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Article

Isolation of bacterial flora from post burn infection: influence of socio-demographic background on the occurrence of burn injury

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Abstract: The objective of this work was to find out the bacteriological profile and risk factor for burn patients. Sixty burn patients were investigated during July 2014 to May 2015. Specimens were collected in the form of wound swabs. The prospective study showed that one third of the patients below 10 years old belong the highest burn infections. The rate of burn infections was higher (61.67%) in female compared to male patients. Respondents under primary level were the major victims (28.33%). On the basis of occupational status, student showed the highest (30%) infection. Maximum (48.33%) patients were injured by first degree burn. Hot liquids (scald) was found as one of the main causes of burn which was 50% prevalent. Forensic background explained maximum burn injuries were accidental (88.34%). Most of the children were the main victims during playing (36.67%) followed by working persons (25%). Pseudomonas spp (73%) was found to be the most common isolated microorganisms followed by Klebsiella spp (6.67%), Staphylococcus aureus (6.67%), Escherichia coli (6.67%), Proteus spp (5%) and Enterobacter (1. 6%). Some medical devices like floor, bed, water etc. were detected as positive sources of organisms. By antibiotic susceptibility tests it was found that Pseudomonas are resistant to ceftriaxone (100%) followed by ceftazidime (93%) and sensitive to colistin (90%). In the case of Staphylococcus aureus, 100% are resistant to amoxyclav and 100% are sensitive to ciprofloxacin.

Keywords: burn; socio-demographic background; bacteria; antibiotic

1. Introduction

Burn injuries constitute a severe emotive, psychosomatic and societal crisis for the affected individuals and their families. It has been estimated that about 75% of the mortality associated with burn injuries is related to sepsis especially in developing countries (Donati et al., 1993). They can be caused by scalds, thermal, electrical, gas or chemical agents (Mirmohammadi et al., 2013). Burns account for 1% of the global burden of diseases and cause more than 7.1 million injuries, a loss of almost 18 million disability-adjusted life years (DALYs), and more than 265,000 deaths worldwide annually (Elsous et al., 2016). Almost 173,000 children in Bangladesh suffered from burn injuries in 2003, making it the 5th leading cause of childhood illness in the country (Mashreky et al., 2008).

Pseudomonas sp, Acinetobacter sp., Proteus mirabilis, Klebsiella sp., Citrobacter sp, Enterobacter sp and Escherichia coli are most common gram negative bacteria associated with burn wounds (Patil et al., 2015). Patients with serious burn injury require immediate specialized care in order to minimize bacterial infection, which is a major cause of morbidity and mortality in burn patients (Rao et al., 2014).

The present study was conducted to determine the bacterial profile of burn wounds, the antimicrobial susceptibility patterns with respect to the source of wound and socio-demographic background of burn injured patients admitted at the National Institute of Burn and Plastic Surgery, Dhaka Medical College Hospital. The
subsequent information may be employed with the aim of burn wound management to lessen the onset and
density of bacterial growth and proliferation within the wounds.

2. Materials and Methods
2.1. Study population
This study included sixty patients who were admitted to the National Institute of Burn and Plastic Surgery,
Dhaka Medical College and Hospital during July 2014 to June 2015 suffering from types of acute burn injuries.

2.2. Sampling
Patients with significant major co-morbid medical conditions, psychiatric disorder, poor morale, severe immune
compromised and short life expectancy, unwilling to take part in the study, burn wound with history of
application of cow dung, toothpaste, egg etc. were excluded from the study. Wound samples were aseptically
collected on day of occurrence (day 1), 5th post burn day and 15th post burn day. Surface swabs were collected
from burn wounds after the removal of dressings and topical antimicrobial agents and cleansing of the wound
surface with 70% alcohol (Church et al., 2006). An area of about 4 cm² will be swabbed using two sterile cotton
swabs. Swab samples were collected from the wound area where the degree of burn was the maximum. Samples
were homogenized in 4 mL sterile saline (Alam et al., 2014).

2.3. Microbiological and biochemical examination
A complete laboratory examination conducted by Alam et al. (2014) was followed in the present study. Samples
were cultured on blood agar and MacConkey agar plates. Pathogenic microorganisms were isolated and
identified following the standard procedures (Forbes et al., 1998). MacConkey agar was used for the isolation of
Gram negative bacteria while the blood agar was used for isolation and identification of Gram positive bacteria.
Nutrient agar was used for the general cultivation and maintenance of bacteria. After inoculation, plates were
kept at 37°C for 24-48 hours A series of several biochemical tests were performed following the standard
protocol to identify the bacteria isolated from the wound samples (Cappuccino and Sherman 1996).

