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Original Research Article

Prevalence of Extended Spectrum ß-lactamases and Metallo B- lactamases in bacterial isolates from burn patients

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K e y w o r d s

Burns; multi-drug resistance; antimicrobial susceptibility.

A B S T R A C T

Infections are one of the common cause of morbidity and mortality following burn injury. The worldwide emergence of antimicrobial resistance, particularly nosocomial isolates reduce the available therapeutic options for effective treatment. Objectives: to evaluate the pattern of bacterial pathogens isolated from wound swabs from patients with burn injury and to determine their antimicrobial susceptibility including MRSA, ESBL and MBL production. During Dec 2010 to Dec 2011, 75 patients were included in this study. Wound swabs were taken on day 1, 3 and 7 of admission in Burns & Plastic surgery ward. Isolation and identification of microorganisms was done using the standard microbiological procedure. Kirby Bauer Disc diffusion test was performed on all isolates for antimicrobial susceptibility. In wound swabs the culture positivity rate was 36.9%, maximum (94.6%) on day 7 of admission. The most common isolate was *P.aeruginosa* followed by *K.pneumoniae* & *A.baumannii.* 53.2% were ESBL producers. 16.88% GNB were identified as MBL producers. *A.baumannii* was the predominant MBL producer followed by *P.aeruginosa* and *K.pneumoniae*. MRSA rate among *S.aureus* was 66.7%, but all were susceptible to glycopeptides and oxazolidinone. Hence Continuous monitoring of microbial spectrum and their antibiogram is absolutely essential to determine changing trends in the type of organisms and their resistance pattern for framing an appropriate hospital antibiotic policy which is mainstay in the management of patients in the burn ward.

Introduction

Infections leading to septicemia are the common cause of morbidity and mortality following burn injury (Heideman, 1992**)** Although burn wound surfaces are sterile immediately following thermal injury, these wounds usually become infected with microorganisms (Weber, 1997).

Hospital acquired infections in burn patients can be endogenous (patient's own flora) or exogenous (hospital environment and personnel). The worldwide emergence of antimicrobial resistance among burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic

options for effective treatment of burn wound infections. Methicillin-resistant staphylococcus aureus (MRSA), vancomycin-resistant enterococci, and multiple resistant Gram-negative bacteria that possess several types of beta-lactamases, including extended spectrum beta-lactamases (ESBL) and metallo beta-lactamases (MBL) have been emerging as serious challenges in hospitalized patients. These organisms can be transmitted easily from one patient to another. Thus,outbreaks are not uncommon in burn units (Falk, 2000).

A well planned surveillance and infection control practices including preventive measures can help reduce the incidence of hospital acquired infections amongst burn patients (Roberts, 2001) As the spectrum of micro-organisms causing infections in burn patients varies not only with geographical location & time but also from hospital to hospital, it is necessary to carry out reviews of the bacterial flora present in burn patients.

The present study was thus planned to evaluate the pattern of bacterial pathogens isolated from wound swabs from patients with burn injury, to determine the antimicrobial susceptibility pattern of the culture isolates by Kirby Bauer disc diffusion method as per CLSI guidelines and to determine the emergence of antimicrobial resistance among organisms isolated with special emphasis on MRSA (disc diffusion method); ESBL (phenotypic confirmatory test) and MBL production (Disc Potentiation test) as per CLSI guidelines. Continuous monitoring of microbial spectrum and their antibiogram is absolutely essential to determine changing trends in resistance pattern, thus helping in judicious use of antibiotics by framing an appropriate antibiotic policy.

Materials and Methods

Study was done over a period of one year from December 2010 to December 2011 in the department of Microbiology. Samples from 75 patients admitted in Burn unit with total burn surface area (TBSA) between 10% - 60% and having age between 10- 60 years were included in the study. Wound swabs were taken on day1, 3 and 7 of admission. The samples received were cultured on 5% sheep Blood Agar and MacConkey Agar plates & also inoculated in BHI broth. Both plates and broth were incubated at 37°C for 24hrs, and then plates were examined for growth. Bacterial isolates were identified according to standard methods and tested against various antibiotics by Kirby Bauer's disc diffusion method & zone diameters were interpretated according to the CLSI guidelines (Santucci, 2003). MRSA was identified using disc diffusion method. 0.5 McFarland standard suspension of organism in broth was prepared and inoculated in Mueller Hinton Agar (MHA) plate with 2%NaCl. 1µg oxacillin disc and 30µg cefoxitin disc (HiMedia) were applied over the inoculated media and incubated at 35°C for 24hrs. Any colony or light film of growth around oxacillin and cefoxitin disc examined under transmitted light was considered MRSA. ESBL production was determined by using phenotypic confirmatory test. Antibiotic disc of ceftazidime 30µg and ceftazidime-clavulanic acid 30/10µg, also cefotaxime 30µg and cefotaxime- clavulanic acid 30/10µg (HiMedia) were placed on inoculated MHA media 30mm apart from centre to centre and incubated at 37°C for 16-18 hrs. The zone of inhibition were recorded and difference in zone size of 5mm or more for either antimicrobial agents tested in combination with

