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ORIGINAL ARTICLE

BACTERIOLOGICAL STUDY OF BURNS INFECTION

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ABSTRACT: A burn is a wound in which there is coagulative necrosis of the tissue, majority of which are caused by heat. Burn injury is a major public health problem in many areas of the world. Burns predispose to infection by damaging the protective barrier function of the skin, thus facilitating the entry of pathogenic microorganisms and by inducing systemic immunosuppression.(1) **OBJECTIVE:** The present study was therefore undertaken to isolate and identify the aerobic bacterial flora in burn patients and its antibiotic susceptibility pattern. **MATERIAL & METHODS:** A total of 100 patients admitted with different degree of burns were studied. Wound swabs were taken with aseptic precautions by dry sterile cotton swab sticks. These swabs were transported to the microbiology laboratory and the isolates were identified based on standard microbiological methods. Antibiotic susceptibility testing was done by Kirby Bauer's disc diffusion method. **RESULT:** A total of 127 bacterial pathogens were isolated from 100 patients. Of these, 69% were monomicrobial in nature and 28% were polymicrobial. The most frequent cause of infection was found to be Staphylococcus aureus (39.4%), followed by Pseudomonas aeruginosa (14.2%), Klebsiella pneumonia (13.4%), E.coli (8.7%) and Acinetobacter species (7.9%).Out of the total Staphylococcus aureus isolates, 19 were Methicillin sensitive and 31 were Methicillin resistant (MRSA). All the MRSA strains were 100% sensitive to Vancomycin and Linezolid. The Pseudomonas aeruginosa isolates were most sensitive to Amikacin (94.4%), Fluroquinolones (61.1%). **CONCLUSION:** Staphylococcus aureus and Pseudomonas aeruginosa were major causes of infection in burn wounds. Therefore it is necessary to implement urgent measures for restriction of nosocomial infections, sensible limitation on the use of antimicrobial agents, strict disinfection and hygiene.

KEYWORDS: Burn wound infection, Antibiotic susceptibility testing, MRSA.

INTRODUCTION: Burn wound infection is one of the most common causes of serious problems and death after thermal injury.(1) A thermal injury causes devitalisation of tissue, produces extensive raw areas and destroys the skin barrier that normally prevents invasion of bacteria, fungi and viruses, making burn wound the most frequent origin of sepsis.(2,3) Infection remains a foremost concern in the management of the burn wound because the large low area with its serous exudates, may act as a huge culture plate on which organisms can establish and multiply, little affected by the body defense mechanisms.(4)

The burned areas were considered to be initially free of major microbial contamination. However, gram positive bacteria in the depths of sweat glands and hair follicles may survive the heat of the initial injury. Unless topical antimicrobial agents are used, these bacteria may heavily colonise the wounds within the first 48 hours after injury.(4) After 5-7 days, the gram negative bacteria and yeast derived from the host's normal GI flora, upper respiratory tract and the hospital environment, get transferred to the wounds through vectors, such as health care workers.(5) Various factors responsible are disruption of the skin barrier, a large cutaneous bacterial load, the possibility of the normal bacterial flora becoming opportunistic pathogens and severe depression of the immune system. All

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these factors contribute towards the sepsis in a burn victim.[6] Despite various advances in infection control measures, like early detection of microorganisms and newer and broad spectrum antibiotics, management of burn septicemia still remains a big challenge and septicaemia continues to be the leading cause of death in burn patients.[3]

Staphylococci are the predominant microorganisms causing infection in burn wounds. They are gaining increasing importance as nosocomial pathogens due to widespread use of broad spectrum antimicrobials. Since 1975, the role of MRSA has gradually increased.[7]

The presence of Pseudomonas infection in burn patients in the late 1950s and early 1960s, considered by many to be a burn specific aberration, was validated by subsequent experience to have been an epidemiological premonition of infection in other critically ill patients.[8] The pattern of infection differs from hospital to hospital; the bacterial flora of infected wound may change considerably during the healing period.[9] Emergence of multi drug resistant pathogens in hospital setting has seriously constrained the available therapeutic options. This necessitates periodic review of the isolation pattern and study of antibiogram of the isolates to strengthen surveillance activities.[10]

Here lies the importance of microbiological investigations and determination of antibiotic susceptibility pattern of the isolates. The present study was undertaken to know the antibiotic susceptibility profile of various aerobic bacterial isolates recovered from inpatients of infected burn wounds which could help in instituting empirical therapy and minimise irrational use of higher antimicrobial agents.

