactamases mediated resistance among gram negative bacteria in burn infection. The aim of the present study is to find the prevalence of β-lactamases mediated resistance among gram negative bacteria in burn infection.

2. Materials and methods

A prospective study was carried out on 50 burn patients over a period of two years at S.S. Institute of Medical College and Research Centre, Davangere 83 gram negative bacterial were isolated and identified by standard laboratory techniques [12]. Antimicrobial sensitivity testing was performed on Mueller-Hinton agar plates with commercially available discs (Hi-Media, Mumbai) by the Kirby-Bauer disc diffusion method [13]. The results were recorded and interpreted as per CLSI recommendations [14].

2.1.Tests for ESBL production

2.1.1.Double disk approximation test for screening:

The test organisms were applied on to a Mueller Hinton agar plate by adjusting turbidity to McFarland no 0.5 tube. Antibiotic discs of Amoxicillin / Clavulanic acid (20/10 μg) and cefotaxime (30 μg) were placed at a distance of 15 mm apart and incubated. Organisms that showed a clear extension of cefotaxime inhibition zone towards the disc containing Clavulanate were considered as ESBL producer [15]. The organisms which were screened and found positive for ESBL production were subjected to confirmatory test.

2.1.2.NCCLS phenotypic confirmatory test

Ceftazidime (30 μg) and ceftazidime plus Clavulanic acid (30/10 μg) were placed on Mueller Hinton agar and incubated. Organism was considered as ESBL producer if there was a ≥ 5mm increase in diameter of Ceftazidime plus Clavulanic disc and that of ceftazidime disc alone [16,17].

2.1.3.Amp C Disk Test

A lawn culture of E.coli ATCC 25922 was prepared on MHA plate. Sterile disks (6mm) was moistened with sterile saline (20 μl) and inoculated with several colonies of test organisms. The inoculated disk was then placed 5mm beside a cefoxitin disc. Plates were incubated overnight at 35°C. A positive test was appeared as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disc [18-20]. A negative test had an undisturbed zone

2.1.4.Metallo β-lactamase (MBL) production

Gram negative organisms that showed resistance to Imipenem were selected for MBL production.

2.2. Imipenem-EDTA combined disc test

This test was performed according to Yong et al. test organisms were inoculated onto Mueller Hinton agar plates as per the CLSI recommendations. Two 10μg imipenem disks were placed on the plate and 10 μl of sterile 0.5 M EDTA solution was added to one of the imipenem disk. The inhibition zones of the imipenem and imipenem plus EDTA disks were compared after inoculation [21]. If the increase in inhibition zone with the imipenem plus EDTA disc was ≥ 7mm than the imipenem disc alone, it was considered as MBL positive.

3. Results

Percentage of resistance exhibited by 83 gram negative bacilli isolated from burn infection to various antimicrobial agents is shown in table-1. All the strains were resistant to more than 2 or more drugs hence all the bacteria were designated as multidrug resistance gram negative bacilli (MDRGNB)

All 83 gram negative bacilli were screened for ESβL production, Amp C β lactamase production. 39.8 % of gram negative bacilli were ESβL producers. Pseudomonas aeruginosa (20.5%) was the predominant ESβL producer followed by Klebsiella pneumonia (7.2%), Acinetobater baumanni (7.2%), Proteus mirabilis (3.6%) and Enterobacter cloacae (1.2%) (Table-2)

22.9% of gram negative bacilli were Amp C producers and Pseudomonas aeruginosa (9.6%) was the predominant AmpC producer followed by Klebsiella pneumonia (4.8%) and Proteus mirabilis (2.4%) (Table 2).

3.1. MBL producers

Not all gram negative bacteria were tested for MBL production. Only those gram negative bacilli resistant to imipenem (Table-2) were screened for MBL production. 21 out of 38 Pseudomonas aeruginosa were resistant to imipenem. Similarly 07 Klebsiella pneumoniae out of 18 were resistant. Among 13 Acinetobacter baumanni 09 were resistant. (Table-3)

Acinetobacter baumanni (6.0%) was the predominant MBL producer followed by *Pseudomonas aeruginosa* (4.8%), *Klebsiella pneumonia* (3.6%) and *Proteus mirabilis* (1.2%)

Table 1: Antibiotic Resistance pattern of Gram negative bacterial isolates from burn infection