2.4. Study of antibiotic susceptibility
The standard agar disc diffusion method known as the Kirby-Bauer method was applied (Alam et al. 2014;
Munshi et al. 2012; Mehta et al. 2007; Bauer et al. 1966). A suspension of the test organisms was prepared by
adjusting the turbidity of the broth in phosphate buffer saline by comparing with McFarland 0.5 solutions.
Constant lawn of bacterial growth was prepared on Muller Hinton agar plates. Beforehand inoculation, the swab
was passed against the wall of the tube to drain out the additional fluid. Commercially accessible antimicrobial
discs (Oxoid, Hampshire, UK) were used aseptically on the surface of the inoculated plates at proper spatial
plan by means of a sterile needle. Susceptibility to the specific antibiotic was interpreted by the presence of
clear zone around the disc (Ferraro et al. 2001).

3. Results and Discussion
3.1. Socio-demographic background and occurrence of burn injury
The present study demonstrates that the percentage of burn infection in patients aged below 10 years was higher
(33.33%) than the other age groups (Table 1). Out of sixty patients, 20 were in Group I (0-10 years), some of
whom were school going children. Children are naturally curious, impulsive and active so they can be victim of
burn easily. This result correlates with the findings reported by Al-Akayleh (1998) which showed that the age
group<10 years had the highest distribution of burn wound infection. Group II (11-20 years) comprising 13
patients, both boys and girls, two boys were caught in fire during playing at the street, rest of the boys and girls
were found to perform household chores such as cooking and babysitting in the absence of their parents. The
authors interviewed 12 patients of Group III (21-30 years) in which most of them were house wives. They were
burnt due to flammable substances which were stored in the home and flammable clothing like georgette or
linen etc. Authors found five patients in Group IV (31-40 years) who were injured in their working places either
by electric voltage or chemicals. Sivamuthu (2019) observed the average age of the burns patient in his study
was 32 years and 30-40 years’ age group was the most commonly affected. In Group V (41-50 years) and Group
VI (51-60 years) we found four patients who got burn injuries because of accidental cases.
In South East Asia; young age, female gender, poor socio-economic status and low educational level are
identified as major risk factors for burn related injuries and death (Wolf and Arnoldo, 2012). In the present
study, percentage of burn injuries were higher in female (61.67%) than in the male (38.33%) (Table 1). This
correlates well with Goswami et al. (2016) but differs with Othman and Kendrick (2010) where male case more
commonly involved. In Bangladesh, traditional dress is sharee or salwar-kamiz or kurta through which fire are spread quickly. A woman usually spends a big time in kitchen where the sources of fire are present. Unfortunately, females are the main victim of acid burning too. The present study found eight female patients who were attacked by their husbands’ family for dowry. Authors studied three other cases which were also homicidal in which patients were injured by “petrol bomb” at the time of political violence in Bangladesh.

In the present study, males were found to be injured accidentally at home or at their working places. At the construction sites in developing countries, it is allowed to do electrical works without protection, which is a major cause of burn injury. Despite significant improvements in product safety, electrical injury is still the cause of many fatalities and of considerable morbidity in developed countries (Koumbourlis, 2002). In the present study, occurrence of burn injury was higher among the patients educated under primary level (28.33%) followed by primary level (23.33%). Among the graduate and above (6.67%), burn injury was the lowest (Figure 1). Low literacy and a lack of safety measures when in dealings with electricity and other sources might be the reason behind high burn occurrence among the poorly educated. Among the 60 patients, 25(41.67%) were students which was the maximum. The lowest rate of burn injury was observed among farmers (6.67%) (Table 1).