MATERIALS AND METHODS: This prospective descriptive study was conducted in the Department of Microbiology at JJM Medical College, Davangere. A total of 100 patients admitted with different degree of burns were studied. All the patients admitted in the Burns ward of the hospital with total burns surface area more than 20%, irrespective of the age and gender differences, were included in the study.

Wound swabs were taken with aseptic precautions using dry sterile cotton swabs in duplicate after moistening with sterile normal saline. The swabs were rubbed onto the burn wound surface and were taken from areas which appeared deep, areas with discharge, thick eschar, etc. The swabs were then sent for culture. One swab was used for gram stain smear and the other for aerobic culture. All samples were collected and processed after obtaining informed consent from the patients and were immediately transferred to the laboratory where they were processed.

The swab for direct smear examination was stained by gram stain method. The swabs for culture were cultured on Blood agar, chocolate agar and MacConkey agar and incubated aerobically overnight at 37^0C. These swabs were also put into liquid media (BHI & Thioglycollate Broth) and sub cultured after overnight incubation onto Blood agar, Chocolate agar and MacConkey agar plates. The isolates were identified based on standard microbiological methods including culture, staining and biochemical tests.[11] The basic aim was to isolate the organisms predominant on the burn wound and determine their sensitivity to various antibiotics for clinical purposes.

Antimicrobial susceptibility testing of isolates was performed by standard Kirby Bauer disc diffusion methods according to CLSI protocol. Depending on the isolate, antibiotic discs were selected from among the following: Penicillin (10U), Ampicillin (10µg), Erythromycin (15µg), Cotrimoxazole (25µg), Cloxacillin (5µg), Oxacillin (1µg), Vancomycin (30µg), Linezolid (30µg), Amoxyclav (20/10µg), Gentamicin (10µg), Cephalexin(30µg), Cefuroxime(30µg), Cefotaxime (30µg), Ceftazidime (30µg), Ciprofloxacin (5µg) and Amikacin (30µg).[12] The Staphylococcus aureus isolates, resistant to Oxacillin

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on Mueller Hinton agar with 4% sodium chloride, were characterised as Methicillin Resistant Staphlococcus aureus.

RESULT: The total number of patients in this study was 100, of which 69 (69%) were females and 31 (31%) were males. Maximum numbers of patients were in the age group 22–45 years (62%). The detailed age distribution is provided in Table 1.

Age	No. of Cases	%
< 13	2	2
14 - 21	17	17
22 - 45	62	62
>46	19	19

Table 1: Age distribution of patients with burn wound infection

Out of the 100 cases, 45 cases (45%) had 25 – 50% degree of burns which was followed by 40 cases (40%) having less than 25% degree of burns. The number of cases in relation to the degree of burns is given in Table 2.

Degree of Burns	Total
<25%	40
25 – 50%	45
51 – 75%	12
>75%	3

Table 2: Burn wound infection in relation to degree of burns

A total of 127 bacterial pathogens were isolated from 100 cases. Of these 100 cases, 3 (3%) showed no growth on culture. 69 cases (69%) were mono microbial in nature and the remaining 28 cases (28%) were polymicrobial.

Among the 127 isolates, 57(44.9%) were gram positive cocci and 70(55.1%) were gram negative bacilli. Of the gram positive cocci, Staphylococcus aureus (39.4%) was the predominant isolate followed by Coagulase Negative Staphylococci (3.9%) and Enterococci (1.6%). The most common gram negative bacilli isolated was Pseudomonas aeruginosa (14.2%) followed by Klebsiella pneumonia (13.4%), E.coli (8.7%) and Acinetobacter species (7.9%). The distribution of organisms is shown in Table 3.