Bacterial isolates	Ps. aeruginosa		Klebsiella pneumoniae		Acinetobacter baumannii		Proteus mirabilis		Enterobacter cloacae		Citrobacter freundii	
Drugs	R	%	R	%	R	%	R	%	R	%	R	%
Amikacin	32	84.2	11	61.1	11	84.6	5	62.5	2	50.0	1	50.0
Ampicillin	38	100	16	88.9	13	100	8	100	4	100	2	100
Aztreonam	19	50.0	7	38.9	9	69.2	6	75.0	2	50.0	1	50.0
Gentamicin	38	100	12	66.7	13	100	7	87.5	4	100	2	100
Cephotaxime	29	76.3	13	72.2	12	92.3	7	87.5	3	75.0	2	100
Ceftriaxone	32	84.2	12	66.7	12	92.3	6	75.0	3	75.0	2	100
Ceftazadime	36	94.7	14	77.8	13	100	8	100	4	100	2	100
Cefoperozone	26	68.4	13	72.2	8	61.5	6	75.0	3	75.0	2	100
Ciprofloxacin	28	73.7	16	88.9	12	92.3	7	87.5	4	100	2	100
Imipenem	21	55.3	7	38.9	9	69.2	4	50.0	2	50.0	1	50.0
Piperacillin-tazobactam	23	60.5	6	33.3	5	38.5	5	62.5	3	75.0	0	0.0
Piperacillin	27	71.1	4	22.2	9	69.2	8	100	4	100	2	100
Amoxycillin	32	84.2	16	88.9	12	92.3	8	100	4	100	2	100
Gatifloxacin	27	71.1	10	55.6	9	69.2	7	87.5	3	75.0	2	100

Table 2. Prevalence of ESBL and Amp C producers in Burn infection

Organisms	No. Tested	ESBL producers		Amp C producers	
		No.	%	No.	%
Pseudomonas aeruginosa	38	17	20.5	08	9.6
Klebsiella pneumonia	18	06	7.2	04	4.8
Acinetobacter baumanni	13	06	7.2	02	2.4
Proteus mirabilis	08	03	3.6	02	2.4
Enterobacter cloacae	04	01	1.2	02	2.4
Citrobacter freundii	02	–	–	01	1.2
Total	83	33	39.8	19	22.9

Table 3. Prevalence of Metallo beta lactamase producers in burn infection

Organisms	No. of GNB isolated	Imipenem resistant	MBL producers	
			No.	%
Pseudomonas aeruginosa	38	21	04	4.8
Klebsiella pneumonia	18	07	03	3.6
Acinetobacter baumanni	13	09	05	6.0
Proteus mirabilis	08	04	01	1.2
Enterobacter cloacae	04	02	–	
Citrobacter freundii	02	01	–	
Total	83	44(53.0)	13	15.7

4. Discussion

The ability to produce β-lactamases enzymes is the major cause of resistance of bacteria to β-lactam antibiotics. Numerous β-lactamases are encoded either by chromosomal genes or transferable genes located on plasmids or transposons [22]. 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 (AmpC β-lactamases) and Class D (Cloxacillin hydrolysing β-lactamases) [23,24].

Extended spectrum β-lactamases are plasmid mediated TEM and SHV derived enzymes isolated for first time in Western Europe in mid 1980s [25]. Initially theses enzymes were commonly found in Klebsiella species and E.coli, [8] but now these enzymes are produced by all the members of Enterobacteriaceae and few other gram negative bacilli [26,27]. These enzymes are capable of hydrolysing broad spectrum cephalosporins and monobactams and inactive against cephamycins and imipenem. In the present study 39.8% of gram negative bacteria were ESBL producers. No studies are available on extended spectrum beta lactamases resistance in burn infection. But few studies in India on different clinical condition have reported the prevalence of ESBL in the range of 58% to 68.1% [9,12,28,29] Our prevalence rate is lesser than other reports compared with different clinical conditions from India and abroad, since the isolates were obtained from infection in burn infections which is not a chronic infection they might be wide disparity in the prevalence rate of ESL producing gram-negative bacteria when compared to other

reports. Pseudomonas aeruginosa was the predominant ESL producer followed by Klebsiella pneumoniae, Acinetobacter baumannii and Proteus mirabilis. In addition to the intrinsic resistance to cephalosporins and aztreonam, ESBL producing organism's exhibit co-resistance to many other classes of antibiotics like quinolones and aminoglycosides resulting in limitation of therapeutic options. In the present study we found such associated resistance with Ciprofloxacin (73.7%). As quinolones are strong selectors of ESBL producers, their use should be restricted as far as possible. 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. However in the present study we could not retrieve the nosocomial pathogens and their sensitivity to reinforce the above arguments. Treatment of ESBL producing strains of Enterobacteriaceae has emerged as a major challenge in hospitalized as well as community patients. There are many factors which determine the choice of antibiotics and the management of burn infections. Although β-lactamases inhibitors have significant activity against ESBL in-vitro, their clinical effectiveness against serious infections due to ESBL producing organisms is controversial. ESBL producing strains might show a false sensitive zone of inhibition in the Kirby Bauer's disc diffusion method. The antibiotics for the treatment include carbapenems, aminoglycoside and β-lactamases inhibitor combinations.

