Original Article

Prospective Study of Burn Wound for Microbial Involvement and Antimicrobial Susceptibility from a Tertiary Center of Gwalior

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Abstract
Background: The major challenge for a burn team is nosocomial infection in burn patients, which is known to cause over 50% of burn deaths.

Aims and Objectives: To assess the pattern of bacterial colonization in a burn wound in patients admitted in Burn Unit.

Materials and Methods: Eighty two burn patients were studied in New Burn Unit of Department of Surgery, Gajra Raja Medical College and JA Group of Hospitals, Gwalior between June 2015 to May 2016. All patients were studied for nature and extent of microbial involvement in burn wounds and antimicrobial susceptibility and isolate pattern. Wound swabs were collected before change of dressing and administration of antibiotics with a swab immersed with normal saline on 0, 3rd, 7th and 14th day of hospital stay.

Results: Most common age group was 31-40 years (28.04%) with female preponderance (56.09%). Most of the patients had total body surface area (TBSA) of burn less than 30% (n=24) followed by 31-40% (n=25). Rate of bacterial growth was more on day 3 and 7 with 63.4% and 94.7% swabs yielding bacterial growth respectively. Bacterial isolates were frequently positive in cases with higher percentage of burn injury and mostly seen at day 7. Most common bacteria observed in burn wound was Staphylococcus aureus (n=96) followed by Pseudomonas species (n=80). A high level of drug resistance was seen with Pseudomonas species.

Conclusion: Burn wound are devastating form of trauma generally affecting female population. Bacterial contamination occurs after 24 hours of initial treat and reaches maximum level at 7 days Staphylococcus aureus and Pseudomonas species are most common organism causing sepsis and wound infection. These organisms were sensitive to use of Norfloxacin, and Amikacin.

Keywords: Nosocomial infection, total body surface area, bacterial infection, burn wound.

Introduction
Infection remains the major cause of morbidity and mortality in burn patients despite of significant advancement of burn care.

Staphylococcus aureus, Pseudomonas aeruginosa, Candida, Enterobacter and Acinetobacter are the classical pathogens affecting burn wound.¹,²
Wound surface culture is an important and useful technique in identifying the organism present on the burn wound and also identifying the predominant culprit of the burn wound flora. Though histological examination of burn wound biopsy is considered most authentic for confirming a diagnosis of invasive burn wound infection, it is time consuming and costly. This cannot be used as a routine diagnostic technique. Hence surface culture becomes the promising methods for diagnosis of infection and distinguishing it from colonization.

From time to time and from place to place the invading micro-organisms vary in their frequency and susceptibility to antibiotics. Hence, it is important to conduct a periodic study to evaluate the infective agents of burn wounds so that preventive measures could be modified accordingly. The aim of this study was to assess the pattern of bacterial colonization in a burn wound in patients admitted in Burn Unit.

**Material and Methods**

Present observational study was performed on 82 patients admitted in New Burn Unit of Department of Surgery, Gajra Raja Medical College and J A Group of hospital Gwalior from June 2015 to May 2016.

Burn patient of either age having age between 10-65 years admitted in burn unit with burn injury >10% and giving consent to take part in study, patients with total body surface area of burn 10-50% and all the cases of thermal burn fulfilling the above mentioned criteria were included in the study. Patient with Total body surface area of burn >50% or <10 %, patient of Age >65yrs or <10 yrs, patient with old infected burn wound, patient suffering from immunodeficiency disorders like AIDS, patient on chemotherapy or steroid therapy and all the patient with type of burn injury being other than thermal such as electric burns, chemical burns, radiation burns, etc. were excluded from the present study.

Ethical Committee approval and written Informed consent was taken from all enrolled patients after detailed counseling. The contents of the consent were read out to the patient in his/her language. Detailed history including age, sex, type of wound / mode of development of wound and any co-morbid condition was recorded in pre-approved performa. Thorough examination of burn including size and site of burn, depth and percentage of burn, any deformity present and presence of slough was done. Complete blood count, hemoglobin, packed cell volume, platelet count, random blood sugar, blood urea level, serum creatinine level, blood grouping, swab culture and sensitivity (0, 3rd, 7th, 14th day) and serum electrolyte level were also measured.

In present study burns are injuries caused by dry heat or scalds by moist heat. Severe burns are also caused by contact with electric wires, and by the action of acids and other chemicals. Nosocomial infection is an infection acquired as a result of hospitalization or treatment received in hospital after 48 hour of admission to hospital or before 30 days of discharge from hospital.

Nature and extent of microbial involvement in burn wounds was assessed as per Church et al. 5

Wound swabs were collected with a swab immersed with normal saline on 0,3rd, 7th and 14th days of hospital stay before dressing changes and administration of antibiotics. Disc diffusion technique was performed to evaluate antimicrobial susceptibility pattern of isolates. 6

The drugs that were tested include, for gram negatives: Amoxicillin + Clavulanic acid (30µg), Amoxicillin(10µg), Ampicillin(10µg), Ceftriaxone (30µg), Cefazidime(30µg), Chloramphenicol (30µg), Doxycyclin, Norfloxacine and Nalidixic acid(30µg). For gram positives: Amoxicillin (30µg), Amikacin, Chloramphenicol (30µg), Clindamycin(2µg), Cephalothin(30µg), Kanamycin(30µg), Methicillin(5µg), Penicillin G (10 IU) and Vancomycin(30µg).

