ORIGINAL ARTICLE

BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF ISOLATES FROM BURN WOUNDS AT A TERTIARY CARE HOSPITAL IN PATNA

Syamal Modi[1], Amit Kumar Anand[2], Smriti Chachan[3], Shankar Prakash[4]

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ABSTRACT: CONTEXT: Infection in burn patients is the leading cause of morbidity and mortality, and remains one of the most challenging concerns for the burn team. The bacteriology of burn wounds is often polymicrobial in nature, and the presence of multi-drug resistant organisms is associated with poor response to antimicrobial therapy, high risk of bacterial sepsis, multi-organ failure and death following burn injury. **AIM:** This study analyzes the bacterial isolates from burn wounds and their antimicrobial sensitivity patterns. **MATERIALS AND METHODS:** Three hundred randomly selected patients with varying degrees of burn injuries, admitted to the burn unit of a tertiary care hospital in Patna, were included in this study. Wound swab/pus/debrided tissue cultures were assessed at weekly intervals. Seven hundred and thirty six samples were eventually collected and analyzed in this study. The samples were cultured on 5% sheep blood agar and Mac Conkey agar for isolation of organisms. Antimicrobial sensitivity test was performed on Mueller Hinton agar by the disk diffusion method. **RESULTS:** Patients between 30-40 years of age were more prone to burn injury. Females outnumbered males as regards prevalence of burn cases. Positive wound cultures were obtained in 631 (85.7%) cases. Staphylococcus aureus (40.4%) was the most common isolate in the first week, but was replaced by Pseudomonas spp. in the second (26.0%) and third (28.8%) post-burn weeks. High level resistance to oxacillin was observed in Staphylococcus aureus and Coagulase negative Staphylococci. Vancomycin was the most effective drug for the gram positive isolates. Pseudomonas and Acinetobacter isolates were resistant to most of the drugs tested. Imipenem was effective against all the gram negative isolates. **CONCLUSIONS:** It is crucial for every burn unit to determine the specific pattern of burn wound microbial colonization, time-related changes in the dominant flora and their antimicrobial sensitivity profiles. This would enable early treatment of imminent septic episodes with proper empirical antibiotics, without waiting for culture reports, thus improving the overall infection- related morbidity and mortality.

KEY WORDS: Antimicrobial sensitivity, Bacterial colonization, Burn wound infection, Sepsis, Severity of burn injury,

INTRODUCTION: Burn wound infections are serious, often life-threatening complications of thermal injury. Burn patients are at the risk for acquiring infection because of their destroyed skin barrier and suppressed immune system, compounded by prolonged hospitalization and invasive diagnostic and therapeutic procedures. [1,2] Despite advances in the use of topical and parenteral antimicrobial therapy, and the practice of early tangential excision, bacterial infection remains a major problem in the management of burn victims today. [3] It is estimated that about 75% of mortality following burn injury is related to infections, rather than osmotic shock and hypovolemia. [3] Therefore, knowledge of the responsible bacterial flora of burn wounds, its prevalence and bacterial resistance, is of crucial importance for fast and reliable therapeutic decisions.

Microorganisms are transmitted to the burn wound surfaces by the hands of personnel, by fomites and possibly by hydrotherapy. [4] 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. [5]

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. [6, 7]

It is known that the spectrum of infective agents varies from time to time and place to place. Hence, it is desirable to carry out periodic reviews of the bacterial flora of burn wounds so that preventive strategies could be modified as necessary.

The aim of this prospective study is to analyze burn patients with respect to the bacterial colonization of burn wounds and the antimicrobial susceptibility patterns of the isolates.

MATERIALS AND METHODS: Three hundred randomly selected patients with varying degrees of burns, admitted to the burn unit of our hospital, during the period November 2011 to December 2012, were included in this study. Patients were assessed as per age, sex, aetiology of burn injury, severity of burn calculated as per percentage of total burnt surface area (%TBSA) [8], duration of hospital stay and the clinical outcome.

