Bacteriological Profile (Aerobic) of Burn Wound Infection with Its Antibiotic Sensitivity Testing in Silchar Medical College and Hospital

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ABSTRACT

Burn wounds are highly susceptible to colonization and infection which creates an obstacle in proper management of burn victims. Since burn wound infection shows changing trends in pathogenicity of microorganisms as well as their antibiotic sensitivity, hence, it is crucial to perform frequent evaluation of the burn wound to ensure early and appropriate therapy in burn patients. The study was conducted to find out the common organisms in infected burn wound samples and their antibiotic sensitivity pattern. The study was conducted in the department of Microbiology and Surgery of Silchar Medical College and Hospital between July, 2015 and June, 2016. Pus samples and wound swabs collected from the hospitalized burn patients were processed according to standard microbiological techniques and Antibiotic sensitivity testing was done using Kirby Bauer’s Disc diffusion technique according to C.L.S.I guideline. Out of 100 pus samples collected from patients admitted in burn unit, 79(79%) cases were culture positive, while 21(21%) were sterile. Out of 79 organisms isolated, 31 (39.24%) were Pseudomonas aeruginosa, 21 (26.58%) were Staphylococcus aureus, 14 (17.72%) were Klebsiella pneumoniae, 8 (10.13%) were Klebsiella oxytoca and 5(6.33%) were Proteus mirabilis. The Gram positive organism showed maximum sensitivity towards Vancomycin and Linezolid (100%) and minimum towards Ampicillin (28.57%) while gram negative isolates showed maximum sensitivity to Imipenem (100%) and minimum towards Ampicillin(17.24%). The high prevalence of antimicrobial resistance emphasizes the need to strengthen the infection control practices along with regular and periodical monitoring and surveillance activities to restrict emerging trend of antimicrobial resistance.

Introduction

Infected burn wounds are not only associated with a delay in epidermal maturation and deep scar formation but also prolongs the hospital stay of the patient and increases the chances of mortality due to sepsis, when compared to non-infected patients.¹ Most of the burn victims, who survive including the initial 24 hours after burns, succumb to burn infection and its complications. Immediately following the thermal injury, the burn wounds are sterile; but eventually get Colonized with microorganisms.² Various factors responsible...
are disruption of the skin barrier, a large cutaneous bacterial load, the possibility of the normal bacterial flora becoming opportunistic pathogens and severe depression of the immune system. All these factors contribute towards the sepsis in a burn victim. The pattern of infection differs from hospital to hospital; the bacterial flora of infected wound may change considerably during the healing period. Microorganisms are transmitted to the burn wound surfaces by the hands of personnel, by fomites and possibly by hydrotherapy. The gastrointestinal tract is a potential reservoir for organisms that infect burn wounds, and it is likely that endogenous microbes are transmitted to burn wound surfaces by faecal contamination. Earlier, *Streptococcus pyogenes* was the most frequent isolate from infected burn wounds. Currently, the common pathogens isolated from burn wounds are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, coliforms, *Acinetobacter* spp., and others like anaerobic bacteria and fungi.

Good infection control practices have a great impact on survival rate of burn patients. Emerging antimicrobial resistance in burn wound bacterial pathogens represent a serious therapeutic challenge for clinicians treating these patients. In order to overcome this problem continuous microbiological surveillance is needed.

So this study is conducted to isolate and identify the common organisms causing burn wound infection and to determine their antibiotic sensitivity pattern to provide empirical treatment for favourable outcome.

**Materials and Methods**

Study area: Silchar Medical College & Hospital (Microbiology & Surgery Department)

Study Period: One year from July 2015 to June 2016.

Inclusion criteria: All pus samples/ wound swabs collected from the hospitalized burn patients.

Exclusion criteria: 1) Patients on antibiotic therapy.
2) Patient with wounds caused by other than burns

Study population: Pus samples in the form of wound swabs were collected from patients admitted in burn unit of Department of Surgery, Silchar Medical College & Hospital. Patients of any age and both the sexes were included in this study.

