



MICROBIAL INFECTION OF BURN WOUNDS

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ABSTRACT Infection is a major cause of morbidity and mortality in burn patients and when it occurs due to multi drug resistant organisms further worsen this situation.

Aim & Objective- To study the rate of isolation of aerobic bacteria in patients with burn wounds, their changing pattern of colonization with hospital stay as well as the sensitivity pattern.

Material & methods- Over a period of one year in a prospective study for 100 patients, two swabs were collected from the infected area of the burn wound every week from the date of admission. These swabs were subjected to direct microscopic examination as well as plated on the suitable culture media. The organisms isolated were identified according to standard protocol for identification and antibiotic sensitivity was performed.

Results- From 161 wound swabs by aerobic bacterial culture, 140 strains were isolated. In the first week of the total swabs, 32.2% of cultures were sterile and percentage of sterile swabs reduced drastically in the subsequent weeks. *Acinetobacter spp.* (42.1%) was the most commonly cultured organism followed by *Pseudomonas spp.* (18.6%) and *Klebsiella spp.* (17.9%). *Staphylococcus aureus* accounted for only 7.9% of isolates and most of these were methicillin resistant. The isolates of *Acinetobacter spp.* and *Klebsiella spp.* showed high degree of resistance to aminoglycosides, third generation cephalosporins and also to carbapenems. Imipenem-cilastatin, colistin and tigecycline were the most effective antibiotics for Gram negative bacteria.

Conclusion- Burn wounds destroy the most important defense mechanism of the body and has high propensity for infection particularly harboring multi drug resistant organisms.

KEYWORDS : burn wounds, microbiology, multi drug resistant organisms

Introduction:

Burn injuries are diverse but are unified in that they all involve liquefactive necrosis of this largest organ of the body, the skin. Infection is a major cause of morbidity and mortality in hospitalized burn patients. About 75% of the mortality associated with this is related to sepsis especially in developing countries. ⁽¹⁾ A florid growth of microbes at the wound site precludes septicemia. Thus, an adequate knowledge of the flora that predominantly colonize the burn wounds in a particular burn ward is essential for premonition and prompt management of imminent clinical sepsis with proper antibiotics, before the results of microbiological cultures become available.

Materials and Methods:

It is a prospective study undertaken at IMS & SUM Hospital, one of the important tertiary care burn centers of eastern Odisha, over a period of 1 year from Jan 2016 to Dec 2016. All the patients admitted in the burn unit consecutively were included in the study.

All patients admitted to the burn unit were primarily stabilized, emphasizing on support of the airway, respiration, and circulation followed by a burn-specific secondary evaluation to determine the site, size and depth of the burn in addition to all investigations that would aid the clinical assessment. All available information was recorded, including the patient demography and the cause of injury. This was followed by the usual regimen of local burn wound care in the form of clothing removal, wound cleaning by washing with sterile saline solution (0.9% NaCl), chemoprophylaxis and dressing.

Surface swabs were collected from all the patients at the time admission and then repeated weekly during their stay in the hospital. The most infected site as per clinical assessment was cautiously included for swabbing. Two swabs were collected from the infected area of the patient and were transported without any delay to the Microbiology Department. One of the swabs was subjected to direct microscopic examination by Gram staining while the other was plated on blood agar and Macconkey agar plates as well as to a nutrient broth. The organisms were identified according to standard identification protocol and antibiotic sensitivity was performed according to Kirby Bauer disk diffusion method as per Clinical Laboratory Standards (NCCLS) guidelines. All *Staphylococci* were screened for methicillin resistance by using oxacillin disk (1 µg).

Results:

From January 2016 to December 2016, 100 patients admitted in the burn unit were included in this study from which 161 wound swabs were collected for culture. Of the total patients, 52% were males and 48 % were females. Mean age of the patients admitted was 30 years. Most of the patients (76%) were in the productive age group of 15-60 years.

Table 1: Demographics of patients

Age group	Male	Female	Total
0-1	1	3	4
2-5	3	2	5
6-14	8	1	9
15-60	38	38	76
>60	2	4	6
Total	52	48	100

There were 2 flash injuries, 70 flame wounds, 9 scald wounds and 19 patients of electrical burns. Mean burnt area was 36.2 %, median being 32%.

