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## Original Article

# Study on Time-related Changes in Aerobic Bacterial Pattern of Burn Wound Infection

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**Abstract**

A prospective study was carried out in 50 burn patients admitted in Burn unit of Dhaka Medical College Hospital over a period of one year from January 2005 to December 2005 to evaluate time-related changes in aerobic bacterial colonization and their sensitivity pattern. Periodic swabs were taken from the burn wound on Day 0, Day 7, Day 14 and Day 21 to see the changing pattern of organisms during hospital stay of patients. In the present study burn injury was highest in the age group 11-20 yrs (34%). Male to female ratio was 1.38:1. The mean percentage of burn was 35.79% of total body surface area (TBSA). Fire was the major cause of burn (38%) followed by electric burn (20%). Among the 200 samples, single organism was isolated in 71% samples and mixed organism in 13.5% and no growth in 14.5%. Among single isolates *Pseudomonas aeruginosa* was leading (28%) followed by *Escherichia coli* (17.5%), *Staphylococcus aureus* (16%), coagulse negative *Staphylococcus* (4.5%) and *Klebsiella* (2%). Among mixed growth *Pseudomonas aeruginosa* was still leading (11%) follow by *E. coli* (9.5%) *Staphylococcus* (5.5%), *Proteus* (1%) and *Klebsiella* (0.5%).There were time -related changes in bacterial isolation from burn wound during hospital stay of patients. On admission 42% of the isolated organisms were *Staphylococcus aureus* and only 6% each *Pseudomonas aerunginosa* and *E. coli* were isolated. No growth was found in 28%. samples. These findings were gradually changing with time and on day 21 *Staphylococcus aureus* were only 4% whereas *Pseudomonas aeruginosa* were 40% and E. coli 28%. Antimicrobial sensitivity test showed that *pseudomonas aeruginosa* was highly resistant to antimicrobial agents. It was most sensitive to Imipenem (98.72%) followed by Aztreonam (33.44%), Ceftazidime (38.32%) and Gentamicin (19.23%). *E. coli* was also found most sensitive to Imipenem (98.15%) followed by Gentamicin (38.95%), Chloramphenicol (37.1%), Ciprofloxacin (35.25%) and ceftraixone (29.70%). *Staphylococcus aureus* was 100% sensitive to Vancomycin followed by Amoxiclav and Oxacillin (53.43% each), Gentamicin (44.70%) and Cloxacillin (39.52%). It is crucial for every burn institution to determine the specific pattern of burn wound microbial colonization, the time-related changes in dominant flora, and the antimicrobial sensitivity profiles. This would enable early treatment of septic episodes with proper empirical systemic antibiotics without waiting for culture results, thus improving overall infection related morbidity and mortality.

## Introduction

Burn injury is a major problem in many areas of the world. It has been estimated that 75% of all deaths following thermal injuries are related to infection[1]. Burn incidences are very common in our country. Burn is the fifth leading cause of child injury in Bangladesh. About 340 children are fatally burnt each year with almost one child dying each day[2]. But unfortunately specialized burn hospital is available only in Dhaka. The increase rate of burn wound infection and sepsis is due to overcrowding, inadequate sterilization and disinfection practices, gross contamination of environment, lack of isolation facilities, inadequate hand washing and absence of barrier nursing[3]. Burn wound allows microbial penetration and burn eschar provides an excellent culture medium for microorganisms with a significant alteration of immune function. Patients have to stay for long period in the hospital and many intravascular and other devices are put in them. Hence they are at greater risk of acquiring hospital-acquired infection. Overcrowding in developing countries increases the risk[3]. The organisms that predominate as causative agents of burn wound infection in any burn treatment facility change over

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time. Gram positive organisms are initially prevalent during hospital stay of patients; then gradually become superceded by gram negative opportunists that appear to have a greater propensity to invade[4]. For every burn institution there should have separate burn management protocol, as the prevalence and type of organisms may vary from centre to centre[5]. Infection in burns is not only important in being responsible for death but it is also an important factor in the prolongation of hospitalization time and delay in skin grafting. It is therefore essential for every burn institution to determine its specific pattern of burn wound microbial colonization, time-related changes in predominant flora and anti microbial sensitivity profiles[6]. This would allow early management of imminent septic episodes with empirical systemic antibiotic before the results of microbiologic culture becomes available thus improving overall infection related morbidity and mortality[6]. In spite of its importance, time-related changes in burn wound microbial flora were not investigated by many burn institutions[5]. In Bangladesh, study on etiology of burn wound infection has been carried out previously where time-related changes in microbial colonization were not included. In view of the above discussion present study was designed.