Number of specimen: A total of 100 pus samples were collected.

A detailed history was taken with reference to name, age, sex, religion, hospital number, chief complaints, past history, underlying disease, antibiotic history etc. and all these informations were recorded in a pre-designed proforma.

The collected samples were processed for identification of organisms using standard microbiological techniques and biochemical test. All strains were tested for antimicrobial susceptibility testing using C.L.S.I guidelines. Under strict aseptic condition pus samples from burn wounds were collected in the form of swabs in sterile test tubes. The collected samples were immediately transferred to Bacteriology section of Department of Microbiology, Silchar Medical College & Hospital for processing. The samples were at
first inoculated into culture media and then direct smears were prepared. The direct smears were then subjected to Gram staining.

Smear were prepared from the specimen in clean grease free glass slides, dried and then heat fixed. Gram staining of the smear was done according to the methods described by Duguid JP(2006). It was examined for the presence of any bacteria and pus cells.

For primary isolation of bacteria the specimens were inoculated into the following media:

1) 5% sheep blood agar media
2) MacConkey agar media

The media were prepared as per methods described by Collee et al., (2006).

The inoculated blood agar and MacConkey agar media were incubated aerobically at 37°C for 24 hours. If no growth was observed after 24 hours incubation then it was reincubated for another 24 hours after which if there was no growth it was considered sterile.

After incubation, identification of bacterium from positive cultures was done with a standard microbiological technique which includes motility testing by hanging drop preparation, gram staining and biochemical reactions such as catalase, coagulase, indole, methylred, Voges-Proskauer, citrate, urease, Phenyl pyruvic acid test and oxidase test. Further biochemical tests done were carbohydrate fermentation test using Lactose, sucrose, mannitol and Maltose, Triple sugar Iron test, Nitrate reduction test, Arginine dihydrolase production, lysine and ornithine decarboxylase test, Hugh and leifson test.

The antimicrobial susceptibility testing were done by Kirby Bauer’s Disk Diffusion method and interpreted as per Clinical Laboratory Standard Institution (CLSI) guidelines. Mueller Hinton agar (MHA) was used as media, it was inoculated with a suspension of organisms equivalent to 0.5 McFarland turbidity standard and discs were applied. Maximum six (6) antimicrobial discs were put in the 100 mm diameter MHA plate and plates were incubated at 37°C overnight.

The antibiotic discs used were purchased from HiMedia Lab Pvt. Ltd. Inhibition zones were measured and reported as sensitive or resistant according to manufacturer’s literature.

*Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 were used as quality control strain. Antibiotic discs were used for determination of sensitivity by Kirby- Bauer disc diffusion test.

For gram positive organisms Vancomycin, Linezolid, Cefoxitin, Cefotaxime and Penicillin and for gram negative organisms Imipenem, Piperacillin/Tazobactum, Aztreonam, Ceftazidine and Cefuroxime were exclusively used in this study.

| Antibiotic concentration/disc | Antibiotic concentration/disc |
|------------------------------|------------------------------|
| IMIPENEM(IPM)-10mcg          | CEPTRIAXONE(CTX)-30mcg       |
| AMPICILLIN(AMP)-10mcg        | AMIKACIN(AK)-30mcg           |
| LINEZOLID(LZ)-30mcg          | AZTREONAM(AM)-30mcg          |
| VANCYMYCIN(VA)-30 mcg        | GENTAMICIN(GEN)-10mcg        |
| AMOXYCLAV(AMC)-30mcg         | LEVOFLOXACIN(LE)-5mg         |
| PIPERACILLIN/TAZOBACTAM      | CIPROFLOXACIN(CIP)-5mg       |
| (PIT)100/10 mcg              |                              |
| CEFOTAXIME(CTX)-30mcg        | CEFUROXIME(CXM)-30mcg        |
| CEFOSITIN(CX)-30mcg          | PENICILLIN(PE)-10 units      |
| CEPTAZIDIME (CAZ)-30mcg      |                              |