Table 2: Distribution of number of patients according to the types and degree of burn

	≤10%	11-20%	21-30%	31-40%	41-50%	51-60%	61-70%	>70%	Total
	No. (%) Total	No. (%) Total	No. (%) Total	No. (%) Total	No. (%) Total	No. (%) Total	No. (%) Total	No. (%) Total	
Electric burn	07 (36.8)	07 (36.8)	02 (10.5)	02 (10.5)	01 (5.2)	00 (0)	00 (0)	00 (0)	19
Scald	01 (11.1)	00 (0)	02 (22.2)	04 (44.4)	02 (22.2)	00 (0)	00 (0)	00 (0)	09
Flame	03 (4.3)	10 (14.3)	12 (17.1)	17 (24.3)	08 (11.4)	08 (11.4)	02 (2.9)	10 (14.3)	70
Flash	00 (0)	01 (50)	00 (0)	00 (0)	01 (50)	00 (0)	00 (0)	00 (0)	02
Total	11 (11)	18 (18)	16 (16)	23 (23)	12 (12)	08 (8)	02 (2)	10 (10)	100

In our study, flame injury had highest propensity to get infected (74%) while other types of burns had lower chances of infection i.e. 47%, 50%, 56% in electric, flash, scald burns respectively.

Table 3: Organism isolation rate in different weeks

	No. of samples	Number of organisms (%)			
		0	1	2	≥3
1 st week	115	37(32.2)	62(53.9)	11(9.6)	5(4.3)
2 nd week	25	1(4.0)	19(76.0)	5(20.0)	0(0)
3 rd week	12	0(0)	7(58.3)	5(41.7)	0(0)
>3 weeks	9	1(11.1)	5(55.6)	3(33.3)	0(0)
Total	161	39	93	24	05

There were 32 swabs obtained at the time of admission in the hospital. Subsequently 83, 25, 12 swabs were obtained in the first, second and third weeks respectively. In the first week of the total swabs 32.2% of cultures were sterile while, 53.9 % grew a single organism and 13.9% grew more than 1 type of organism. The percentage of sterile swabs reduced drastically in the subsequent weeks i.e. 4%, 0%, 11.1% in the 2nd, 3rd and 4th week respectively. Accordingly 41.7 of swabs in 3rd week yielded multiple organisms.

Acinetobacter spp. was the predominant organism in the first three weeks which was replaced by *Klebsiella spp.* in the 4th week of hospital stay. Four samples grew yeast cells on cultures. Infection by two organisms was in 24(14.9%) culture collections and most of them (87.5%) were by a combination of different types of Gram negative bacilli.

Table 4: Types of organisms isolated in various weeks

Type of Organism	No.(% total in the week)				
	1 st week	2 nd week	3 rd week	4 th week	Total
<i>S. aureus</i>	10(12.1)	0(0)	1(5.9)	0(0)	11(7.9)
<i>CONS</i>	1(1.2)	0(0)	0(0)	0(0)	01(0.7)
<i>Enterococcus spp.</i>	6(7.2)	2(6.9)	0(0)	0(0)	08(5.7)
<i>E.coli</i>	1(1.2)	1(3.4)	0(0)	0(0)	02(1.4)
<i>Klebsiella spp.</i>	11(13.3)	5(17.2)	3(17.6)	6(54.5)	25(17.9)
<i>Enterobacter spp.</i>	2(2.4)	0(0)	0(0)	0(0)	02(1.4)
<i>Citrobacter spp.</i>	0(0)	1(3.4)	0(0)	1(9.1)	02(1.4)
<i>Pseudomonas spp.</i>	10(12.0)	5(17.2)	7(41.2)	4(36.4)	26(18.6)
<i>Acinetobacter spp.</i>	40(48.2)	13(44.8)	6(35.3)	0(0)	59(42.1)
<i>BYC</i>	2(2.4)	2(6.9)	0(0)	0(0)	04(2.9)
Total	83	29	17	11	140

Acinetobacter spp. isolated was mostly sensitive to tigecycline (89.8%) and colistin (77.9%). *Pseudomonas spp.* was more sensitive (42.3%) to imipenem than *Acinetobacter* strains (28.8%). Colistin was another effective anti-pseudomonal drug. The isolates of Enterobacteriaceae family members showed lesser degree of resistance than the non-fermenters like *Acinetobacter spp* and *Pseudomonas spp.* Apart from *Klebsiella spp* other Enterobacteriaceae were sensitive to aminoglycosides like amikacin and netilmicin. Imipenem, colistin and tigecycline were the most effective antibiotics for Gram negative bacteria.