AmpC β-lactamases are clinically important cephalosporinases encoded on chromosomes of many of the Enterobacteriaceae and a few other organisms [19], where they mediate resistance to cephalothin cefazolin, cefoxitin, most of the penicillins and β-lactamase inhibitor [18,19]. In many bacteria Amp C enzymes are inducible and can be expressed at high levels by mutation [19,21]. Over expression confers resistance to broad spectrum cephalosporins. In the present study 22.9% were AmpC producers and Pseudomonas aeruginosa was the predominant Amp C producer followed by Klebsiella pneumonia, Acinetobacter baumannii and Proteus mirabilis. There are no reports to compare the incidence of Amp C mediated resistance among burn infection in India and aboard.

Metallo β-lactamase (MBL) is a group of carbapenem hydrolysing β-lactamase [30]. They have been reported from many countries, as well as from different parts of Indian subcontinent, particularly in multidrug resistance pathogens like Pseudomonas aeruginosa and Acinetobacter species. The MBLs are inhibited in-vitro by CuCl3, FeCl3, EDTA and thiol compounds like 2 mercaptopropionic acid, sodium mercaptoacetoic acid and 2 mercaptoethanal, but not by β-lactamase inhibitors like Clavulanic acid, sulbactum or tazobactam [6]. Detection of MBL production in MDR organisms from burn infection has tremendous therapeutic consequences, as the treatment option for such isolates are aztreonam or potentially toxic polymyxin B and colistin. In the present study not all gram negative bacteria were tested for MBL production. Only those gram negative bacilli resistant to imipenem were screened for MBL production and 29.5% of them were metallo β-lactamase producers. Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus mirabilis were the predominant MBL producers.

5. Conclusion

In conclusion, the present study highlights the high prevalence of β-lactamases among the multi drug resistant gram negative isolates in burn infection. It also reflects grim future of the treatment options available for these notorious pathogens. The high incidence of β-lactamases production due to multiple mechanisms in burn infection is alarming and urgent action needs to be taken from both the therapeutic and infection control perspective. Clinical microbiology laboratories should perform the screening techniques to detect these β-lactamases routinely so that the suitable antimicrobial therapy can be instituted and the dissemination of these isolates may be prevented by employing appropriate control measures.

6. References

[1] Agnihotri N,Gupta V, Joshi RM., Aerobic bacterial isolate from burn wound infections and their antibiograms- a five- year study. Burns. 2004; 30: 241-243.

[2] Mc Manus A.T., Mason A.D..jr, McManus W.F., Pruitt B.A,Jr: A decade of reduced Gram -negative infections and mortality improved isolation of burned patients. Arch Surg. 1994 ; 129: 1306-1309.

[3] Donati I, Scammazo F, Gervasoni M, Magliano A, Stankov B, Fraschini F . Infection and antibiotic therapy in 4000 burned patients treated in Milan, Italy, between 1976 and 1988. Burns. 1993; 19: 345-348.

[4] Fridkin SK, Steward CD, Edwards JR, Pryor ER, McGowan JE Jr, Archibald LK, et al. Surveillance of antimicrobial use and antimicrobial resistance in United States hospitals: project ICARE phase2. Clin Infect Dis. 1999; 29 (2):245–52.

[5] Pfaller MA, Jones RN. MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) results from the Americas: resistance implications in the treatment of serious infections. J Antimicrob Chemother. 2000; 46:25–37.

[6] Goossens H. MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) results from Europe: comparison of antibiotic susceptibilities between countries and centre types. J Antimicrob Chemother. 2000;46:39–52.

[7] Jerestin BH, Vandana Agarwal Pathat M. Extended spectrum -lactamases mediated resistance to third generation cephalosporins in Klebsiella pneumoniae in Nagpur, central India. Indian Journal Med Res. 1997;105:158-161.