Results and Discussion

Out of 100 samples collected, 79(79%) cases were culture positive, while 21(21%) were sterile. Out of 100 patients 30 burn patients
(30%) are in the age group of 20-29 years which is the most common age group found in this study followed by 22 patients between 30-39 years, 19 patients between 0-9 years, 13 patients between 40-49 years, 10 patients between 10-19 years, 4 patients above 59 years and 2 patients between 50-59 years respectively. Out of 79 culture positive cases, 21 (26.58%) cases were caused by gram positive organisms, while 58 (73.42%) cases were caused by gram negative organisms. Out of 79 culture positive cases, *Pseudomonas aeruginosa* 31(39.24%), *Staphylococcus aureus* 21(26.58%), *Klebsiella pneumoniae* 14 (17.72%), *Klebsiella oxytoca* 8 (10.13%) and Proteus mirabilis 5(6.33%). Gram positive isolate showed maximum sensitivity towards Vancomycin and Linezolid (100%) followed by Ciprofloxacin (85.71%), Cefoxitin (80.95%), Gentamicin (76.19%), Levofloxacin (71.43%), Amikacin (71.43%), Amoxicillin-Clavulanic acid (66.67%), Ceftriaxone (61.9%), Penicillin (42.85%), Cefotaxime (38.09%) and Ampicillin (28.57%). MRSA detected was 19.05%. The gram negative isolates showed maximum sensitivity to Imipenem (100%) followed by Ciprofloxacin (84.48%), Levofloxacin (81.03%), Ceftriaxone (67.24%), Aztreonam (67.24%), Piperacillin/Tazobactum (65.52%), Amikacin (62.06%), Ceftazidime (58.62%), Cefuroxime (56.89%), Gentamicin (53.45%), Amoxycillin-Clavulanic acid (41.38%) and Ampicillin (17.24%).

Infection is the most important problem in the treatment of burns. Burns become infected because the environment at the site of the wound is ideal for the multiplication of infecting organisms. The immune-suppressive status of the patient, immediate lack of antibodies, plentiful supply of moisture and nutrients in the physical environment; the temperature and gaseous requirements etc. are ideal for the growth of microorganisms.\(^6,7\)

Burn wound infections are showing changing trends in the relative importance and cyclic Pathogenicity of microorganisms as well as their antimicrobial sensitivities. To ensure early and appropriate therapy in burn patients, a frequent evaluation of the wound is necessary. Thus, a continuous surveillance of microorganisms and their antibiotic susceptibility patterns is essential to maintain good infection control programmes in the burn unit, thus improving the overall infection related morbidity and mortality.\(^14\)

### Table 1 Age wise distribution of burn patients

| AGE GROUP     | NO. OF CASES | CULTURE POSITIVE |
|---------------|--------------|------------------|
| 0-9 YEARS     | 19           | 12               |
| 10-19 YEARS   | 10           | 9                |
| 20-29 YEARS   | 30           | 21               |
| 30-39 YEARS   | 22           | 20               |
| 40-49 YEARS   | 13           | 11               |
| 50-59 YEARS   | 2            | 2                |
| >59 YEARS     | 4            | 4                |
**Table 2** Distribution of isolates based on gram staining

| Isolates         | Number | Percentage |
|------------------|--------|------------|
| Gram positive    | 21     | 26.58%     |
| Gram negative    | 58     | 73.42%     |
| **Total**        | **79** | **100%**   |

**Table 3** Different organisms isolated

| ORGANISM                     | NUMBER | PERCENTAGE |
|------------------------------|--------|------------|
| *Pseudomonas aeruginosa*     | 31     | 39.24%     |
| *Staphylococcus aureus*      | 21     | 26.58%     |
| *Klebsiella pneumoniae*      | 14     | 17.72%     |
| *Klebsiella oxytoca*         | 8      | 10.13%     |
| *Proteus mirabilis*          | 5      | 6.33%      |
| **TOTAL**                    | **79** | **100%**   |