Table 5: Sensitivity pattern of the isolated organisms

	E.coli n=2	Klebsiella spp n=26	Citrobacter spp n=2	Enterobacter spp n=2	Acinetobacter spp n=59	Pseudomonas spp n=26	S.aureus n=11	CONS n=1	Enterococcus spp n=8
OX	NA	NA	NA	NA	NA	NA	36.4	0	NA
AMC	0	0	0	100	0	0	9.1	0	25
CTR	0	0	50	100	0	7.7	36.3	0	NA
CFS	50	0	100	100	11.9	7.7	72.7	100	NA
CPT	50	11.5	100	100	3.4	3.8	81.8	100	NA
PIT	50	7.7	50	50	3.4	23.1	27.3	100	12.5
LE	50	23.0	50	50	10.2	7.7	45.5	0	12.5
AK	0	0	50	50	0	7.7	72.7	100	12.5
NET	50	3.8	100	100	15.3	NA	NA	100	NA
IC	100	50	100	100	28.8	42.3	27.3	100	50
CL	100	84.6	100	100	77.9	73.1	NA	NA	NA

TGC	100	80.7	100	100	89.8	NA	NA	NA	NA
CAC	NA	NA	NA	NA	NA	7.7	NA	NA	NA
LZ	NA	NA	NA	NA	NA	NA	90.9	100	87.5
V	NA	NA	NA	NA	NA	NA	90.9	100	87.5
TEI	NA	NA	NA	NA	NA	NA	100	100	NA
MO	NA	NA	NA	NA	NA	NA	27.3	0	NA

OX-Oxacillin, AMC-Amoxycillin clavulanic acid, CTR-Ceftriaxone, CFS-Cefoperazone sulbactam, CPT-Cefepime Tazobactam, PIT-Piperacillin tazobactam, LE-Levofloxacin, AK-Amikacin, NET-Netilmicin, IC- Imipenem cilastatin, CL- Colistin, TGC-Tigecyclin, CAC- Ceftazidime clavulanic acid, LZ- Linezolid, V-Vancomycin, TEI- Teicoplanin, MO- Moxifloxacin

Only 36.4 % of *S.aureus* strains isolated were methicillin sensitive. Cefepime- tazobactam, cefoperazone-sulbactam, linezolid and teicoplanin were the most useful antibiotics for these MRSA strains. Only 9.1% strains of *S.aureus* were vancomycin resistant. *Enterococcus* strains were mostly sensitive to linezolid, vancomycin and imipenem-cilastatin.

Discussion:

The burn wound surface is a protein-rich environment consisting of avascular necrotic tissue (eschar) that provides a favorable niche for microbial colonization and proliferation. (2-4) The nature and extent of the thermal injury along with the types and amount of microorganisms colonizing the burn wound appear to influence the future risk of an invasive wound infection.(5) Clinical diagnosis of burn wound infection is often hampered by thermal injury-induced hyperpyrexia, immune suppression and systemic inflammatory response syndrome (6), thus requiring a microbiological aid. A prior knowledge of burn wound microbiota also aids in a presumptive therapy.

Bacteria can be introduced onto this surface from a number of exogenous and endogenous sources, leading to colonization. (7) These are mainly derived from the patient's gastrointestinal, upper respiratory tracts as well as from the hospital environment. (8,9) Following colonization, depending on the invasive capacity of the organism, local wound factors, and the degree of immunosuppression of the patient the microorganism on the surface start to penetrate the burn eschar and progressing to invasive infections. (9)

There have been varying opinions regarding the method of obtaining optimal sample for culture of burn wounds. Tissue biopsy to provide quantitative assessment of burn wounds have been advocated by certain studies, but superficial swabs are the most convenient and least invasive approach and also provide an adequate sampling of the microbial flora present on the wound surface. (10) Other than in 3rd degree burns, the use of burn wound biopsies to determine burn wound bacteriology requires local anaesthesia and leads to unwanted scarring while not being otherwise of much practical use, as the time needed for processing limits their predictive and therapeutic value. (11) Thus, in the present study, surface swabbing of the most probable area was advocated for noting the microbiota as well as assessment of its change with time.