The study was approved by our Institutional Ethics Committee. Informed consent was obtained from the patients or their relatives for inclusion in the study.

Clinical samples comprised of wound/pus/debrided necrotic tissue for culture. Sterile swabs were used for surface sampling, which were sent to our bacteriology laboratory in Stuart's transport media. Pus was collected directly into a sterile container or by using sterile swabs. Debrided tissue was collected using forceps into a sterile container containing normal saline.

Seven hundred and thirty six samples were collected from the patients on weekly basis over a period of three weeks or eventual outcome (death/discharge) of the patients, whichever earlier. The samples were cultured on 5% sheep blood agar and Mac Conkey agar. All isolates were identified by standard bacteriologic, biochemical and serologic methods. [9] Antimicrobial sensitivity test was performed using commercially available disks (Hi Media) on Mueller Hinton agar, by the Kirby Bauer disk diffusion method. [10]

RESULTS: Majority of patients (n=86, 28.6%) belonged to the age group 30-40 years indicating that this group was maximally prone to burn injuries. Females (n=186, 62.0%) were found to sustain burn injuries more frequently than males (Table 1).

Analysis of aetiology showed that the most common cause (67.0%) of burn injury was the flame of kerosene stove. Other causes of burn injuries were LPG, hot water, chemicals, cooking oil and electrical (Figure 1).

The patients were grouped on the severity of burn injury. Majority of patients (n=171, 57.0%) had ≥ 60 % TBSA (Table 2).

First week samples were obtained from all 300 (100.0%) patients, the second week samples could be obtained from 286 (95.3%) patients, while in the third week, samples could be collected in only 150 (50.0%) patients. Hence, a total of 736 samples were analyzed for bacterial growth and antibiotic sensitivity.

The positive and sterile results of culture are depicted in Table 3. Of the 631 (85.7%) sample positive for culture, single isolates were observed in 563 (89.3%) cases while 65 (10.3%) and 3 (0.5%) cases showed two and three isolates respectively. Altogether, 732 isolates were identified and subjected to antimicrobial sensitivity test.

Results showed that during the first week post-burn, Staphylococcus aureus (40.4%) was the predominant isolate, followed by Pseudomonas spp. (18.6%), Coagulase negative Staphylococci(CONS) (11.1%), Escherichia coli (10.0%), Klebsiella spp. (6.7%), Streptococcus pyogenes and Enterococcus faecalis (4.4% each) while Proteus spp. and Acinetobacter spp. were isolated in 2.2% cases each. However, Pseudomonas spp. was the dominant isolate during the second (26.0%) and third (28.8%) weeks post-burn (Table 4).

Table 5 shows the antimicrobial sensitivity patterns of the gram positive isolates from burn wounds. 45.5% strains of Staphylococcus aureus were resistant to oxacillin while the resistance in Coagulase negative Staphylococci was found to be 40.0%. Staphylococcus aureus showed sensitivity to a wide range of antibiotics whereas in Coagulase negative Staphylococci, the susceptibility to the antibiotics tested was much lower. However, all Staphylococci were susceptible to Vancomycin.

Of the gram negative isolates from wound culture, Pseudomonas spp. invariably showed high level resistance to most of the antibiotics tested. High level resistance was also observed in

Acinetobacter spp. Other isolates such as Escherichia coli, Klebsiella spp., and Proteus spp. were relatively more sensitive to the drugs used in the tests. All gram negative isolates were found to be sensitive to Imipenem (Table 6).

The mean duration of hospital stay was much lower (14.1 ± 2.7 days) amongst the non-survivors who developed infection, as compared to those who had infection but survived (21.9 ± 2.2 days) (Figure 2).

DISCUSSION: 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.[4,6] Contamination of burn wounds is almost the rule rather than an exception.