ORGANISMS	No. of Cases	%
Staphylococcus aureus	50	39.4
Pseudomonas aeroginosa	18	14.2
Klebseilla pneumoniae	17	13.4
E. coli	11	8.7
Acinetobacter species	10	7.9
Coagulase Negative Staphylococci	5	3.9
Proteus mirabilis	5	3.9

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Klebseilla oxytoca	3	2.4
Proteus vulgaris	3	2.4
Providencia stuartii	3	2.4
Enterococci	2	1.6

Table 3: Organisms isolated in the study group

In relation to the depth of burns wound, the most common organism isolated both in superficial and deep burns was Staphylococcus aureus (41.3% and 37.5% respectively). This was followed by Pseudomonas aeruginosa (15.9%) in superficial burns and Klebsiella pneumoniae (15.6%) in deep burns. Burn wound infection in relation to depth is given in Table 4.

	Staphylococcus aureus	**Pseudomonas aeruginosa**	**Klebsiella pneumoniae**	**E. coli**	**Acinetobacter species**	**Coagulase Negative Staphylo-**	**Proteus mirabilis**	**Enterococci**
Superficial	26(41.3%)	10(15.9%)	7(11.1%)	5(7.9%)	6(9.5%)	4(6.3%)	2(3.2%)	1(1.6%)
Deep	24(37.5%)	8(12.5%)	10(15.6%)	6(9.4%)	4(6.3%)	1(1.6%)	3(4.7%)	1(1.5%)

Table 4: Burn wound infection in relation to depth

Amongst the 50 isolates of Staphylococcus aureus, 36 random isolates were sent for phage typing to the Staphylococcal Phage Typing Center at Maulana Azad Medical College, Delhi. Of these, 24 (66.6%) were phage typable and belonged to phage III. Among phage III, phage type 83 was the commonest followed by phage type 47 Depicted in Table 5.

	NUMBER	**%**
Typable	24	66.6
a) Group I	0	0
b) Group II	3	8.3
c) Group III	6	16.7
d) Group IV	3	8.3
e) Mixed	12	33.3
Non typable	12	33.3

Table 5: Phage typing of Staphylococcus aureus isolates

Out of the total 50 isolates of Staphylococcus aureus, 31(62%) were Methicillin Resistant Staphylococcus aureus (MRSA) and 19 (%) were Methicillin Sensistive Staphylococcus aureus (MRSA). All the MRSA strains were 100% sensitive to Vancomycin and Linezolid. Details of the antibiogram is depicted in Table 6.

DOI: 10.14260/jemds/2015/2021

Total(50)	MRSA*(31)		MSSA*(19)	
	S	R	S	R
Penicillin	0	31(100%)	0	19(100%)
Cloxacillin	0	31(100%)	18(94%)	1(6%)
Erythromycin	3(9%)	28(91%)	12(63%)	7(37%)
Ciprofloxacin	2(7%)	29(93%)	12(63%)	7(37%)
Gentamicin	0	31(100%)	5(27%)	14(73%)
Amikacin	13(42%)	18(58%)	18(94%)	1(6%)
Chloramphenicol	31(100%)	0	19(100%)	0
Vancomycin	31(100%)	0	19(100%)	0
Linezolid	31(100%)	0	19(100%)	0
Cefuroxime	15(48.4)	16(51.6%)	17(89.5%)	2(10.5%)

Table 6: Antibiotic Susceptibility pattern in MRSA and MSSA Isolates

*MRSA- Methicillin Resistant Staphylococcus aureus
*MSSA- Methicillin Sensitive Staphylococcus aureus

All of the Coagulase Negative Staphylococci isolated were 100% sensitive to Penicillin and Cloxacillin. The Enterococcus species were 100% sensitive to Vancomycin, Amikacin, Cefuroxime and Erythromycin. Details of the antibiogram are depicted in Table 7.

Total	Coagulase Negative Staphylococci(5)		Enterococci(2)	
	S	R	S	R
Penicillin	5 (100%)	0	0	2 (100%)
Cloxacillin	5 (100%)	0	NT	NT
Erythromycin	5 (100%)	0	2 (100%)	0
Ampicillin	3 (60%)	2 (40%)	0	2 (100%)
Gentamicin	2 (40%)	3 (60%)	NT	NT
Amikacin	4 (80%)	1(20%)	2 (100%)	0
Cotrimoxazole	5 (100%)	0	NT	NT
Vancomycin	5 (100%)	0	2 (100%)	0
Cephalexin	1 (20%)	4 (80%)	0	2 (100%)
Cefuroxime	5 (100%)	0	2 (100%)	0

Table 7: Antibiotic Susceptibility pattern in Coagulase Negative Staphylococci and Enterococcus Isolates

*NT- Not Tested.

