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Bacteriological and Antimicrobial Sensitivity Profile of Burn Wound Infections in a Tertiary Care Hospital of Uttarakhand

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ABSTRACT

Introduction: Burn wound injuries are one of the most common, invasive and devastating forms of trauma. Despite the recent advances in burn wound management, bacterial infections persist as an important complication and leading cause of morbidity and mortality among burnt patients. Identification and antimicrobial susceptibility pattern of bacterial pathogens associated with burn wounds can help clinicians to plan patient management effectively and efficiently.

Materials & Methods: This prospective study was conducted for a period of one year (July 2016- June 2017). A total of 160 specimens (wound swabs and pus exudates) from burn wound patients received at microbiology department for culture and sensitivity were included in the study. Once received the samples were processed immediately as per the standard operating procedures of our laboratory. Identification and antimicrobial sensitivity testing of the bacterial isolates was performed on VITEK®2 COMPACT automated identification and antimicrobial susceptibility testing (ID/AST) system (bioMérieux, France) and interpreted as per Clinical Laboratory Standards Institute guidelines. The use of automated VITEK®2 COMPACT system for ID/AST ensures accurate results for most of the clinical isolates and eliminates the requirement of human analysis and error of results.

Results: A total of 160 samples were received from burn wound patients, out of which 113 (70.6%) were culture positive. *P.aeruginosa*, *A.baumannii* and *Proteus mirabilis* were the most predominant gram-negative isolates whereas *S.aureus*, Coagulase-negative staphylococcus and *Str.pyogenes* were the most commonly isolated gram-positive organisms.

Antimicrobial sensitivity profile of bacterial isolates revealed Piperacillin-tazobactam, imipenem, cefoperazone-sulbactam and colistin to be the most effective antimicrobials against gram-negative isolates, whereas linezolid, teicoplanin, vancomycin and amikacin were the most effective drugs against gram-positive isolates.

Conclusion: Due to the increased morbidity and mortality associated with the burn wound infections, early detection of the causative agents and the intervention are a prerequisite for better clinical outcomes of burnt patients. Data extrapolated from our study can be helpful for primary care physicians to optimize the treatment modalities, articulating policies for empiric antimicrobial therapy and to minimize the rate of infection among burn wound patients.

Key Words: Multi drug resistant, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Total body surface area, VITEK

INTRODUCTION

Burns injury, one of the most common, invasive and devastating forms of trauma is a global public health concern. It

causes damage to the largest organ in the human body, the skin, which functions to provide homeostasis, thermoregulation, sensation, immunological defense and acts as a formidable barrier against various infections.¹ World Health

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Organization (WHO) has estimated that burn injury results in 265,000 deaths annually, with nearly half of these occurring in the WHO Southeast Asia Region, ²more so in low and middle-income countries, which are least equipped to provide timely and comprehensive care. ³ Millions of those who survive are left with lifelong disabilities and disfigurements, often with resulting social stigma and rejection. In India, over 1,00,000 people are affected by burns annually and over 20,000 of them die. ⁴

Different cultural and social factors and the availability of healthcare facilities result in diverse epidemiology of the burn wound injury. ⁵ The most common cause of the burns are due to chemicals, hot liquids followed by electricity and molten or hot metals. ⁶ The severity of a burn depends on the degree of heat, duration of exposure, and thickness of the involved skin. The treatment of burns requires a multidisciplinary approach and it is often cost-intensive. Of note, majority of burn injuries in India occur in rural areas where the unavailability of quality healthcare facilities and financial constraints can further complicate the effective and efficient management of such cases.

Despite the recent advances in burn wound management, microbial infections persist as an important complication and leading cause of morbidity and mortality among burn patients. ^{1,7} Most of the times such infections are the reason for a prolonged hospital stay which eventually account for overwhelming pharmacotherapeutic and pharmacoeconomic implications not only on patients but on healthcare facility as well. Predominant risk factors for burn wound infection are the size of burn wound, i.e., the percentage of total body surface area (TBSA) burnt and the duration of hospitalization. ⁸ Burn wound itself provides a conducive environment for the microorganisms to colonize, which eventually leads to infection. The primary insult from a burn is the wound itself with three characteristic areas of involvement, a) Zone of coagulation: first associated area of the wound, nearest to the heat source and includes dead tissue forming the burn eschar; b) Zone of stasis: is the area adjacent to the zone of coagulation and is viable but at risk of ischemia due to perfusion defects; c) Zone of hyperemia: it is the third area, which consists of relatively normal skin, with increased blood flow and vasodilatation and minimal cellular injury. ⁸ Primarily the burn wound injuries have the moist, protein-rich eschar, which is an ideal environment for microbial colonization and infection. The avascular zone of coagulation diminishes the immunological defenses, particularly neutrophils, from attacking pathogens via their respiratory burst mechanisms, which requires oxygen. ⁹ The inflammation also impairs wound healing due to the release of proteases from macrophages.