Our study revealed that females sustain burn injuries 1.63 times more frequently than males. Proportion of female burn cases were also found higher in the studies of Narlawar et al and Ahuja et al. [11, 12]

Kerosene stove flames were singled out as the most common cause of burn injury, as shown in other studies in India. [13, 14]

We found 87.0% patients to develop burn wound infections in the first week following injury, similar to the findings of de Macedo et al and Agnihotri et al. [3, 15]

Our finding that Staphylococcus aureus was the most predominant isolate (40.4%) in the first post-burn week is in correlation with the results of other workers. [3,16,17] but is in contrast to the findings of Agnihotri et al and Singh et al who have reported Pseudomonas spp. as the predominant organism in the first week following burn.[15,18] However, similar to our observations, Pseudomonas spp. was reported as the predominant isolate in the second and third post-burn weeks by other workers as well. [19,20] Other isolates found less commonly were Coagulase negative Staphylococci, Escherichia coli, Klebsiella spp., Streptococcus pyogenes, Enterococcus faecalis, Proteus spp. and Acinetobacter spp. The isolation profile is in accordance with the results of de Macedo et al, Taylor et al and Vindenes et al. [3, 16, 17]

The antimicrobial susceptibility pattern as observed by us was in contrast to other similar studies. We found 45.5% strains of Staphylococcus aureus to be oxacillin resistant, the figures being dissimilar to that found by others. [3,6] However, Staphylococci showed moderate to high sensitivity to amikacin, gentamycin, co-trimoxazole, amoxicillin-cloxacillin, azithromycin, cefaclor, levofloxacin, vancomycin and clindamycin similar to that found by other workers.[3,6,21]

The gram negative sensitivity pattern, particularly the high level resistance of Pseudomonas and Acinetobacter as observed by us was in accordance with the results of other workers. [15, 22] The high prevalence of multi-drug resistant isolates is probably due to empirical use of broad-spectrum antibiotics. Other gram negative isolates were relatively more sensitive to amikacin, gentamycin, co-trimoxazole, cefotaxime, netilmicin, cefixime, levofloxacin, imipenem, piperacillin-tazobactam and aztreonam, as also reported in other studies. [3, 23]

CONCLUSIONS: 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.

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Table 1: Age and Sex distribution of patients (n=300)

Age (years)	Number of patients		Total
	Male	Female	
< 10	0 (0)	0 (0)	0 (0)
10-20	14 (12.2)	43 (23.1)	57 (19.0)
20-30	14 (7.5)	15 (8.1)	29 (9.6)
30-40	29 (25.4)	57 (30.6)	86 (28.6)
40-50	18 (15.8)	25 (13.4)	43 (14.3)
50-60	13 (11.4)	15 (8.1)	28 (9.5)
60-70	26 (23.8)	31 (16.7)	57 (19.0)
> 70	0 (0)	0 (0)	0 (0)
Total	**114 (38.0)**	**186 (62.0)**	**300 (100.0)**

Figures in parentheses indicate percentage

Table 2: Distribution of patients based on the severity of burn injury (%Total Burn Surface Area, TBSA) (n=300)

% TBSA	Number of patients		Total
	Male	Female	
< 20	7 (6.1)	7 (3.8)	14 (4.6)
20-40	14 (12.3)	14 (7.5)	28 (9.3)
40-60	19 (16.7)	68 (36.6)	87 (29.0)
60-80	46 (40.4)	68 (36.6)	114 (38.0)
≥ 80	28 (24.6)	29 (15.6)	57 (19.0)
Total	**45 (71.4)**	**18 (28.6)**	**63 (100.0)**

Figures in parentheses indicate percentage

Table 3: Percentage of bacteriological culture results of burn patients (n=736)

Result	Time of sampling(weeks)		
	1st(n=300)	2nd(n=286)	3rd(n=150)
Sterile	13.0	18.0	9.9
Positive	87.0	82.0	90.1

Table 4: Percentage of isolates from burn wounds (n=702)