The gram negative pathogens like E.coli, Klebsiella pneumonia and Acinetobacter species, showed maximum sensitivity to Fluroquinolones (Ofloxacin), Aminoglycosides (Amikacin) and IIIrd generation Cephalosporins (Cefotaxime) (Table 8). Among the other gram negative pathogens isolated, Proteus mirabilis was 100% sensitive to Amikacin, 80% sensitive to Ofloxacin and Ceftazidime. Proteus vulgaris was 100% sensitive to Amikacin, Ofloxacin and Ceftazidime.

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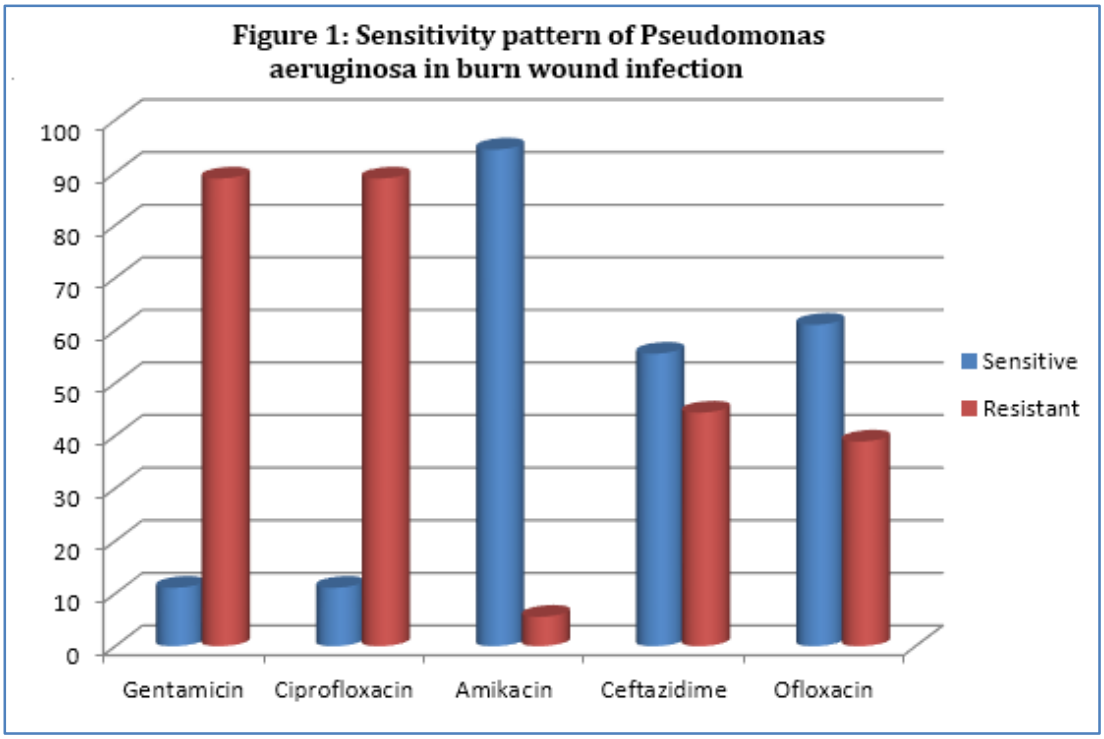
Pseudomonas aeruginosa isolates were most sensitive to Amikacin (94.4%), Ofloxacin (61.1%) and Ceftazidime (55.6%) and showed resistance to Gentamicin (88.9%) and Ciprofloxacin (88.9%). (Figure 1)

Total	E. coli(11)		K.pneumoniae(17)		Acinetobacter sp.(10)	
	S	R	S	R	S	R
Amikacin	11(100%)	0	17(100%)	0	10(100%)	0
Cefotaxime	9 (81.9%)	2(18.1%)	10(58.9%)	7(41.1%)	3 (30%)	7(70%)
Ceftazidime	8 (72.7%)	3(27.3%)	10(58.9%)	7(41.1%)	6(60%)	4(40%)
Ciprofloxacin	6 (54.5%)	5(45.5%)	13(76.4%)	4(23.6%)	1(10%)	9(90%)
Gentamicin	2 (18.1%)	9(81.9%)	5(29.4%)	12(70.6%)	3 (30%)	7(70%)
Cotrimoxazole	3 (27.3%)	8(72.7%)	4(23.6%)	13(76.4%)	NT*	NT*
Ofloxacin	6 (54.5%)	5(45.5%)	11(64.7%)	6(35.3%)	2(20%)	8(80%)