## Materials and Methods

The microbial colonization of wounds was studied weekly from the date of admission to the 21st day of hospitalization. So total number of samples of 50 patients was 200. On admission, the sampling procedure included collection of swab from clinically deep area of burn wound site prior to any cleansing. Later swabs were taken on occasions of surgical debridement or surgical excision and grafting. In each sampling procedure, the bandages were removed, the remnants of topical antimicrobial agents were scraped away and the wounds were swabbed before washing and applying new topical antimicrobial agents. Swabs were collected by using sterile cotton tipped swabs. Specimens were immediately transferred to sterile test tube. In case of collection of sample from dry surface, swabs were moistened with sterile normal saline. After collection, tubes were plugged properly, labeled and carried promptly to the microbiology laboratory of Dhaka Medical College. Wound swabs obtained from the burn patients were subjected to microbiological analysis. The isolates were identified by standard microbiological techniques and their antibiotic susceptibility was determined by using Stokes disc diffusion technique.

**Objectives:**

1. To perform aerobic culture of four samples from each burn wound case and their sensitivity pattern at a regular interval of seven days.
2. To develop a guideline for empirical treatment on the basis of time-related changes and antimicrobial sensitivity pattern of aerobic bacteria causing burn wound infection

**Ethical issues:** Written or verbal consent of patient or legal guardian and permission of the respective authority of burn unit were taken.

**Inclusion criteria:** Patients admitted within 24 hours of burn injury.

**Exclusion criteria:**
1. Patients admitted after 24 hours of burn injury.
2. Referred from other hospital.
3. Patient with more than 70% total body surface area burn (TBSAB).

**Results**

Among the total isolates single organisms were isolated in 71% samples, mixed organisms in 13.5% samples and no growth in 15.5% samples Among single isolates *Pseudomonas aeruginosa* was leading (28%) followed by *Escherichia coli* (17.5%), *Staphylococcus aureus*, (16%), Coagulase negative *Staphylococcus* (4.5%) and *Klebsiella* (2%). Prospective study revealed time-related changes in organism isolation. Gram positive organisms were initially prevalent then were gradually superceded by Gram negative organisms (Table- II). Mixed organisms were absent on admission culture which were gradually increasing up to Day 21. Isolation of *Staphylococcus aureus* was 42% on admission and was gradually decreasing to 4% on Day 21. On the other hand single isolation of *Pseudomonas aeruginosa* and *Esch. coli* were 6% each on admission culture which were gradually increasing upto 40% and 28% respectively on Day 21. The antibiogram of Gram negative organisms isolated from burn wound is shown in Table III. *Pseudomonas aeruginosa* was highly sensitive to Imipenem (98.72%) followed by Aztreonams (33.44%) and Ceftazidime (28.32%) but resistance to Ampicillin and Tetracycline was 100% followed by Cotrimoxazole (97.28%), Cephalexin (94.72%), Ciprofloxacin (92.16%), Ceftriaxone (84.48%) and Gentamycine (80.77%). Similarly *Esch. coli* was highly sensitive to imepenem (98.15%). Its resistance to Ampicillin was 88.8% followed by Tetracycline (85.1%), Cephalexin (79.55%), Cotrimoxazole (75.85%), Ciprofloxacin (64.75%), Chloramphenicol (62.9%) and Gentamicin (61.05%).*Klebsiella, Proteus, Enterobacter* and *Acinetobacter* was 100% Sensitive to Imipenem. Antimicrobial Sensitivity Pattern of Gram positive organisms isolated from burn wound is shown in Table IV. *Staphylococcus aureus* were 100% resistant to penicillin and conversely 100% sensitive to vancomycin followed by Oxacillin and Amoxiclav (53.47% each), Ceftriaxone (46.5%), Gentamicin (44.7%), Cloxacillin (39.52%), Cephalexin (37.2%), Ciprofloxacin (34.88%), Tetracycline (30.22%), Cotrimoxazole (20.92%) and Ampicillin (9.3%).

