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Bacteriological Profile of Burns Wound Isolated from a Teaching Hospital in Telangana, India

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A B S T R A C T

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimise morbidity and mortality present study is carried out to determine the bacteriological profile and antimicrobial resistance patterns among the patients admitted in burns ward at Osmania General Hospital, Hyderabad. The study included 150 patients admitted in burns unit at Osmania General Hospital, Hyderabad over a period of six months from March 2015 to August 2015. The wound swabs collected were sent to Microbiology lab for Grams stain, Culture and Antimicrobial susceptibility. Majority of the cases were in the age group 20-30 yrs. Females outnumbered males. Burns due to flames were the most common cause of the wound. Among 150 samples, 143 were culture positive of which, *Staphylococcus aureus* (*S. aureus*) was the predominant isolate followed by *Klebsiella*. Vancomycin was the most common sensitive drug among gram positive and Carbapenems for gram negative. To ensure early and appropriate therapy routine microbiological surveillance and a regular update of their antimicrobial susceptibility pattern could help in prevention and development of multidrug resistance.

Keywords

Burns Wound, surgical ward, *Klebsiella* app.,

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Introduction

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimise morbidity and mortality.[1]

Burn wounds are highly vulnerable to colonization and infection by microorganisms and this is a major problem in the management of burn victims. Immediately following the thermal injury, the burn wounds are sterile, but later, they eventually get colonized with microorganisms. The bacterial flora of infected wounds may change considerably during the healing period.[2]

It has been estimated that 75% of the deaths following thermal injuries are related to infections.[3]

Despite various advances in infection control measures, like early detection of

microorganisms and use of newer broad spectrum antibiotics, management of burn septicemia still remains a big challenge and it continues to be the leading cause of death in burn patients. [2]

As patients have to stay for long period in the hospital and exposed to many intravascular devices, they are at greater risk to hospital-acquired infection.[4]

Staphylococcus aureus is recognized as one of the most important bacterial pathogens seriously contributing to the problem of hospital infections all over the world. [5]

The increasing rate of burn wound infection and sepsis is due to overcrowding, inadequate sterilization and disinfection practices and gross contamination of environment, which predispose to secondary infections and in addition to lack of isolation facilities, inadequate hand washing and absence of barrier nursing.[4]

Complicating this high rate of infections is the fact that the spectrum of bacterial isolates and their antibiogram varies with time and geographical area. [3]

In view of the above facts, the present study is carried out to determine the bacteriological profile and antimicrobial resistance patterns among the patients admitted in burns ward at Osmania General Hospital, Hyderabad

Materials and Methods

The study was carried out at department of Microbiology, Osmania General Hospital, Hyderabad over a period of six months from March 2015 to August 2015.

Inclusion criteria

1. Wound swabs taken from 150 patients admitted in burns ward in OGH.

2. Swabs taken at the time of admission.

Exclusion criteria

Immunocompromised patients; patients with documented/ proven source of infection.

Specimen collection and processing

The area around the burn wound was cleaned with 70% ethyl alcohol and the wound swab was collected from the depth of the wound using two sterile cotton swabs. The sample was transported immediately to the laboratory for further processing. Samples were collected from the wound site, to rule out hospital acquired infections.

Direct Microscopy

The first swab was subjected to gram stain. The stained smear was screened carefully for presence or absence of pus cells and bacterial morphology, arrangement and their Gram reaction.

Gram Positive Control: *Staphylococcus aureus* ATCC 25923

Gram Negative Control: *Escherichia coli* ATCC 25922

Inoculation on culture media

The second swab was inoculated onto pre-incubated plates of MacConkey agar and 5% sheep blood agar by rolling the swab over the agar to make a primary well and then streaking from the primary inoculum using a sterile bacteriological loop to form secondary, tertiary and quaternary streak lines then these plates were incubated at 37°C for 24 hours in the incubator. Plates were observed for growth the next day. Plates showing no growth were incubated further aerobically at 37°C for next

24 hrs. All the isolates were identified by standard biochemical tests.[6] Antimicrobial susceptibility was done using Kirby Bauer disc diffusion method as per CLSI guidelines[7].