**Table 4** Distribution of patients based on total burn surface area

| Total Burn Surface Area(TBSA) | NO. OF CASES | PERCENTAGE |
|--------------------------------|--------------|------------|
| <25%                           | 44           | 44%        |
| 25- 50 %                       | 38           | 38%        |
| 51- 75%                        | 11           | 11%        |
| >75%                           | 7            | 7%         |
| **TOTAL**                      | **100**      | **100%**   |

**Table 5** Distribution of patients based on type of burn

| TYPE OF BURN    | NO. OF PATIENTS | PERCENTAGE |
|-----------------|-----------------|------------|
| FLAME BURN      | 71              | 71%        |
| SCALD BURN      | 14              | 14%        |
| ELECTRIC BURN   | 15              | 15%        |
| **TOTAL**       | **100**         | **100%**   |

**Table 6** Sensitivity pattern of Gram positive isolate (*Staphylococcus aureus*)

| ORGANISM   | TOTAL NO | CIP   | LE   | GEN | AMP | CTR | CTX | CX  | AMC | PE | AK | LZ | VA |
|------------|----------|-------|------|-----|-----|-----|-----|-----|-----|----|----|----|----|
| S.aureus   | 21       | 18    | 15   | 16  | 6   | 13  | 8   | 17  | 14  | 9  | 15 | 21 | 21 |

**PERCENTAGE**

|             |          |       |      |     |     |     |     |     |     |    |    |    |    |
|-------------|----------|-------|------|-----|-----|-----|-----|-----|-----|----|----|----|----|
|             | 85.71    | 71.43 | 76.19| 28.57| 61.9%| 38.09| 80.95| 66.67| 42.85| 71.43| 100 | 100 |
Table 7: Sensitivity pattern of Gram negative isolates

| Organism       | Total No | CIP | LE | GEN | AMP | AK | CTR | PIT | CAZ | CXM | AMC | AT | IMP |
|----------------|----------|-----|----|-----|-----|----|-----|-----|-----|-----|-----|----|-----|
| P. aeruginosa  | 31       | 27  | 25 | 15  | 5   | 16 | 22  | 19  | 18  | 17  | 10  | 20 | 31  |
| K. pneumoniae  | 14       | 11  | 12 | 9   | 2   | 11 | 9   | 10  | 8   | 8   | 8   | 10 | 14  |
| K. oxytoca     | 8        | 7   | 6  | 5   | 2   | 6  | 5   | 6   | 5   | 5   | 4   | 6  | 8   |
| P. mirabilis   | 5        | 4   | 4  | 2   | 1   | 3  | 3   | 3   | 3   | 3   | 2   | 3  | 5   |
| Total no       | 58       | 49  | 47 | 31  | 10  | 36 | 39  | 38  | 34  | 33  | 24  | 39 | 58  |
| Percentage     | 84.48%   | 81.03% | 53.45% | 17.24% | 62.06% | 67.24% | 65.52% | 58.62% | 56.89% | 41.38% | 67.24% | 100% |

Fig. 1 Pie diagram showing culture results

Culture result

Culture positive 79%
Sterile 21%

79%
21%
In this study out of 100 samples from burn patients 79(79%) samples were culture positive. This finding is comparable to findings of Kaushik et al.,\textsuperscript{15} AL –Bdour et al.,\textsuperscript{16} Idomir et al.,\textsuperscript{17} Dash et al.,\textsuperscript{18} Saxena et al.,\textsuperscript{5} Modi et al.,\textsuperscript{14} Magnet et al.,\textsuperscript{19} and Sharma et al.,\textsuperscript{20} In Kaushik et al.,\textsuperscript{15} culture positivity was 293 out of 336 samples i.e. (87.2%). Culture positivity of AL-B dour MN et al.,\textsuperscript{16} was 84.6%, Idomir et al.,\textsuperscript{17} was 86.2%, Dash et al.,\textsuperscript{18} was 88.6%, Saxena et al.,\textsuperscript{5} was 70.33% i.e. 147 out of 209 samples showed culture positivity. Modi et al.,\textsuperscript{14} was 85.7%, Magnet et al.,\textsuperscript{19} showed 66.66% i.e. 100 out of 150 samples showed positive growth and Sharma et al.,\textsuperscript{20} showed 87.96% positive culture.