clavulanic acid vs its zone when tested alone were considered ESBL. Strains resistant to carbapenems were screened for Metallo beta lactamase (MBL) production by using Disc Potentiation test. (Figure 1) In this test Imipenem disc 10µg and Imipenem - EDTA disc (Hi Media) were placed on inoculated plates. After 16-18 hrs incubation at 35ºC, the zone of inhibition were recorded and an increase in zone size of atleast 7mm around the Imipenem- EDTA disc compared to Imipenem disc alone was considered as MBL producers (Ami Varaiya 2008).

Results and Discussion

Wound swabs

On Day one, none of the samples were culture positive, on Day three, 12 (16%) and on day seven, 71 (94.6%) samples were culture positive. In twelve patients same organisms were isolated on day 3 and day 7. (Table 1) The most common isolate was *P. aeruginosa* – 39 (47.0%), followed by *K. pneumoniae* - 21 (25.3*%), A. baumannii - 15 (*18.07%), *S. aureus*- 6 (7.2%) and *E. cloacae* - 2 (2.4%). Most of the isolates were found to be multi-drug resistant as shown in table 2. Isolation of MRSA was 66.7% (4 out of 6), but all the strains were susceptible to glycopeptides and Linezolid. Forty one (53.2%) gram negative isolates were ESBL producers as determined by phenotypic confirmatory tests while thirty three (46.75%) out of eighty one isolates showed no zones for both cefotaxime (30µg) and ceftazidime (30µg) alone and in combination with clavulanic acid. (Table 3). Thirteen (16.88%) out of seventy seven gram negative bacilli were identified as MBL producers (Table 3). The main goal of studying pattern of micro-organisms isolated from burn patients and their antibiogram is the rationalisation of management of infections in these patients. The collection of samples on different days was planned to observe the possible effect of time-related changes in the rate of isolation of micro-organisms. On day one, none of the samples grew any organism while on day three and seven, twelve (16%) & seventy one (94.6%) samples respectively were culture positive. The findings of the study suggest that with increase in duration of hospital stay, the rate of isolation of the organisms multiplied almost six times between days three and seven. In study conducted by Ezzatollah, 2011 and Altoparlak, 2004 the rate of isolation increased 3-4 times with increase in duration of hospital stay. This finding suggests the need for effective management of the burn patients through proper use of antimicrobial agents and strict implementation of hospital infection control practices to minimise the duration of stay in the hospital.

The most frequently isolated organism from burn wounds in our study were *P. aeruginosa* (47.0%), followed by *K. pneumoniae* (25.3*%), A. baumannii (*18.07%), *S. aureus* (7.2%) and *E. cloacae* (2.4%) which is in agreement with the findings of other studies (Karimi, 2002; Kaushik, 2001 and Rastegar, 2000). Isolation of high frequency of *Pseudomonas aeruginosa* (47%) and *Klebsiella pneumoniae* (25.3%) might be because these agents are found frequently in hospital environments, and burn wounds are an ideal medium for their survival. *Pseudomonas spp*. are inherently resistant to commonly used antibiotics and can even survive in common antiseptics; therefore, eradication of organisms from patients and the environment is difficult (Oncul, 2009). As reported in other studies *Acinetobacter baumannii* (18.4%) have emerged as a significant cause of wound infections in our burn unit.

Table.1 Micro-organisms isolated from wound swabs collected from 75 patients

Micro-organisms	**Wound Swabs**		
	Day 1	**Day 3**	**Day 7**
P. aeruginosa	-	7	32
K. pneumoniae	-	1	20
A. baumannii	-	1	14
Enterobacter cloacae	-	1	1
S. aureus	-	2	4
Total		**12**	**71**

Table.2 Resistance pattern of various isolates from disc diffusion method

Organisms	**Antimicrobial Resistance pattern**						
Pseudomonas(39)	**G**	**Ak**	**Cf**	**Ca**	**Pc**	**PT**	**Imp**
Disc diffusion (Kirby Bauer)	35 89.7%	27 69.2%	27 69.2%	35 89.7%	27 69.2%	14 35.9%	2 5.1%
Klebsiella (21)	A	G	Ak	Cf	Ci	PT	Imp
Disc diffusion (Kirby Bauer)	19 90.4%	19 90.4%	19 90.4%	15 71.4%	19 90.4%	13 61.9%	1 4.7%
Acinetobacter(15)	A	G	Ak	Cf	Ci	PT	Imp
Disc diffusion	15 100%	15 100%	15 100%	15 100%	15 100%	12 80%	10 66.7%
Enterobacter (2)	A	G	Ak	Cf	Ci	PT	Imp
Disc diffusion (Kirby Bauer)	2 100%	2 100%	2 100%	2 100%	2 100%	0	0
S.aureus (6)	Ox	T	E	Cf	Co	Va	Lz
Disc diffusion (Kirby Bauer)	4 66.7%	4 66.7%	4 66.7%	6 100%	4 66.7%	0	0