The following antibiotics were used (Hi-Media) -

Gram positive cocci

Vancomycin (VA) 30 µg, Cefoxitin (CX) 30 µg, Amikacin (AK) 30 µg, Amoxyclav (AMC) µg, Ofloxacin (OF) 5 µg, Tetracyclin (TE) 30 µg Penicillin (P) 10 units, Cotrimoxazole (COT) 25 µg

Gram Negative Bacilli

Imipenem (IPM) 10 µg, Ciprofloxacin (CIP) 5 µg**,** Piperacillin and Tazobactum (PIT) 30 µg, Amikacin (AK) 30 µg, Cefaperaxone and Sulbactum (CFS), Ceftazidime (CAZ) 30 µg, Ceftriaxone (CTR), Cotrimoxazole (COT) 25 µg.

Result and Discussion

In this study, wound swabs were taken from 150 isolates, admitted in burns ward, Osmania General hospital, Hyderabad over a period of six months, that is from March 2015 to August 2015. Majority of the cases were between the ages of 20 – 30 years. Children less than 10 years contributed to 8.66 % and elders above 70 years contributed to 3.33 % of the total cases. The youngest patient was 6 months old and the oldest patient was 78 years of age. There was a female proponderance (87.58%) Out 63 (42%) were male and 87 (58%) female

Burns due to flames (63.3%) were the most common cause of burn wounds (Figure 1). The total burned surface area (TBSA) was less than 30% in 12 (85.71%) of these 36 cases.

Out of 150 isolates, 143 were culture positive and 7 were culture negative. Among the culture positive isolates, 131 (91.6%) showed monomicrobial growth and 12 (8.3%) polymicrobial growth. About 56.6% were Gram positive and 43.6% were Gram negative.

Of these, *Staphylococcus aureus* was the predominant isolate 51.3% followed by Klebsiella species 16% (Table1)

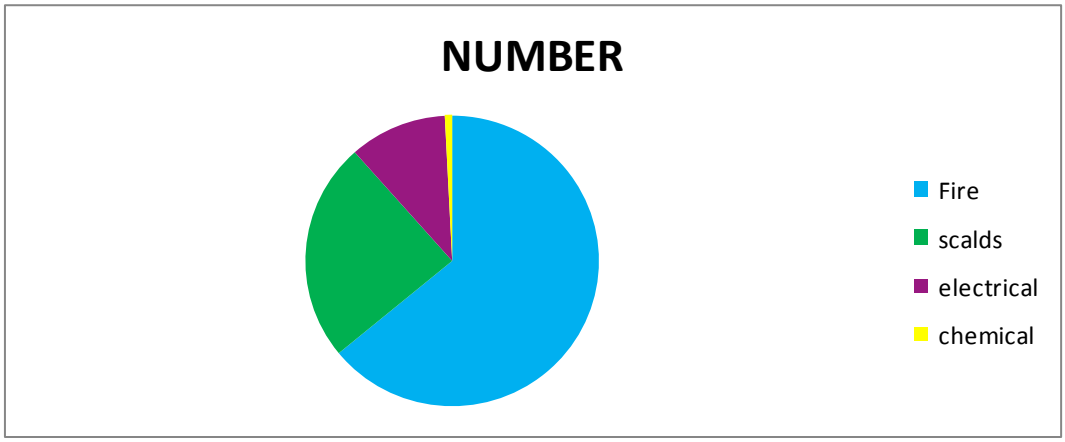
Table.1 Bacteriological profile of burns wound

Organism	number	percentage
S.aureus	77	51.3%
Klebsiella	24	16
Pseudomonas	12	9.3
CoNS	4	2.6
Proteus sps	8	4
E.coli	4	2.6
Citrobacter sps	2	1.3
Polymicrobial growth	12	8
No bacterial growth	7	4.6%
Total	150	100