Typically the burn surface is sterile immediately after the thermal injury and after a period of about 48-72 hrs (relative sterility), the wound is initially colonized by the surrounding

microbes from the skin, hair follicles and sebaceous glands (endogenous flora), ¹⁰ which are usually gram-positive bacteria. After around fifth to sixth day the gram-positive organisms are often replaced by gram-negative organisms ¹¹ which are either present in hospital environment or are transmitted from the hands of healthcare workers and fomites (exogenous flora). Moreover, the gastrointestinal tract is also one of the predominant potential reservoirs for endogenous gram-negative organisms that colonize the burn wound surface. ⁸ Among the gram-positive organisms, *Staphylococcus aureus* and *Streptococcus pyogenes*, and among the gram negative organisms *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus spp.* and *Citrobacter spp.* are the most predominant organisms involved in burn wound infection. These pathogens, particularly the gram-negative organisms are known for their increasing resistance to a variety of broad-spectrum antibiotics and can further complicate the clinical outcome of the patients. Moreover, infection with multi drug-resistant organisms or polymicrobial infections are associated with worse clinical outcomes. Nearly 73% of post-burn deaths occurring within five days have been reported to be sepsis-related. ¹²

The use of prophylactic antibiotics is common practice with burnt patients. ¹³ Drug resistant bacteria with intrinsic resistance towards antibiotics, the ability to survive longer in the hospital environment and hand-to-hand transmission of bacteria reflect their easy spread and the possible causes of outbreak. ^{14,15} The bacterial infections in burnt patients vary both with time and place. ^{16,17} Thus, continuous surveillance and update of antibiotic resistance pattern of microorganisms is imperative for infection control programs and accurate antibiotic treatment in the burnt patients. With the above background, the present study was undertaken to identify the bacteriological profile of infected burn wounds and their antimicrobial sensitivity pattern among the patients admitted in a tertiary care hospital of Uttarakhand. This study will be helpful to determine the predominant bacterial agents causing burn wound infection in our healthcare setup and their antimicrobial profile will be helpful to tailor the existing guidelines for better patient management and care.

MATERIALS AND METHODS

This prospective study was conducted in Dept. of Microbiology of Shri Mahant Indiresch Hospital Dehradun for a period of one year (July 2016- June 2017). A total of 160 specimens (wound swabs and pus exudates) from burn wound patients received at microbiology department for culture and sensitivity were included in the study.

Following aseptic conditions, all the samples were collected and transported to the microbiology laboratory without delay. Once received the samples were processed immediately

as per the standard operating procedures of our laboratory. Samples were plated on 5% sheep blood agar (BA) and MacConkey's agar (MA), and were incubated at 37°C for 48 hours before being reported as sterile. Plates were examined for the growth and the preliminary identification was made based on colony morphology, gram staining, and the battery of biochemical tests (catalase, coagulase and oxidase tests). Based on the aforesaid tests the organisms were broadly classified as gram-positive cocci (in clusters or chains) and gram-negative bacilli (oxidase positive or oxidase negative). Further identification (ID) and antimicrobial sensitivity testing (AST) was done using VITEK®2 COMPACT automated ID/AST system (bioMérieux, France). The use of automated VITEK®2 COMPACT system for ID/AST ensures accurate results for most of the clinical isolates and eliminates the requirement of human analysis and error of results.

For gram-negative bacteria, ID-GNB, AST-N280 and AST-N281 cards (bioMérieux, France) and for gram-positive bacteria ID-GPB, AST-P628 (bioMérieux, France) were used following the manufacturer's instructions. Briefly, a single bacterial colony (pure growth) was taken and the suspension was made in normal saline. The optical density of the bacterial suspensions was adjusted to 0.5 McFarland. Finally, the Vitek Tubes were shaken well before putting into the Vitek machine to maintain homogenous suspension. The AST card contained following antibiotics: ampicillin, amoxicillin/clavulanic acid, amikacin, ceftriaxone, ciprofloxacin, clindamycin, co-trimoxazole, cefoperazone/sulbactam, colistin, cefuroxime, erythromycin, gentamicin, imipenem, linezolid, piperacillin/tazobactam, teicoplanin, tigecycline, and vancomycin. *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 were used as controls. Results were interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines (M100-S24 and M100S, 26th Ed.). The MIC breakpoint used to identify bacteria susceptible for colistin was 2 mg/l and tigecycline was 1 or 2 mg/l.¹⁸

RESULTS

Out of total 160 patients, the majority of the cases were seen in age group between 31 years to 40 years (27.5%). Males (60.7%) were more commonly affected than females (39.3%) and the male: female ratio was 1.5:1. Table 1 depicts the gender and age-wise distribution of burn wound patients. Among the causes of burn, thermal burns (36.2%) were found to be the most predominant followed by electric burns (26.9%) and scald burns (16.2%). Table 2 shows the distribution of the type of burns.