3.2. Cause and degree of burn injury

In the present study, 1-10% burn showed the highest occurrence (41.67%). Above 30% burn displayed the lowest occurrence (Table 2). Out of 60 respondents, most were injured by scald (29), followed by flame (13), electric (11), chemical (7). Forson et al. (2017) found 33(66%) of the studied patients had flame injury caused by gas, while 14 (28%) had scald injury and 3 (6%) had electrical injuries. This correlates with a study conducted by Shahzad et al. (2012) in Pakistan, which reported the predominant burn agent to be gas flame (76%), followed by scald (14%), contact (6%), electrical (3%) and chemical (1%). Furthermore, the study reveals that most of the cases were accidental (88.33%) followed by suicidal (6.67%) and homicidal (5%) (Table 3). Sivamuthu (2019) found that etiologies of injuries were flame burns (60%), scalds (25%), electrical burns (10%) and chemical burns (5%). Most of the burn injuries in his study were accidental (65%), followed by suicidal (15%), industrial (12%) and homicidal (8%). In the present study, 36.67% children were playing at the time of injury. 25% patients were injured during working followed by 13.33% patients who were involved in cooking, bathing with hot water (13.33%) and other works (8.33%) while burning (Table 3). The study reveals that 66.67% injuries occurred at home and surrounding areas followed by construction area (25%) and street (8.33%) (Table 3).

3.3. Sources of organism

Samples were collected from trolley used for patients but no growth of organisms was found. The floor of emergency and ward were also checked. After 5 pm, growth of organisms from floor was notified as it was the last time for wiping the floor with disinfectants. Authors found growth of organisms at the bed of patients (Table 4). Bed covers were not regularly changed. Patient housing in single bed in a room with a separate sink facility to wash hands and change in staffing pattern has been shown to prevent infection and reduce mortality (Shirani et al., 1986). Authors checked the curtain of emergency, cotton used for dressing, plaster, silicream, gloves, stethoscope; hand of surgeon, ward boy and nurse before attending patients but found no growth of organisms. Patients body seemed as major source of infection in the present study. The floor and bed of OT complex were found to be free from any growth of organisms because OT complex is a highly protective area (Table 4).

3.4. Antibiotic sensitivity and resistance

Usually fresh burn wound are sterile. But with the passing of time it would be infected. We took sample from patients at the 1st day of the incident and cultured but found no growth of organisms. At the 5th day of admission we took sample from each patient and then we found types of bacterial growth in wound. Out of 60 samples we found Pseudomonas sp. in 44 samples (73.33%) which is a gram negative bacteria followed S. aureus (6.67%), E. coli (6.67%), Klebsiella sp. (6.67%) Proteus sp. (6.67%) and Enterobacter sp. (1.67%). Deshpande et al. (2012) found Klebsiella was the most common organism isolated from the culture studies followed by Pseudomonas and methicillin resistant Staphylococcus aureus. Al-Aali (2016) found that the bacterial infection at least once reached 100% by the end of the 4th week of admission. In his study, S. aureus, Klebsiella pneumoniae and coagulase negative Staphylococci were the most frequently isolated organisms (20.2%) followed by Pseudomonas aeruginosa (14.6%) and E. coli (10.1%) (Table 5).
In the present study, we found that after taking antibiotics the growth of bacterial flora was decreased. At 15\textsuperscript{th} post burn day we found no growth of bacteria in 38 samples (63.33%), rest 12 isolates were \textit{Pseudomonas} sp. (20%), followed by \textit{S. aureus} (5%), \textit{E. coli} (3.33%), \textit{Klebsiella} sp. (3.33%) and \textit{Proteus} sp. (3.33%) (P=0.035) (Table 5). Taneja \textit{et al.} (2013) observed that colonization rates were 33% on first day, 94% on 7th day and 100% by 14th day. 42% swabs grew gram negative bacteria. Overall \textit{Staphylococcus aureus} was the predominant isolate (45%) followed by \textit{Pseudomonas aeruginosa} (13.9%). In case of \textit{Pseudomonas} sp. 100% were found resistant to ceftriaxone followed by ceftazidime (93%) and doxycycline (86.36%) (Figure 2). 90% \textit{Pseudomonas} were found sensitive to colistin followed by imipenin (84.1%) and are sensitive to piperacillin (88.63%). \textit{S. aureus} were resistant to amoxyclave and sensitive to ciprofloxacin (100%). In case of \textit{E. coli}, 83.3% were resistant to piperacillin (Figure 2). In a study conducted by Forson \textit{et al.} (2017), most of the \textit{Pseudomonas} sp. were resistant to ampicillin, cotrimoxazole, cefuroxime and ceftriaxone. Whilst most of the \textit{Proteus mirabilis} were resistant to ampicillin, cotrimoxazole, gentamicin, cefuroxime and ceftriaxone. \textit{Klebsiella} sp. and \textit{Klebsiella oxytoca} were both found to be resistant to ampicillin, tetracyclin, cotrimoxazole and ciprofloxacin (Figure 2).