Table I. Organisms isolated from 200 samples (50 burn patients)

| Organisms | Number of isolation | | Total |
|---|---|---|---|
| | Single | Mixed | |
| *Pseudomonas aeruginosa* | 56 (28) | 22(11) | 78(39) |
| *Esch. coli* | 35(17.5) | 19(9.5) | 54(27) |
| *Staph. aureus* | 32(16) | 11(5.5) | 43(21.5) |
| *Coagulase –ve Staphylococcus* | 9(4.5) | - | 9(4.5) |
| *Klebsiella* | 4(2) | 1(0.5) | 5(2.5) |
| *Proteas* | 2(1) | 2(1) | 4(2) |
| *α-haemolytic Streptococcus* | 2(1) | - | 2(1) |
| *Enterobacter* | 1(0.5) | - | 1(0.5) |
| *Acinetobacter* | 1(0.5) | - | 1(0.5) |
| No growth | 31(15.5) | - | 31(15.5) |
| Mixed | - | 27(13.5) | 27(13.5) |

Table II. Time-related changes in organism isolation from burn wound

| Microorganisms | On admission n=50 | | 7th day n=50 | | 14th day n=50 | | 21st day n=50 | | Total n=200 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Pseudomonas* | 3 | 6 | 15 | 30 | 18 | 36 | 20 | 40 | 56 | 28 |
| *Esch. Coli* | 3 | 6 | 8 | 16 | 10 | 20 | 14 | 28 | 35 | 17.5 |
| *Staph. aureus* | 21 | 42 | 6 | 12 | 3 | 6 | 2 | 4 | 32 | 16 |
| *CoNS* | 8 | 16 | 1 | 2 | - | - | - | - | 9 | 4.5 |
| *Klebsiella* | - | - | 1 | 2 | 1 | 2 | 2 | 4 | 4 | 2 |
| *Proteus* | - | - | | | 1 | 2 | 1 | 2 | 2 | 1 |
| α *-Haemolytic Streptococcus* | 1 | 2 | 1 | 2 | | | | | 2 | 1 |
| *Enterobacter* | - | - | - | - | 1 | 2 | - | - | 1 | 0.5 |
| *Acinetobacter* | - | - | - | - | - | - | 1 | 2 | 1 | 0.5 |
| No growth | 14 | 28 | 10 | 20 | 7 | 14 | - | - | 31 | 15.5 |
| Mixed Organisms | - | - | 8 | 16 | 9 | 18 | 10 | 20 | 27 | 13.5 |

Table III. Antimicrobial sensitivity pattern of Gram negative organisms isolated from burn wound

| Antimicrobial agent | Sensitivity | *Pseudomonas Aeruginosa n=78* | | *Esch.coli n=54* | | *Klebsiella n=5* | | *Proteus n=4* | | *Enterobacter n=1* | | *Acientobacter n=1* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| AMP | S | 0 | 0 | 6 | 11.2 | 0 | 00 | 0 | 00 | 0 | 00 | 0 | 00 |
| | R | 78 | 100 | 48 | 88.8 | 5 | 100 | 4 | 100 | 1 | 100 | 1 | 100 |
| CN | S | 15 | 19.23 | 21 | 38.95 | 2 | 40 | 1 | 25 | 1 | 100 | 0 | 00 |
| | R | 63 | 80.77 | 33 | 61.05 | 3 | 60 | 3 | 75 | 0 | 0 | 1 | 100 |
| CRO | S | 12 | 15.52 | 16 | 29.7 | 3 | 60 | 2 | 50 | 00 | 0 | 00 | |
| | R | 66 | 84.48 | 38 | 70.3 | 2 | 40 | 2 | 50 | 1 | 100 | 1 | 100 |
| CIP | S | 6 | 7.84 | 19 | 35.25 | 2 | 40 | 2 | 50 | 0 | 00 | 0 | 00 |
| | R | 72 | 92.16 | 35 | 64.75 | 3 | 60 | 2 | 50 | 1 | 100 | 1 | 100 |
| CL | S | 4 | 5.28 | 11 | 20.45 | 1 | 20 | 1 | 25 | 0 | 00 | 0 | 00 |
| | R | 74 | 94.72 | 43 | 79.55 | 4 | 80 | 3 | 75 | 100 | 1 | 100 | |
| T | S | 0 | 00 | 8 | 14.9 | 1 | 20 | 0 | 0 | 00 | 0 | 0 | 00 |
| | R | 78 | 100 | 46 | 85.1 | 4 | 80 | 4 | 100 | 1 | 100 | 1 | 100 |
| SXT | S | 2 | 2.77 | 13 | 24.15 | 0 | 00 | 0 | 00 | 0 | 00 | 0 | 00 |
| | R | 76 | 97.28 | 41 | 75.85 | 5 | 100 | 4 | 100 | 1 | 100 | 1 | 100 |
| IPM | S | 77 | 98.72 | 53 | 98.15 | 5 | 100 | 4 | 100 | 1 | 100 | 1 | 100 |
| | R | 1 | 1.28 | 1 | 1.85 | 0 | 00 | 0 | 00 | 0 | 00 | 0 | 00 |
| CAZ | S | 22 | 28.32 | | | | | | | | | | |
| | R | 56 | 71.68 | | | | | | | | | | |
| ATM | S | 26 | 33.44 | | | | | | | | | | |
| | R | 52 | 66.56 | | | | | | | | | | |
| C | S | | | 20 | 37.1 | | | | | | | | |
| | R | | | 34. | 62.9 | | | | | | | | |