In other studies, conducted by Agnihotri et al.,\textsuperscript{21} Begum et al.,\textsuperscript{22} Mamani et al.,\textsuperscript{23} Kulkarni et al.,\textsuperscript{24} Shrivastava et al.,\textsuperscript{25} rate of culture positivity were high compared to present study. Culture positivity showed by Agnihotri et al.,\textsuperscript{21} was 96%, Begum et al.,\textsuperscript{22} was 92.85%, Mamani et al.,\textsuperscript{23} was 93.3%. In Kulkarni et al.,\textsuperscript{24} 83 out of 91 samples i.e. 91.2% showed positive growth and in Shrivastava et al.,\textsubscript{25} 109 out of 118 samples i.e. 92.37% showed positive culture.

While studies conducted by Vaez et al.,\textsuperscript{26} and Mohamed et al.,\textsuperscript{27} found comparatively low rate of culture positivity of 31% and 60 %respectively.

In the present study, gram negative organisms were the predominant pathogens constituting 73.42% case.

This finding is in concordance with Kulkarni et al.,\textsuperscript{24} Vaez et al.,\textsuperscript{26} and Sharma et al.,\textsuperscript{20} However studies conducted by Komolafe et al.,\textsuperscript{28} and Idomir et al.,\textsuperscript{17} found higher percentage of gram positive organisms compared to gram negative organisms.

In the present study the most common organism isolated was \textit{Pseudomonas aeruginosa} which constituted 39.24\% of total organisms followed by \textit{Staphylococcus aureus} (26.58\%). This finding correlates with studies conducted by Kaushik et al.,\textsuperscript{15} Agnihotri et al.,\textsuperscript{21} Rajput et al.,\textsuperscript{4} Dash M et al.,\textsuperscript{18} Saxena et al.,\textsuperscript{5} and Magnet et al.,\textsuperscript{19}.

However in study conducted by Srinivasan et al.,\textsuperscript{29} the most common organism was \textit{Klebsiella} (33.91\%), in Vindenes et al.,\textsuperscript{30} the most common organism was \textit{Coagulase – negative Staphylococcus} (21.5\%) and in study conducted by Bayram Y et al.,\textsuperscript{31} the most common organism was \textit{Acinetobacter baumannii} (23.6\%) which is dissimilar to present study.

Among the gram positive isolates, Linezolid and Vancomycin are found to be most effective drugs showing 100\% sensitivity to all isolates. Similar observation was made by Sharma et al.,\textsuperscript{20} where gram positive isolates were 100 \% sensitive to Vancomycin and Linezolid.

In studies conducted by Ahsan et al.,\textsuperscript{32} and Bhama et al.,\textsuperscript{33} \textit{Pseudomonas aeruginosa} was 100\% sensitive to Imipenem which is similar to present study. However studies like Dash et al.,\textsuperscript{18} Saxena et al.,\textsuperscript{5} and Behesti et al.,\textsuperscript{34} low rate of sensitivity was found which were 90.8\%, 95.77\% and 38.9\% respectively. Since burn wound infection shows changing trends in pathogenicity of microorganisms as well as their antibiotic sensitivity, hence, it is crucial to perform frequent evaluation of the burn wound to ensure early and appropriate therapy in burn patients. Also, the high prevalence of antimicrobial resistance emphasizes the need to strengthen the infection control practices along with regular and periodical monitoring and surveillance activities to restrict emerging trend of antimicrobial resistance.
This study concludes that in vitro testing of antibiotics prior to its use may help to prevent multidrug resistant organisms in burn infection which will help in reducing morbidity and mortality of burn patients.

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