Table.2 Antimicrobial spectrum of Gram Negative isolates

Isolate	IMI	CR	TZP	AM	CFS	CB	CTX	CT
Klebsiella **(n=24)**	24	16/8	12/12	13/11	10/14	9/15	8/16	12/12
P.aeruginosa **(n=12)**	11/12	8/4	9/3	10/2	3/9	8/4	5/7	3/9
Proteus **sps (n=8)**	8	6/2	5/3	6/2	4/4	4/4	3/5	4/4
E.coli **(n=4)**	4	2/2	3/1	3/1	2/2	2/2	1/3	2/2
Citrobacter **sps (n=2)**	2	2	1	1	2	1	1	2

Fig.1 Aetiology of burns



Among gram positive cocci, vancomycin was the most sensitive drug. About 57.1% of *S. aureus* strains were resistant to Cefoxitin. Among Gram negative isolates Imipenem was the most sensitive drug (Table 2). Bacterial infections of the burn wound still remains a major cause of morbidity and mortality in thermally injured patients.[8] The burned patient is prey for a wide variety of microorganisms as burns present an extensive surface with a large mass of dead tissue and free exudation of serum which is favourable for bacterial growth.[9] The burn site initially becomes colonized with microorganisms which if uncontrolled progresses to invasion and give rise to bacteremia and sepsis, which is a major cause of mortality in burn patients.[8]

Although the diagnosis of burn wound infections can be made clinically additional microbiological evidence is needed for instillation of proper therapy. Among the various microbiological methods available, the swab culture technique was used because it is a simple, convenient and effective method for the identification of all potential pathogens and their antimicrobial sensitivity.[10.]

Thermal injury creates a breach in the surface of the skin and is the hallmark of thermal

injury. Several important physiological functions are altered by thermal injury. The body tries to maintain homeostasis by initiating a process of contraction, retraction, and coagulation of blood vessels immediately after a burn injury. Three distinct zones have been defined within the burn wound: [10]

(i) the zone of coagulation, which comprises the dead tissues that form the burns is located at the center of the wound nearest to the heat source;

(ii) the zone of stasis; which comprises tissues adjacent to the area of burn necrosis that is still viable but still at risk for ongoing ischemic damage due to decreased perforation

(iii) zone of hyperaemia, which comprises normal skin with minimal cellular injury that has a predominant vasodilation and increased blood flow as a response to injury.

The histopathology of cutaneous burns shows initial changes due to direct effects of thermal injury and the subsequent inflammatory response. Cytologic and histologic alterations in the epidermis and skin appendages include nuclear pyknosis, perinuclear halo, disintegration of nucleoprotein (karyorrhexis) and condensation of eccrine sweat ducts and the pilo-sebaceous apparatus with disruption of sebaceous glands. Intercellular edema and separation of dermo-epidermal junction are responsible for vesiculation of partial-thickness burns and blister fluid is believed to represent a transudate from the well-developed capillary plexus in the papillary dermis. Dermal injury is recognized by the refractile eosinophilia and fusion of necrotic collagen fibres. Vessel wall show similar changes with coagulative erythrocytic and fibrin thrombi. Oedema, long recognized as an important phenomenon in thermal inflammation, is caused by transudation of fluid and protein from damaged vessels within the wound. [11]

About 50.6% of the patients were in the age group of 20 to 40 years which shows majority of the burn wound infections are seen during this age group According to Sadeghi-Bazargani *et al.,* the average age of the patient varies from 19 to 35 in the different studies they reviewed[12]. Similar results were seen by Chakraborty *et al.,* who reported that 56.6% of the cases were of 20-39 years age. 8 Likewise, Jaiswal *et al.,* that most of the cases were between 21 – 30 years of age. [13]

Incidence was more in females than males. In the present study the percentage of female and male is 58 % female and 42 % males. This is similar to findings by Kaur *et al.,* Rajput *et al.,* Ganesamoni *et al.,* [13, 14, 15] In contrast, Agnihotri *et al.,* Ramakrishnan *et al.,* reported that the incidence was higher in males in their study. [16, 17] High incidence of burns in females is probably due to occupational hazards of working in the kitchen as the kitchen is the most common place to receive a burn.