All the data were analyzed using IBM SPSS ver. 20 software. Frequency distribution and cross tabulation was used to prepare the tables. Significance was assessed at 5% level.
Results
Maximum patients belong to the age group of 31-40 years [23 (28.04%)] with female [46(56.09%)] preponderance compared to male [36 (43.9%)]. Based on total body surface area (TBSA) involved, patients were grouped in 31-40% (n=25) of body surface area with burn injuries, 23 had 41-50% of burn area and 34 patients had involvement of less than 30% of their TBSA.
A total of 223 microbial isolates were identified from 328 wound swabs. It was found that swabs collected on day of admission were generally sterile, with only 6 of the 82 swabs yielding bacterial growth. While 76 (92.6%) burn wound swabs were sterile on day 0, microbial colonization reached 94.7% within the first week. Contamination of swabs on day 3 and day 7 with 63.4% and 94.7% swabs yielding bacterial growth respectively. Swabs collected on day 14 of admission showed significant decrease (p<0.05) in bacterial contamination owing to use of better and higher antibiotics according to antibiotic susceptibility.

Table 1: Showing relation of TBSA with Bacterial Growth*

| TBSA (%) | Day 0 | Day 3 | Day 7 | Day 14 |
|----------|-------|-------|-------|--------|
|          | Positive | Negative | Positive | Negative | Positive | Negative | Positive | Negative |
| 10-20    | 0      | 15     | 3      | 12     | 11      | 4       | 4        | 11      |
| 20-30    | 0      | 18     | 9      | 9      | 18      | 0       | 10       | 8       |
| 30-40    | 2      | 28     | 19     | 8      | 27      | 0       | 18       | 9       |
| 40-50    | 4      | 18     | 1      | 1      | 22      | 0       | 17       | 5       |
| Total    | 6      | 76     | 52     | 29     | 78      | 4       | 49       | 33      |

Data is expressed as no of patients, TBSA; total body surface area, *A total of 223 microbial isolates were identified

Table 2: Showing Isolation pattern of bacteria from burn wound*

| Bacteria isolated | Pus 1 isolates | Pus 2 isolates | Pus 3 isolates | Pus 4 isolates | Total |
|-------------------|---------------|---------------|---------------|---------------|-------|
| S. aureus         | 0             | 34            | 45            | 24            | 96    |
| Pseudomonas spp.  | 0             | 19            | 43            | 22            | 80    |
| Proteus spp.      | 0             | 6             | 2             | 2             | 10    |
| Klebsiella spp.   | 5             | 4             | 2             | 0             | 11    |
| E. coli           | 1             | 2             | 2             | 0             | 5     |
| Citrobacters spp. | 0             | 2             | 2             | 0             | 4     |
| S. pyogenes       | 0             | 2             | 0             | 0             | 2     |
| No growth         | 76             | 30             | 4             | 33           | 142   |
| Total isolates    | 6             | 71             | 98            | 48            | 223   |

Data is expressed as no of patients, TBSA; total body surface area, *A total of 223 microbial isolates were identified

Table 3: Showing antibiotic susceptibility of bacteria

| Antibiotic            | Bacterial isolates from burn wound resistant to particular antibiotic (%) |
|-----------------------|---------------------------------------------------------------|
|                       | Pse       | Sau       | Pro       | Cit       | Kle       | Eco       | Pro       | Spy       |
| Ampicillin            | 100       | -         | 50        | 100       | 100       | 40        | 100       | -         |
| Amoxicillin+ clavulanic acid | 100       | -         | 10        | 100       | -         | 100       | -         | -         |
| Amoxicillin          | 100       | 20.8      | 50        | 100       | 100       | 60        | 100       | -         |
| Chloramphenicol      | 87.5      | -         | 20        | 100       | -         | 100       | 100       | -         |
| Cefazidime           | 100       | 39.6      | 30        | 100       | 100       | 100       | 100       | -         |
| Ceftriaxone          | 37.5      | -         | 10        | 100       | 54.5      | -         | -         | -         |
| Doxycycline          | 97.5      | -         | 50        | 100       | 100       | 40        | 100       | -         |
| Nalidixic acid       | 95        | -         | 50        | 50        | -         | -         | -         | -         |
| Norfloxacin          | 15        | -         | 50        | -         | -         | -         | -         | -         |
| Cephalothin          | -         | 37.5      | -         | -         | -         | -         | -         | -         |
| Methicillin          | -         | 31.3      | -         | -         | -         | -         | -         | -         |
| Penicillin G         | -         | 100       | -         | -         | -         | -         | -         | -         |
| Amikacin             | 0         | 18.8      | -         | -         | -         | -         | -         | -         |
| Clindamycin          | -         | 4.2       | -         | -         | -         | -         | -         | -         |
| Vancomycin           | -         | 8.3       | -         | -         | -         | -         | -         | -         |
| Kanamycin            | -         | 25        | -         | -         | -         | -         | -         | -         |
| Total isolate        | 80        | 96        | 10        | 4         | 11        | 5         | 1         | 2         |