AMP =Ampicillin , CN = Gentamicin , CRO = Ceftriaxone , CIP = Ciprofloxacin ,CL = Cephalexin , T= Tetracycline , SXT = Cotrimoxazole, IPM = Imipenem , CAZ =Ceftazidime , ATM =Aztreonam , C = Chloramphenicol .

Table IV. Antimicrobial sensitivity pattern of Gram positive organisms isolated from burn wound

| Antimicrobial agent | Sensitivity | Staphylococcus aureus n=43 | | CoNS N=9 | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| P | S | 0 | 00 | 0 | 00 |
| | R | 43 | 100 | 9 | 100 |
| AMP | S | 4 | 9.3 | 1 | 11.12 |
| | R | 39 | 90.73 | 8 | 88.88 |
| CRO | S | 2 0 | 46.5 | 5 | 55.55 |
| | R | 23 | 53.5 | 4 | 44.44 |
| CL | S | 16 | 37.2 | 5 | 55.55 |
| | R | 27 | 62.8 | 4 | 44.44 |
| CIP | S | 15 | 34.88 | 4 | 44.44 |
| | R | 28 | 65.12 | 5 | 55.55 |
| OB | S | 17 | 39.52 | 3 | 33.33 |
| | R | 26 | 79.48 | 6 | 66.66 |
| SXT | S | 9 | 20.92 | 2 | 22.22 |
| | R | 34 | 79.08 | 7 | 77.77 |
| E | S | 10 | 23.25 | 3 | 33.33 |
| | R | 33 | 76.75 | 6 | 66.66 |
| T | S | 13 | 30.22 | 5 | 55.55 |
| | R | 30 | 69.78 | 4 | 44.44 |
| CN | S | 20 | 44.7 | 6 | 66.66 |
| | R | 23 | 55.3 | 3 | 33.33 |
| OX | S | 23 | 53.47 | 5 | 55.55 |
| | R | 20 | 46.53 | 4 | 44.44 |
| VA | S | 43 | 100 | 9 | 100 |
| | R | 00 | 00 | 0 | 00 |
| AMC | S | 23 | 53.47 | 5 | 55.55 |
| | R | 20. | 46.53 | 4 | 44.44 |

P = Penicillin , AMP = Ampicillin , CRO = Ceftriaxone , CL = Cephalexin , CIP = Ciprofloxacin , OB = Cloxacillin , SXT = Cotrimoxazole , E = Erytromycin , T = Tetracycline , CN = Gentamicin , OX = Oxacillin ,VA = Vancomycin , AMC = Amoxiclav .