Isolate	Time of sampling(weeks)		
	1st(n=261)	2nd(n=235)	3rd(n=135)
Staphylococcus aureus	40.4	20.3	18.2
Pseudomonas spp.	18.6	26.0	28.8
CONS	11.1	15.1	14.9
Escherichia coli	10.0	10.6	8.6
Klebsiella spp.	6.7	7.0	6.7

Streptococcus pyogenes	4.4	2.2	2.0
Enterococcus faecalis	4.4	2.2	2.0
Proteus spp.	2.2	6.7	6.2
Acinetobacter spp.	2.2	9.9	12.6

Total number of patients	300
Total samples analyzed	736
Total positive samples	631

Table 5: Percentage sensitivity of Gram positive isolates from burn wounds (n=300)

Antibiotic	S. aureus (n=178)	CONS (n=84)	S. pyogenes (n=19)	E. faecalis (n=19)
Oxacillin	55.5	60.0	66.7	80.0
Amikacin	66.7	70.2	66.7	86.3
Gentamycin	83.3	86.3	76.7	76.7
Co-trimoxazole	77.8	52.3	66.7	66.7
Amoxyclav	70.0	76.7	80.0	56.1
Cefaclor	50.0	56.6	83.3	50.0
Azithromycin	66.7	75.0	76.0	79.3
Levofloxacin	83.3	80.0	86.0	82.6
Vancomycin	100.0	100.0	100.0	100.0
Clindamycin	86.7	82.3	88.9	88.0

Table 6: Percentage sensitivity of Gram negative isolates from burn wounds (n=331)

Antibiotic	Pseudomonas spp. (n=149)	Acinetobacter spp. (n=47)	E.coli (n=63)	Klebsiella spp. (n=42)	Proteus spp. (n=30)
Amikacin	52.2	33.3	62.5	76.7	70.0
Gentamycin	48.2	43.6	71.4	26.3	56.0
Netilmycin	51.4	46.7	71.4	71.4	48.0
Co-trimoxazole	18.9	20.0	54.4	46.8	23.2
Cefotaxime	39.1	30.0	42.8	52.6	52.1
Cefixime	45.7	13.3	57.1	60.0	60.0
Levofloxacin	80.4	40.0	71.4	80.0	72.6
Piper-Tazobact	53.3	66.7	63.4	70.7	66.5
Imipenem	100.0	100.0	100.0	100.0	100.0
Aztreonam	25.6	20.3	75.9	73.2	61.2

Figure 1: Percentage distribution of burn cases according to the cause of burn (n=300)