Table 1. Occurrence of burn injury based on socio-demographic background.

| Gender  | No. of patients | Prevalence (%) |
|---------|----------------|----------------|
| Male    | 23             | 38.83          |
| Female  | 37             | 61.67          |

| Age Group | No. of patients | Prevalence (%) |
|-----------|----------------|----------------|
| Group I   | 20             | 33.33          |
| Group II  | 18             | 30             |
| Group III | 13             | 21.67          |
| Group IV  | 5              | 8.33           |
| Group V   | 2              | 3.33           |
| Group VI  | 2              | 3.33           |

| Profession | No. of patients | Prevalence (%) |
|------------|----------------|----------------|
| Business   | 5              | 8.33           |
| Service    | 10             | 16.67          |
| Day laborer| 9              | 15             |
| Farmer     | 4              | 6.67           |
| Student    | 18             | 30             |
| House wife | 8              | 13.33          |
| Other      | 6              | 10             |

Figure 1. Burn injury and level of literacy.
Table 2. Degree of burn injury.

| Group | Percentage of burn | No. of patients | Prevalence (%) |
|-------|--------------------|-----------------|---------------|
| I     | 01-10%             | 29              | 48.33         |
| II    | 11-20%             | 16              | 26.67         |
| III   | 21-30%             | 11              | 18.33         |
| IV    | 31-40%             | 2               | 3.33          |
| V     | 41-50%             | 2               | 3.33          |

Table 3. Background of burn injury.

| Cause of burn   | No. of patients | Percentage (%) |
|-----------------|-----------------|----------------|
| Scald           | 30              | 50             |
| Flame           | 10              | 16.67          |
| Electric        | 9               | 15             |
| Chemical        | 8               | 13.33          |
| Others          | 3               | 5              |
| Forensic background |           |               |
| Accidental      | 53              | 88.34          |
| Suicidal        | 2               | 3.33           |
| Homicidal       | 5               | 8.33           |
| Place           |                 |                |
| Construction area | 12            | 20             |
| Kitchen         | 17              | 28.33          |
| Bathroom        | 6               | 10             |
| Other places in home | 18          | 30             |
| Street          | 2               | 3.33           |
| Others          | 5               | 8.34           |
| Activity        |                 |                |
| Working         | 15              | 25             |
| Cooking         | 9               | 15             |
| Playing         | 22              | 36.67          |
| Bathing         | 8               | 13.33          |
| Others          | 6               | 10             |

Table 4. Source of organisms.

| Medical devices                  | Growth of microorganisms |
|----------------------------------|----------------------------|
|                                  | Yes | No  |
| Trolley                          |     | √   |
| Floor(Emergency)                 | √   |     |
| Floor( Ward)                     | √   |     |
| Bed                              | √   |     |
| Curtain                          |     | √   |
| Cotton used for dressing         |     | √   |
| Plaster                          |     | √   |
| Silcream                         |     | √   |
| Gloves                           |     | √   |
| Stethoscope                      |     | √   |
| Hand of ward boy                 |     | √   |
| Patient body                     |     | √   |
| Hand of surgeon                  |     | √   |
| Hand of Nurse                    |     | √   |
| OT complex                       |     | √   |
Table 5. Bacterial flora in burn wound.

| Day | Pseudomonas sp. | S. aureus | E. coli | Klebsiella sp. | Proteus sp. | Others | Absent |
|-----|-----------------|-----------|---------|----------------|-------------|--------|--------|
| 5th | 44              | 73.33     | 4       | 6.67           | 6.67        | 3      | 5      | 1      | 1.67 | 0     | 0     |
| 15th| 12              | 20        | 3       | 5              | 3.33        | 2      | 3.33   | 1      | 1.67 | 38    | 63.33 |

Figure 2. Antibiotic sensitivity.

4. Conclusions
Burns provide a suitable site for bacterial growth and contamination, mainly because of the greater area involved and lengthier period 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. Mass education and household environmental adjustment should be considered to prevent burn injury and infection. Special attention should be given to handle unsafe fire and other injury sources.

Conflict of interest
None to declare.

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