Out of total 160 samples, processed bacterial growth was seen in 113 (70.6%) samples while 47 (29.4%) samples showed no growth. Among the 113 samples with bacterial

growth, gram-negative organisms (68; 60.2%) outnumbered the gram-positive organisms (45; 39.8%). *P. aeruginosa*, *A. baumannii* and *Proteus mirabilis* were the most predominant gram-negative isolates whereas *S. aureus*, Coagulase-negative staphylococcus (CONS) and *Str. pyogenes* were the most commonly isolated gram-positive organisms. Table 3 shows the distribution of various bacterial isolates grown from burn wounds of the patients.

Antimicrobial sensitivity testing was carried out for all 113 bacterial isolates. Piperacillin-tazobactam, imipenem, cefoperazone-sulbactam and colistin showed maximum activity for gram-negative isolates, whereas linezolid, teicoplanin, vancomycin and amikacin were the most effective drugs against gram-positive isolates. Table 4 depicts the antimicrobial sensitivity profile of all the bacterial isolates.

DISCUSSION

In the present study a total of 160 samples were collected, out of which, 113 samples showed growth with an isolation rate of 70.6%, a finding which was in tandem with the previous studies by Srinivasan *et al.*, Dutta *et al.*, and Richcane *et al.* who reported the isolation rate to be as high as 86.28%, 88.23% and 90.7% respectively.^{8, 19, 20}

Regarding the sex distribution of the patients in the present study, males (60.7%) outnumbered the females (39.3%) with male: female ratio of 1.5: 1. The possible reasons for this male preponderance can be related to socio-economic and cultural habits of earning the livelihood primarily by males and also to their adventurous nature and the greater desire to be active in comparison to their female counterparts.^{21,22} Our findings were in concordance with previous studies by Aali *et al.*, Ghaffaret *et al.* and Richcane *et al.*^{20,23,24} but were in contrast to the studies by Khurram *et al.* and Latikasharma *et al.*^{25,26}

Among the causes of burn, thermal burns (36.2%) were found to be the most predominant followed by electric burns (26.9%) and scald burns (16.2%). A study by Shahzadet *et al.* also reported thermal burns to be the most common cause of burn injuries.²⁷ Various other studies by Richcane *et al.*, Agbenorku *et al.*, and Mahalakshmyet *et al.*, have reported scald burns as the most common cause of burn injuries.^{20,28,29} The most affected age group in our study was between 31 to 40 years (27.5%) of age, a finding that is in parallel to the various other studies.^{8,25,26,30,31} The probable reasons for this are, the active involvement of this age group in outdoor work and more common exposure to fire-related work (household and occupational). In contrast to our findings, various other studies reported age group of 0-5 years as the most common age group suffering from burns.^{20,28,32,33} Underdevelopment of the cognitive function, tendency of being more active during early developmental stages and to pull or push objects

containing hot liquids have been reported to be the common reasons for the same.²⁸

Nosocomial infection in burnt patients is a major challenge for clinicians. A previous study reported that 75% of all deaths among burnt patients were associated with infection.¹⁹ Our study results revealed a high isolation rate of about 70.6% with gram-negative isolates comprising the predominant bacterial etiology. The presence of gram-negative organisms in the majority of the cases suggests that most of such wounds may either have resulted due to prolonged hospital stay or due to prolonged time between the injury and the hospital admission. The predominance of gram-negative bacteria in burns has been documented in several studies where they have been shown to be an independent predictor of mortality among burnt patients.^{34,35} Studies by Bessa *et al.* and Hwee *et al.* also support our view by stating that the long hospital stay is directly proportional to high incidence of burn wound infections particularly of gram-negative etiology and is inversely proportional to the positive clinical outcome of the patients.^{36,37} One of the major factors adding to the complication of burn wound patients is a multi drug resistant (MDR) organism. Any MDR strain if sets in the hospital environment, can persist for months. Robust microbiological surveillance as well as restrictive antibiotic policy can be helpful in prevention and treatment of such MDR isolates. Moreover, overcrowding in burn ward is an important cause of cross-infection and must be avoided to prevent any nosocomial infection among patients.