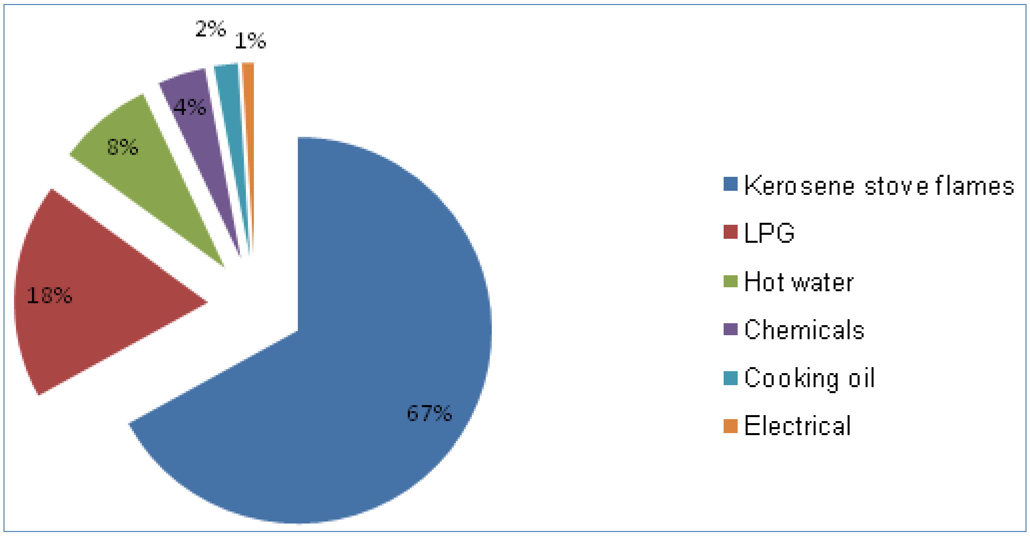
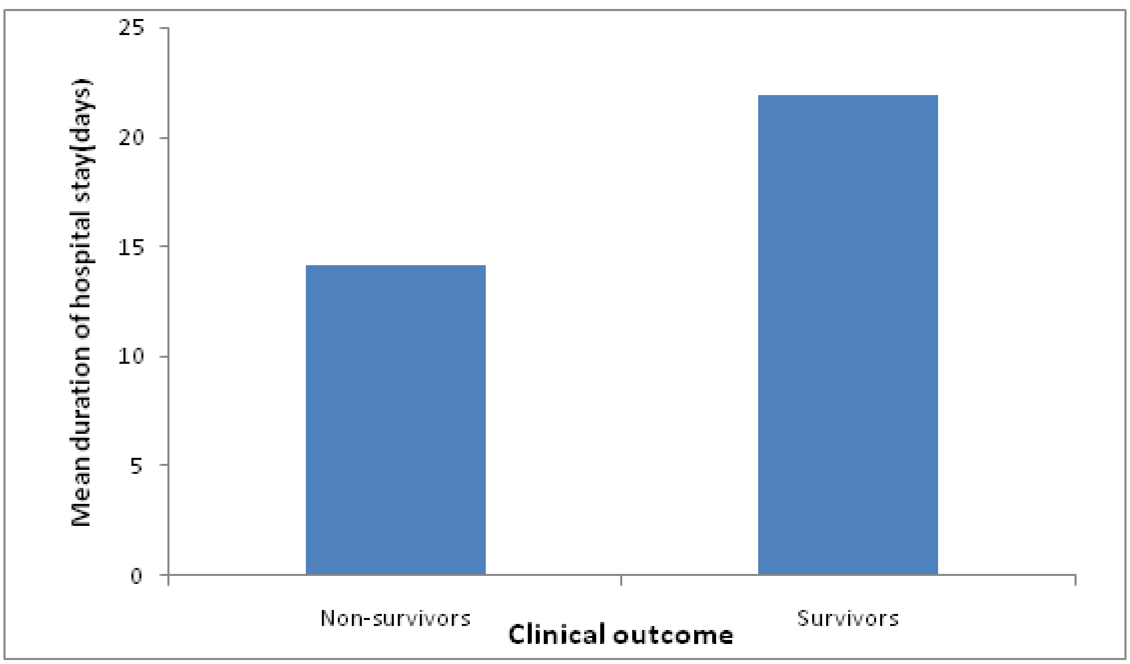


Figure 2: Mean duration of hospital stay in relation to clinical outcome of burn patients



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AUTHORS:

1. Syamal Modi
2. Amit Kumar Anand
3. Smriti Chachan
4. Shankar Prakash

PARTICULARS OF CONTRIBUTORS:

1. Tutor, Department of Microbiology, Patna Medical College & Hospital, Patna & Director and Consultant Microbiologist, Modi Diagnostics, Patna.
2. Tutor, Department of Microbiology, Patna Medical College & Hospital, Patna.
3. DNB Trainee (Family Medicine), Apollo Gleneagles Hospital, Kolkata & Visiting Consultant, Modi Diagnostics, Patna.
4. Professor and Head, Department of Microbiology, Patna Medical College & Hospital, Patna.

NAME ADRRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Syamal Modi,
Road Number 11, Rajendra Nagar,
Patna – 800016, Bihar.
Email – syamalmodi@gmail.com

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