According to most studies, flame burns are the most common type of burn injuries.[18,19] In the present study as well, flame burns were the commonest type of burns accounting for 63.3% of all the cases. It was followed by burns due to scalds seen in 24 % of the cases, while this while this type of burn injury is most common in children, in the present study it is more common in the age group below 10 years of age. As the age increases, frequency of scalds decreases and flame burns increase in number. [20]

In this study, the overall isolation rate was found to be 95.4%. This was comparable with findings of isolation rates such as 93% by Ramakrishnan *et al.,* 95% by Kaur *et al.,* and 97.01% by Mehta *et al.,* [16] In contrast with the lower isolation rates findings of Srinivasan *et al.,* (86.3 %).[21]

The present study noted that monomicrobial growth was more common (88%) than polymicrobial. This is comparable to other studies by Jefferson Lessa Soares de Macedo et al, Ramakrishnan *et al.,* and Kaushik *et al.*, who reported monomicrobial growth rates of 89.3%, 84%, 78% respectively. [17, 22] In this study, the most common isolate is the *Staphylococcus aureus*, this is similar to some studies especially from developed countries which report *Staphylococcus aureus* as the most important organism in burn patients and also in comparison with the study of VG Bhat et al, Alghalibi *et al.,* and Naveen Saxena *et al.,* [23, 24] The second most common organism isolated is Klebsiella species (16%),Whereas, in some studies, it is *Pseudomonas aeruginosa*, which is the most common isolate in burns patients. [8,13.] Prevalence of Pseudomonas species in the burn wards may be due to the fact that the organism thrives in a moist environment.[14] As for *Pseudomonas aeruginosa*, they accounted for 9.3 % of all the organisms isolated in our study. Our results were comparable with those of Kauretal *et al.*, Agnihotri *et al.*, Ekrami *et al.*, Komolafe *et al.,* who also reported a low incidence. [18,25]

The prevalence of MRSA is 57.1%.Similar findings were seen by Rastegar Lari *et al.*, and Kaushik *et al.,* [14] The high prevalence of MRSA in our environment calls for routine testing of all *S. aureus* isolates from wound specimens for methicillin resistance. This will serve to curb the spread of this organism and also to institute early and appropriate therapy with ultimate reduction in the cost of management of these patients.

The Gram negative isolates, namely *Escherichi coli*, *Proteus* species and *Citrobacter* species showed good sensitivity to imipenem and piperacillin/tazobactam and moderate sensitivity to ceftazidime and ceftriaxone. Mehta M *et al.,* saw a significantly high percentage of resistance among gram negative bacilli to aminoglycosides, ciprofloxacin, carbenicillin, tobramycin and ceftriaxone. [26] But in comparison, Imipenem and combination drugs like cefoperazone/sulbactam were found to be effective. Jefferson Lessa Soares de Macedo *et al.,* Rastegar Lari *et al.*, reported a high degree of resistance to antimicrobial agents.[22, 14] Resistance to antibiotics in burn isolates reported previously has shown a gradual increase in resistance over time. [18]

The resulting antibiograms give some cause for concern because the predominant bacterial isolates were relatively resistant to the commonly available, more economical antimicrobials. However, this was not entirely un-expected as hospitals are an important breeding ground for the development and spread of antibiotic resistance. Many studies have shown that most of the organisms causing infection in burn patients are highly resistant to routinely used antibiotics and of exposing to heavy antibiotic use, a high density of patient population in frequent contact with health care staff and patient attendant increase the risk of cross-infection. [27.]

Burn wound infections are showing changing trends in the relative importance and bacterial colonization patterns as well as their antimicrobial sensitivities. To ensure early and appropriate therapy in burn patients, a frequent evaluation of the wound is necessary.

In conclusion, to ensure early and appropriate therapy routine microbiological surveillance and a regular update of their antimicrobial susceptibility pattern could help in prevention and development of multidrug resistance.

Our results may be helpful in providing useful information regarding the pattern of burn

wound microbial colonization, the dominant flora and antimicrobial resistance in our burn unit and thus will help in formulation of effective guidelines for therapy, thus improving overall infection related morbidity and mortality.

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