[8] Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum beta lactamase producing gram negative bacteria in a tertiary care hospital. Indian J Med Res. 2002; 115:153-7

[9] Chaudhary U, Aggarwal R. Extended spectrum β-lactamases and emerging threat to clinical therapeutics. Indian J Med Micrbiol. 2004;22(2):75-80

[10] Abigali S, Mathur, Jesudasan MV. Ceftazidime resistance among Klebsiella pneumoniae in South India. Indian Journal Med Research. 1995;120:53-55

[11] Cunha BA. Antibiotic selection for diabetic foot infections: a review. J Foot Ankle Surg. 2000;39:253–57.

[12] Baird D. Staphylococcus: Cluster-forming gram-positive cocci. In : Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14 th ed. New York: Churchill Livingstone; 1996. p. 245-61.

[13] Bauer A W, Kirby W M M, Sherris JC, Jurek M Antibiotic susceptibility testing by a standardized disc method. American Journal Clinical Pathology. 1966;45:493-496.

[14] Clinical laboratory Standards Institute (CLSI) performance standards for antimicrobial disk susceptibility testing 16th informational supplement. CLSI document M2-A9. Wayne (PA), 2006..

[15] Brun-Buisson C, Legrand P, Philippon A, Montravers F, Ansquer M, Duval J et al. Transferable enzymatic resistance to third-generation cephalosporins during a nosocomial outbreak of multiresistant Klebsiella pneumoniae. Lancet. 1987; 8(2): 302-306.

[16] Coudron PE, Moland ES, Sanders CC. Occurrence and Detection of Extended-Spectrum ß-lactamases in members of the family Enterobacteriaceae at a Veterans Medical Center: Seek and You May Find. J Clin Microbiol. 1997; 35(10):2593-2597.

[17] Bhattacharya S. Extended spectrum β-lactamases from petridish to the patient. Indian J Med Microbial. 2006;24(1):20-24

[18] Singhal S, Mathur T, Khan S, Upadhyay DJ. Evaluation of method for Amp C β-lactamases in gram negative clinical isolate from tertiary care hospital. Indian Journal of Medical Microbiology. 2005;23(2):120-124

[19] Black JA, Moland ES, Thomson KS. Amp C disk test for detection of plasmid mediated Amp C β-lactamases. Journal of Clin Microbiol. 2005;43(7):3110-13

[20] M Sinha, H Srinivasa. Mechanisms of resistance to carbapenems in meropenem resistant Acinetobacter isolates from clinical samples. Indian Journal of Medical Microbiology. 2007;25(2);121-125

[21] Leek, lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double disk synergy test for differentiation of metallo- β-lactamases producing clinical isolates of Pseudomonas spp and Acinetobacter spp. J Clin Microbiol. 2003;41:4623-29

[22] Mary VJ, Kandathi AJ, Balaji V. Comparison of methods to detect carbapenamase and metallo β-lactamases production in clinical isolates. Indian J Med Res. 2005;121:780-83

[23] Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob Agent Chemother. 1995;39:1211-33

[24] Jacoby GA. Extended-spectrum b-lactamases and other enzymes providing resistance to oxyimino--lactams. Infect Dis Clin North Am. 1997; 11(4):875-87.

[25] Livermore DM, Yuan M. Antibiotic resistance and production of extended spectrum b-lactamases amongst Klebsiella spp. from intensive care units in Europe. J Antimicrob Chemother. 1996; 38(3):409-24.

[26] Albertini MT. Surveillance of methicillin-resistant Staphylococcus aureus (MRSA) and Enterobacteriaceae producing extended-spectrum beta-lactamase (ESBLE) in Northern France: a five-year multicentre incidence study. J Hosp Infect. 2002; 52(2): 107-13.

[27] Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum β-lactamases among Enterobacteriaceae species isolated at a tertiary care institute. Indian J Med Microbial. 2006;24(3):208-11

[28] Zorgani A., Zaidi M., Ranka R., Shahen A.:The pattern and outcome of septicaemia in a burns intensive care unit.Ann.Burns Diasters. 2002;15: 179-182.

[29] Ananthakrishnan AN, Kanungo R, Kumar A, Badrinath S. Detection of extended spectrum β-lactamase producers among surgical wound infections and burn patients in JIPMER. Indian Journal of Medical Microbiology. 2004;18:160-165.

[30] Chu, Y.-W., M. Afzal-Shah, E. T. S. Houang, M.-F. I. Palepou, D. J. Lyon, N. Woodford, and D. M. Livermore (2001) IMP-4, a novel metallo-β-lactamase from nosocomial Acinetobacter spp. collected in Hong Kong between 1994 and 1998. Antimicrob. Agents Chemother. 2001;45:710–714