In the present study, *P.aeruginosa* was the most commonly isolated gram-negative bacteria followed by *A.baumannii* and *Pr.mirabilis*. Similar findings, with *P.aeruginosa* being the predominant isolate among burn wound patients have been reported previously.^{25,38,39} High prevalence of these pathogens is associated with their ability to flourish well in a moist environment and their prolonged persistence in hospital environment,⁴⁰ which eventually can result in a high level of antibiotic resistance among such pathogens, particularly in *Pseudomonas* spp.⁴¹ Moreover, the local practices like application of cow dung, toothpaste, fountain pen ink and mud paste over the burn wound can also be the possible reason for isolation of these organisms from the burn wound patients.²⁵

Among the gram-positive organisms, *S.aureus* was the most predominant followed by CONS and *Str.pyogenes*. Although various other studies have shown *S.aureus* to be the most predominant etiological agent in burn wound patients,⁴² but in the present study it was found to be the second most common isolate after *P.aeruginosa*. Similar observations have been reported by previous studies as well.^{38,39,43} CONS accounted for 8.9% of the total organisms isolated from the burn wounds, a finding which is in tandem with previous studies by Mama *et al.* and Richcane *et al.* who reported CONS in 14.5% and 2.3% respectively from the burn wound

infections.^{20,44} CONS, although is a normal skin flora, but is a common contaminant of the burn wounds.

The antimicrobial profile of our study indicates the emergence of MDR strains in our hospital set up. The gram-negative isolates exhibited a high degree of resistance to the commonly used antibiotics like ampicillin, amoxy-clav, ceftriaxone, ciprofloxacin, cefuroxime, gentamicin and penicillin, the observation which was in contrast to the previous studies.^{15,16,41,42} Various predisposing factors documented for the acquisition of MDR strains include prolonged/previous hospitalization, invasive procedures, comatose state and advancing age. In addition to the above, indiscriminate use of broad-spectrum antibiotics and poor compliance with hospital antibiotic policy can also provide a conducive environment for MDR strains. Piperacillin-tazobactam, imipenem and cefoperazone-sulbactam showed maximum activity against gram-negative isolates. Similar findings were reported by Mundhada *et al.* and Guggenheim *et al.* who also observed imipenem and meropenem to be the most effective drugs against gram-negative isolates.^{42,45} Colistin and tigecycline are among the antibiotics used as last resort for the treatment of MDR or extensively drug resistant (XDR) isolates, and both these drugs were found to be effective against majority of the gram-negative isolates in our study. However, resorting to both these drugs can impose a considerable financial burden on the patient. In comparison to gram-negative isolates, the degree of resistance observed among gram-positive organisms was less. In addition to vancomycin, teicoplanin and linezolid, which showed 100% sensitivity to gram-positive isolates, amikacin and co-trimoxazole, were also found to be effective against gram-positive organisms.

The use of automated VITEK®2 COMPACT system for identification and antimicrobial susceptibility testing ensures accurate results for most of the clinical isolates and eliminates the requirement of human analysis and error of results.

CONCLUSION

The most common isolate from burn wound patients in our study was *P.aeruginosa*, *S.aureus* and *A.baumannii*, majority of them being resistant to commonly prescribed antimicrobials. The emerging drug-resistant strains and the scarcity of any newer antibiotic in the pipeline make active microbial surveillance in the clinical settings more imperative. Due to the increased morbidity and mortality associated with the burn wound infections, early detection of the causative agents and the intervention are a prerequisite for better clinical outcome of burnt patients. Although completely eliminating such infections seems to be difficult, but reducing the rate of burn wound infections to minimal will surely be beneficial in reducing patient morbidity and mortality, as well as in preventing the pharmacotherapeutic and pharmacoeco-

nomic losses.

A multidimensional approach in this regard ensuring close clinical liaison between the surgical team, the microbiologist and the infection control team can surely turn the tide in favor of the patients as well as the clinicians. We realize that data extrapolated from our study may not be representative of the whole Indian scenario and must be interpreted cautiously. However, the findings of our study can serve as a template to optimize hospital antimicrobial policy and antimicrobial prescribing guidelines. The relevant and regular policy and protocol changes can definitely overcome the burn wound infection rate in any healthcare facility. Given the considerable clinical and economic consequences of burn wound infections, the goal of a healthcare system should be “zero tolerance” to such infections and the associated adverse events.