Sau; S. aureus, Pse; Pseudomonas spp., Pro; Proteus spp., Kle; Klebsiella spp., Eco; E coli, Cit; Citrobacters spp., Spy; S. pyogenes
On antibiotic susceptibility testing of Gram-negative bacteria, we found the most susceptible to imipenem (93.67%) and amikacin (75.94%). The susceptibility for gentamicin, ciprofloxacin, and trimethoprim sulfamethoxazole was 53.79%, 55.69%, and 42.45%, respectively. They were found to be resistant to cefotaxime (69.62%) and Tetracycline (69.81%).

Discussion
Burn patients are at a high risk of infection as a result of the nature of the burn injury itself, the immune-compromising effects of burns, prolonged hospital stays, and intensive diagnostic and therapeutic procedures. In present study, most common age group involved was 31-40 years (28.04%) with female (56.09%) preponderance. In agreement to present study Mundhada et al reported most common age group as 20-30 years with females (54%) preponderance. This may be due to the fact that females are probably more prone to occupational hazards of working in the kitchen. Mundhada et al studied 50 burn patients and reported that most of the patients had TBSA of burn in between 36% and 40, similarly in present study most of the patients had TBSA between 31-40% (n=25). Evidences have also shown that the chances of mortality increase with increase in TBSA of burn.

In a similar study by Mundhada et al where 202 swabs were studied, 181 swabs revealed growth while 21 swabs showed no growth. In agreement to this in present study a total of 223 microbial isolates were identified and bacterial isolates were more frequently positive in cases with higher involvement of TBSA with burn injury mainly at day 7. A Brazilian study by de Macedo et al found that the bacterial colonization of burn wounds reached 86.6% within the 1st week. Our study showed that there was gradual increase in rate of positive burn swab from periodic culture obtained which is supported by Erol et al. An Egyptian study by Ibrahim et al on 158 surface swabs of 66 burn patients reported that Staphylococcus aureus, Klebsiella pneumoniae and coagulase negative staphylococci were the most frequently isolated organisms. Present study findings that, Staphylococcus aureus was the most common bacteria observed in burn wound coincide with many previous reports. In contrast to present study Revathi et al and some other workers indicated P. aeruginosa as predominant organism in burn wound. A recent study from Maharashtra reported Klebsiella pneumoniae (34.40%) followed by Pseudomonas aeruginosa (23.94%), Staphylococcus aureus (22.94%) as the most common isolates from burn wound. Other studies by Srinivasan et al., Kehindeet al., and Mohammed et al. also recorded that Klebsiella species is the most common isolate from burn wounds. Lakshmi et al noted that most common bacteria isolated from the periodic swab culture were of pseudomonas species (33.6%) followed by E. coli (20%), Klebsiella species (17%), etc. however, contrary to other studies, Lakshmi et al reported low incidence of Staphylococcus aureus growth in periodic swab cultures.

The main forces driving the increase in antimicrobial resistant bacteria are poor infection control practices and inappropriate use of antibiotics. Sewnet et al studied 50 burn patients reported that the bacterial isolates identified were resistant to the commonly used drugs. High resistance was observed for Ampicillin (77.4%), Doxyecylone (74.0), Nalidixic acid (70.5%), Penicillin G (68.2%), tetracycline (67.5%), Methicillin (29.5%), Gentamycin (19.1%) and Ceftriaxone (18.5%). Similar results were depicted in the present study. In another study by Bayram et al, 43% of P. aeruginosa isolates were multidrug-resistant. Meropenem, amikacin, ciprofloxacin and cefepime were found to be most active antimicrobial agents against P. aeruginosa. In present study, we observed high level of drug resistance among gram negative isolates especially Pseudomonas species. The same was moderately resistant to Ceftriaxone (37.5%) whereas resistance was more marked for other
antibiotics. All isolates of *Pseudomonas* species were completely resistant for ampicillin, amoxicillin + clavulanic acid, amoxicillin and ceftazidime. Similar results were reported by Negeri et al. 25

**Conclusion**

Present study data showed that burns provide a suitable site for bacterial multiplication and infection mainly because of the larger area involved and longer duration of patient stay in the hospital. To ensure early and appropriate therapy in burn patients, a frequent evaluation of the wound is necessary, a strict antibiotic policy should be followed by all burn institutions and a rotation program for topical antimicrobial may retard the development of resistance. Therefore, a continuous surveillance of microorganisms and a regular update of their antibiotic resistance pattern are essential to maintain good infection control programs in the burn unit, thus improving the overall infection-related morbidity and mortality.

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