***G-** Gentamicin(10µg), Ak- Amikacin(30µg), Cf-Ciprofloxacin(5µg), Ca-Ceftazidime(30µg), Pc- Piperacillin(100µg), PT- Piperacillin-Tazobactam(100/10µg) , Imp-Imipenem(10µg) , A- Ampicillin(10µg), Ci- Ceftriaxone(30µg), Ox- Oxacillin(1µg), T-Tetracycline(30µg), E- Erythromycin(15µg), Co- Cotrimoxazole(1.25/23.75µg), Va-Vancomycin(30µg) & Lz- Linezolid(30µg)

Table.3 Results of ESBL and MBL production by gram negative organisms

Organisms	Total isolates	ESBL producers		MBL producers	
		No.	No.	No.	%age
P. aeruginosa	39	20	2	2	48.7%
K. pneumoniae	21	17	1	1	19.04%
A. baumannii	15	2	10	10	86.67%
E. cloacae	2	2	Nil	Nil	-
Total	**77**	**41**	**13**	**13**	**46.75%**

Presence of *Acinetobacter* spp. as normal skin flora, its easy transmissibility, ability to remain viable in a hospital environment due to its multi-drug resistant status and tendency to adhere to abiotic surfaces have been implicated in the increased incidence of nosocomial infections due to this organism Also *Acinetobacter* is a hydrophilic organism and preferentially colonizes in aquatic environments (Singh, 2003; Sengupta, 2001).

The pattern of bacterial resistance is The ability to produce β-lactamases is one of the most important mechanism of resistance of bacteria to β-lactam antibiotics. ESBL belonging to groups SHV, TEM, CTX-M have mainly been implicated in the transfer of drug resistance in gram negative organisms (Kumar, 2006). 53.2% of gram negative bacteria were identified as ESBL producers. Singh *et al*., (2003) from Delhi, have reported 61% ESBL production while 39.8% ESBL production was quoted from Karnataka (Bandekar, 2011). *Klebsiella pneumoniae* (80.9%) was the predominant ESBL producer followed by *Pseudomonas aeruginosa* (51.28%) and *Acinetobacter baumannii* (13.3%). In addition to the intrinsic resistance to cephalosporins and aztreonam, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics like quinolones and aminoglycosides resulting in limitation of therapeutic options. Treatment of ESBL producing strains has emerged as a major challenge in hospitalised as well as community patients. Although β- lactamases inhibitors have significant activity against ESBL in vitro, their clinical effectiveness against serious infections is controversial.

extremely important for epidemiological and clinical purposes in hospital settings. The antibiogram profile observed in the isolates raises a serious cause for concern because the predominant bacterial isolates were highly resistant to the commonly used antimicrobials. The incidence of MRSA was 66.7% (4 out of 6 isolates) but all the strain were sensitive to glycopeptides (Vancomycin) and oxazolidinone (Linezolid) which is similar to reports particularly from India (Singh, 2003; Sengupta, 2001).

Amongst the options that are available for the treatment of these MDRO, Metallo β-lactamases (MBL) are a group of carbapenem hydrolysing β-lactamase(Chu 2001). They have been reported from many countries, as well as from different parts of Indian subcontinent, particularly in MDR pathogens like *Pseudomonas aeruginosa* and *Acinetobacter* species. Detection of MBL production in MDRO from burn infection has tremendous therapeutic consequences, as the treatment option for such isolates are very limited

i.e. polymyxin B and colistin. In our study, 16.88% of gram negative bacilli were metallo β-lactamase producers. *Acinetobacter baumannii* was the predominant MBL producer followed by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. This is in accordance with another study from India (Bandekar *et al.*, 2011) showing 15.6% of gram negative bacilli as MBL producers.

In light of the findings of this study, it appears absolutely essential that all the isolates should be screened for drug resistance parameters as per the standard recommended methodologies and fully operational scientifically designed antibiotic policy for burn unit should be framed. Further strict infection control strategies should be implemented to prevent the spread of drug resistant clones among the hospital pathogens to achieve the ultimate objective of improving infection related morbidity and mortality in burn patients.

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