Table 8: Antibiotic Susceptibility pattern in the gram negative isolates

*NT- Not Tested



DISCUSSION: In our study, the total number of isolates obtained were 127 of which Staphylococcus aureus was the commonest organism isolated, accounting for 39.4% of the total isolates. This is similar to the study by Lesseva et al (36.9%),[7] and Yemul et al (41.5 %).[13] This was followed by Pseudomonas aeruginosa which accounted for 14.2 % of the total isolates. Prevalence of Pseudomonas species in the burn wound may be due to the fact that the organism thrives well in a moist environment. Pseudomonas aeruginosa is a well recognised cause of nosocomial infections among patients with

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burns. It is usually spread from patient to patient by direct contact , via staff involved in direct patient care or through contact with contaminated surfaces.[3]

Staphylococcus aureus was most sensitive to Amikacin, Cloxacillin, Chloramphenicol and Cefuroxime. None of the Staphylococcus aureus isolates were sensitive to Penicillin. This was consistent with the study done by Pandit et al.[14] We observed that 62% of the Staphylococcus aureus isolates in our study were Methicillin Resistant Staphylococcus aureus (MRSA) which was more than that reported by Lesseva et al (23.8%),[7] but less compared to that of Song et al (98%).[15] All the MRSA isolates were uniformally sensitive to Vancomycin, as was observed by Lesseva et al (100%).[7] The higher incidence of MRSA strains in our study may probably be due to the fact that MRSA either developed in the wound during antibiotic therapy or would have entered the wound after colonising the patient prior to the infection .

36 random isolates of Staphylococcus aureus were sent for phage typing. Of these 66.6% were phage typable of the group III. The most common phage type was 83. Phage typing is a powerful epidemiological tool for analysis of the source of nosocomial infections.

The gram negative bacilli isolated in the present study were more sensitive to Amikacin, Cefotaxime, Ofloxacin, Ceftazidime and were resistant to Gentamicin and Cotrimoxazole. Pseudomonas aeruginosa, the second common isolate, was most sensitive to Amikacin (94.4%), Ofloxacin (61.1%) and Ceftazidime (55.6%). This was similar to studies done by Rastegar Lari et al[1] and Revathi et al.[16]

The antibiotic sensitivity patterns of strains isolated from burns also support the view that a large proportion of these organisms are hospital acquired and many of the burns wound become colonized by several species of gram negative bacilli which would provide opportunities for resistance to be transferred and also to increase even when antibiotics are not used.[17,18,19]

Overcrowding, prolonged hospital stay and irrational use of antibiotics serve as major risk factors for nosocomial burn wound infection with resistant organisms.[20]

Adequate bacteriological surveillance and monitoring from the moment of admission into the burn care unit, in order to diagnose any infection and to study the colonising flora, is an important measure in the assessment of the more pathogenic or multi drug resistant organisms.[21]

CONCLUSION: Staphylococcus aureus was the most common isolate in our study followed by Pseudomonas aeruginosa. Amikacin, Ofloxacin and Ceftazidime were found to be more effective for Pseudomonas aeruginosa. We concluded that the composition of bacterial flora in burns is dependent not only on the depth and extent of the burn but also on the duration of burn and the age of the patient. High numbers of patients in our study were infected with resistant micro-organisms probably due to loss of primary defense as well as cross infection. Gram negative infection may be due to endogenous route while gram positive infection might be from surface contamination. Multiple invasions of organisms are very common and that also provides opportunities for transfer of resistance among organisms.[20]

Burn wound monitoring requires the study of changing bacterial flora and the antibiotic sensitivity reports. Repeated swab cultures and antibiograms are advised for proper selection of antibiotics to control sepsis. The development of resistance to a particular antibiotic is dependent on the use of that antibiotic in society at large. Overuse of any antibiotic predisposes to development of resistance.

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