## Discussion

Infection is an important cause of morbidity and mortality in burns. Severe burn patients are very susceptible to infection because of wide exposed raw areas, the presence of necrotic tissue, protein rich exudates, inability of blood to reach the colonized areas of wounds and other host defense mechanisms. The colonization and later invasion of tissues is from patient's normal flora of skin or from gastrointestinal tract or more usually by cross infection[7-10]. In the face of high mortality because of bacteraemia in burned patients, it is important to select antibiotics or combination of antibiotics with broad coverage for the usual pathogens. In a large number of patients this has to be empirical pending results of cultures[11]. As the type of bacteria and their sensitivity vary from place to place analysis of burn wound microbial colonization is to be performed so that the prophylactic and therapeutic regimens could be rationalized. There are also time-related changes in burn wound microbial colonization. Different types of study on burn wound infection have been carried out in different countries of the world. Among them few were regarding time-related changes in bacterial colonization. In Bangladesh time-related changes in burn wound infection were not included in previous studies. Infection with one or more organisms was present in 84.5% cases in our study. Single organism was isolated in 71% and mixed organism in 13.5% and no growth in 15.5% swabs. Incidence of infection varies from place to place and country to country due to different therapeutic and preventive policy[12].

*Pseudomonas aeruginosa* isolation was maximum in our study in both single (28%) and mixed (11%) infection (Table I). These findings were consistent with those of other centers of different countries[5,10,13]. But in a previous study in our country isolation of *Staphylococcus aureus* was leading[14]. In our study *Esch. coli* was the second most common organism isolated singly (17.5%) or in combination with other organisms followed by *Staphylococcus*, *Klebsiella* and others. Beta-haemolytic *Streptococcus* was not found in the wounds of any patient. Similarly the complete absence of B-haemolytic *Streptococcus* was also reported by some workers[1,10,13,15,16,17]. But some other workers found the organism in post burn infection[3,5,18].

Analysing the results of four wound swabs taken from burn wound of each patient it was observed that by day 21 all the samples yielded growth, number of mixed growth was highest and Gram negative organisms were predominant. All these changes were gradual from the starting to the end of sample collection (Table II). On Day 0 colonization by *Staphylococcus aureus* was 42% followed by *Pseudomonas aeruginosa* 6% and *Esch. coli* 6%. On Day 7 it was 12%, 30%and 16%; on Day 14 the percentage was 6%, 36% and 20%; on Day 21 it was 4%, 40% and 28% respectively. On Day 0 there was no mixed growth which was 20% on Day 21. All the (100%) swabs yielded growth on Day 21. Present study revealed that Gram positive cocci *(Staphylococcus aureus)* were initially prevalent then were gradually superceded by Gram negative bacilli specially *Pseudomonas aeruginosa* throughout patients hospital stay of 21 days. The study results of various worker revealed that the bacteriology of burn infection has been changing from time to time and also the antimicrobial sensitivity pattern. There are also time- related changes in burn wound microbial colonization. Gram positive cocci are initially prevalent then are gradually superceded by Gram negative bacilli throughout the patients hospital stay[1,4,19,20] that have a greater propensity to invade[21]. These time-related changes have also been found in our study (Table II). Periodic reviews of patterns of isolation and susceptibility profiles of organisms infecting burn wounds are needed in order to modify the preventive and therapeutic strategies[13]. It is therefore essential for every burn institution to determine its specific pattern of burn wound microbial colonization, time-related changes in predominant flora and antimicrobial resistance profile. This would allow early management of septic episode with proper empirical systemic antibiotics before the results of microbiological cultures become available thus improving the overall infection related morbidity and mortality[5].

**Conclusion and recommendation**

There were time-related changes in microbial colonization during hospital stay of patients. Initially *Staphylococcus* was predominant organism isolated from burn wound which was gradually superceded by Gram negative organism specially *Pseudomonas aeruginosa.*

For empirical treatment of wound infection in the first week when *Staphylococcus aureus* is predominant, Amoxyclav and from second week onward as Gram negative organism *specially Pseudomonas* aeruginosa is predominant, Gentamicin may be applied which will be cost-effective. Vancomycin and Imepenem are costly as well as reserve drugs. For imminent septic episode in the first week, Vancomycin and in the second week onward Imepenam is recommended before culture and sensitivity report. Prompt energetic effort is needed for comprehensive care of the patient so that hospital stay can be shortened thereby improving overall infection related morbidity and mortality.

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