Although burn wound surfaces are sterile immediately following thermal injury, these wounds gradually become colonized with microorganisms (2) Gram-positive bacteria that survive the thermal insult, such as Staphylococci located deep within sweat glands hair follicles, heavily colonize the wound surface within the first 48 hour unless topical antimicrobial agents are used (2,12) After a week of burn, these wounds are subsequently colonized with other microbes, including Gram positive bacteria and Gram negative bacteria, and yeasts derived from the host's normal gastrointestinal and upper respiratory flora and/or from the hospital environment. (2-4, 12) In the present study, highest number of *Staphylococcus aureus* was found in the first week swabs which was drastically replaced in second and third week. The cultures in 3rd and 4th week had a predominance of Gram negative organisms. Our findings are consistent with those of other centers where Gram positive cocci were gradually superseded by Gram negative bacilli throughout the patients' hospital stay. (4,5,13-15)

Acinetobacter spp. accounted for the highest proportion of organisms present in the burn wound surfaces. Similar studies by Chim *et al* (16) and Singh *et al* (17) have also demonstrated an increasing prevalence of this organism in burn wards. It has been suggested that human skin could be the source of this organism. (18) It is being hypothesized that

Acinetobacter baumannii is said to be more prevalent in warm climates, and is present as a normal skin flora thus leading to increase in colonization and nosocomial infections by it.⁽¹⁵⁾ More over this organism being resistant to multiple drugs can thrive well in hospital environment.

Pseudomonas spp. is the second most predominant organism in our study. They are also prevalent as shown by other studies^(19, 20) and are due to the fact that it can thrive well in a moist environment of burn wounds.⁽¹⁹⁾ The variations in the organisms are due to differing local conditions, such as climate or microbial prevalence differing prevention protocols, topical and systemic treatment of burn wounds, sampling regimens as well as study lengths.

In the present study the *Acinetobacter* isolates were mostly resistant to commonly used antibiotics, thus necessitating the use of reserve drugs like tigecycline and colistin. A high degree of carbapenem resistance was also noted in these strains. Study by Chim *et al*⁽¹⁶⁾ also show a higher percentage of resistance (78.7%) of *Acinetobacter* isolates to imipenem. *Acinetobacter spp.* is now being commonly isolated not only from burn units but also from other Intensive care units^(16, 21) and they are generally resistant to the carbapenems which were thought to be the last resort of antibiotics. The widespread use of broad spectrum antimicrobials in the Burn wards and ICU as well as the increased susceptibility of these critically ill burn patients to infection provides a fertile ground warranting acquisition of resistance mechanisms and transformation to form new strains.

Klebsiella spp. account for 17.9% of the total organisms isolated in the study. These strains also show very high degree of resistance to third generation cephalosporins, aminoglycosides and carbapenems. Increasing resistance of *Klebsiella* strains has also been noted in another study.⁽²²⁾

In our study, only 7.9% of isolates were *S. aureus* of which only 36.4% strains were methicillin sensitive. Similar lower rate of isolation have also been noted with Rezai *et al*⁽²¹⁾, while other studies^(12, 23-25) report it as the leading cause of burn wound infections.

Conclusion:

The infection of burn wounds account for a high proportion of mortality in burn patients. Microbiota responsible for it often changes with time, locality and with the procedures used for treatment. *Acinetobacter spp.* and *Pseudomonas spp.* account for the highest proportion of infective agents and they are often resistant to commonly used antibiotics as well as carbapenems. Gram positive isolates often colonize the burn wounds in the first week of burn hospitalization giving way to Gram negative isolates in the later weeks. Burn wounds are harbinger of multi drug resistant *Acinetobacter* strains particularly in tropical areas. Thus the knowledge of the shift in the spectrum of organism is essential for an empiric treatment regimen.

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