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Table 1: Gender and age-wise distribution of burn wound patients. (n=160)

Age group (in years)	Males	Females	Total
0 - 10	07	13	20
11 - 20	14	11	25
21 - 30	17	07	24
31 - 40	29	15	44
41 - 50	19	10	29
51 - 60	11	07	18
Total	97	63	160

Table 2: Distribution of the type of burns observed in the study. (n=160)

Type of burns	No. of cases	Percentage
Thermal burn	58	36.2
Electric burn	43	26.9
Scald burn	26	16.2
Flame burn	22	13.8
Chemical burn	11	6.9
TOTAL	160	100%

Table 3: Distribution of the various bacterial isolates grown in clinical samples from burn wound patients. (n=113)

Organism	Number (%)
Gram-negative organisms (n=68)	
<i>Pseudomonas aeruginosa</i>	23 (20.3)
<i>Acinetobacter baumannii</i>	17 (15)
<i>Proteus mirabilis</i>	14 (12.4)
<i>Klebsiella pneumoniae</i>	07 (6.2)
<i>Escherichia coli</i>	04 (3.6)
<i>Enterobacter cloacae</i>	03 (2.7)
Gram-negative organisms (n=45)	
<i>Staphylococcus aureus</i>	19 (16.8)
CONS	10 (8.9)
<i>Streptococcus pyogenes</i>	09 (7.9)
<i>Enterococcus faecalis</i>	07 (6.2)
Total	113

CONS: Coagulase negative staphylococcus

Table 4: Antimicrobial sensitivity profile of bacterial isolates from the burn wound patients.

Antibiotics	Organisms									
	<i>P.aeruginosa</i> n=23	<i>A.baumannii</i> n=17	<i>P.mirabilis</i> n=14	<i>K.pneumoniae</i> n=07	<i>E.coli</i> n=04	<i>En.cloacae</i> n=03	<i>S.aureus</i> n=19	CONS n=10	<i>Str. Pyo- genes</i> n=09	<i>Ent. faecalis</i> n=07
AMK	8.7	17.6	21.4	71.4	75.0	66.7	89.5	90.0	NT	NT
AMP	8.7	5.9	14.3	14.3	25.0	0.0	10.5	20.0	NT	NT
AMC	4.3	5.9	14.3	28.6	50.0	66.7	63.1	80.0	NT	NT
CTX	13.0	11.8	7.1	28.6	50.0	0.0	NT	NT	NT	NT
CIP	34.8	5.9	14.3	28.6	25.0	0.0	31.6	70.0	NT	NT
CLD	NT	NT	NT	NT	NT	NT	57.9	60.0	NT	71.4
COT	8.7	11.8	35.7	28.6	50.0	33.3	57.9	80.0	33.3	42.8
CFS	86.9	64.7	64.3	57.1	50.0	33.3	NT	NT	NT	NT
COL	86.9	94.1	NT	85.7	100	100	NT	NT	NT	NT
CEF	56.5	17.6	35.3	14.3	25.0	0.0	NT	NT	NT	NT
ERT	NT	NT	NT	NT	NT	NT	68.4	70.0	77.8	71.4
GEN	8.7	17.6	21.4	57.1	50.0	33.3	78.9	70.0	NT	NT
GEN-HL	NT	NT	NT	NT	NT	NT	NT	NT	NT	42.8
IMI	91.3	82.3	78.6	85.7	100	100	NT	NT	NT	NT
LNZ	NT	NT	NT	NT	NT	NT	100	100	100	100
PIP-TZ	95.6	64.7	92.8	85.7	100	100	NT	NT	NT	NT
PN-G	8.7	5.9	0.0	0.0	0.0	0.0	10.5	20.0	100	85.7
TEC	NT	NT	NT	NT	NT	NT	100	100	100	100
TIG	86.9	94.1	92.8	85.7	100	100	63.1	90.0	44.4	57.1
VAN	NT	NT	NT	NT	NT	NT	100	100	100	100

Sensitivity pattern shown in the table is the percentage of the isolates sensitive to the antibiotic. Intermittently sensitive isolates were considered as resistant.

AMK: Amikacin; AMP: Ampicillin; AMC: Amoxycillin-clavulanate; CTX: Ceftriaxone; CIP: Ciprofloxacin; CLD: Clindamycin; COT: Co-trimoxazole; CFS: Cefoperazone-sulbactam; COL: Colistin; CONS: Coagulase negative staphylococcus; CEF: Cefuroxime; ERT: Erythromycin; GEN: Gentamicin; GEN-HL: Gentamicin-high level; IMI: Imipenem; LNZ: Linezolid; PIP-TZ: Piperacillin-tazobactam; PN-G: Penicillin; TEC: Teicoplanin; TIG: Tigecycline; VAN: Vancomycin