Research Article

Evaluation of Burn Wound Microbes and their Antibiotic Susceptibility Pattern

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ABSTRACT

Introduction and Aim: Nosocomial bacterial infections have reached an alarming state in the healthcare system. Majority of the morbidity and mortality in burn patients are caused by microbial infections, particularly those brought on by multidrug-resistant (MDR) organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA). This study aimed to investigate microbiological diversity among burn patients and their antibiotic susceptibility profiles at SUM hospital, Odisha, India.

Methodology: This prospective analysis was carried out from 2023 to 2024 on burn patients who were admitted to intensive care units/burn wards owing to either industrial or domestic accidents. Numerous variables were included for data collection such as the extent of burns, clinical and demographic data, laboratory results and treatment details. The data was analysed using various statistical tools.

Results: It was determined that a total of 741 isolates were collected from 181 hospitalized burn patients with total body burnt surface area of 29.6%. The predominant microbes found were *Pseudomonas aeruginosa* (88.63% of 741) followed by *Klebsiella pneumoniae* (67.04%; of 741) and *Acinetobacter baumannii* with 62.50% in male patients, while in female patients the *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were found as 84.94% followed by *Acinetobacter baumannii* with 58.06%. There were 24 different type of bacterial isolates and 5 fungal isolates found in both the genders across various age groups.

Conclusions: The occurrence of microbial infection was more often in male patients than females, while multidrug resistance microbial infection was more common among female patients than males. The pattern of infection acceleration was observed in positive correlation with patients total body surface area (TBSA) and prolonged hospital stay.

Keywords: *P. aeruginosa, K. pneumoniae, A. baumannii*, Burns, MDR, MRSA.

1. INTRODUCTION

Microbial infection is the primary cause of morbidity and mortality in burn injuries of all kinds, accounting for 51% of burn patient fatalities. Infections due to burn injuries are defined as the accumulation of high bacterial concentrations (>105 CFU/g of tissue) on the surface of the burn wound [1]. Burn injuries represent a significant healthcare challenge worldwide, often resulting in complex medical conditions that require comprehensive management. While initial treatment focuses on stabilizing the patient and wound care, one of the most pressing concerns in burn injury care is the risk of infection. Burn wounds provide an ideal environment for microbial proliferation due to compromised skin barriers and tissue necrosis. Due to the breakdown of the natural skin barrier and subsequent suppression of immunological responses, patients with burn injuries are more vulnerable to infection. Moreover, the burn surface area offers a rich growth substrate due to its abundance of necrotic tissue and protein-rich wound exudate. In such cases, infection continues to be the leading cause of death [2] [3]. Microorganisms typically colonize the surface of a burn wound before they invade viable subcutaneous tissue. Within the first 48 hrs., colonization takes place. The primary sources of colonizing microorganisms are the hospital environment and the flora on the patient's body. The range of agents that colonize or infect may vary throughout different areas and communities, within the same area and community at different times, and in relation to the length of hospital stay [2] [4]. Burn area, burn depth, inhalation injury, and burn severity scores are all strongly correlated with burn infection, according to a number of studies. As a result, individuals with severe and non-severe burns should receive alternative infection management and treatment approaches [5]. Before the findings of cultures are available, early treatment of suitable empirical antibiotic medication can be implemented in a burn unit through surveillance of wound colonization. Nevertheless, most of the earlier research had solely examined colonization in relation to burn injuries [6] [7]. Infection not only delays wound healing but also increases morbidity and mortality rates among burn patients. Understanding the dynamics of burn wound infections is crucial for healthcare providers to implement effective preventive strategies and prompt treatment interventions.

This study provides an overview of the microbial pathogens involved, the factors affecting infection, and the implications of burn wound infection on patient's health. By addressing these aspects, healthcare professionals can better navigate the burn-related morbidities and improve patient's health. We think that the development of infections in burned individuals may involve some microorganisms colonizing additional body locations in addition to the burn wound. Finding bacterial infections and how they relate to the type, location, and severity of burns is the goal of this study. Furthermore, the purpose of this research is to assess how long it takes for bacterial infections to heal in response to various medications and to ascertain whether antibiotic resistance and hospital stays are related. By concentrating on common bacterial flora that infect burn patients, this study could aid in the development of a uniform protocol for the administration of antibiotics in burn units at SUM Hospital Bhubaneswar, Odisha, India..

2. MATERIALS & METHODS

A prospective study was conducted in the burn ward of SUM Hospital Bhubaneswar, Odisha, India. During the six-month study period, patients who spent at least fifteen days in the hospital in 2023 were selected for this investigation. Patients who were transferred from another medical facility, admitted more than 12 hrs. after a burn injury or spent fewer than two weeks in the burn unit were excluded from the study. At the time of admission and every seven days after that, patients' burn wounds, nasal, axillary, inguinal, and umbilical regions were routinely monitored and sampled. A total of 181 patients were admitted over the course of the research period.

The process of collecting samples for burn wounds involved taking swabs from different parts of the wound, cleaning it, and then using a topical antimicrobial treatment right away. Subsequent samples were taken both after the topical agent residue was scraped away and before daily wound washing or debridement. Sampling techniques for various body parts: if the patient's skin was still intact, swabs were obtained from the anterior nares, axilla, inguinal area, and umbilicus. Samples were regarded as burn wounds if the skin was burnt. Single swabs were utilized to sample two separate sites (the nares, axilla, and groin on both sides), excluding the umbilicus. Nasal and skin samples were taken using swabs that had been soaked in sterile saline right before the sample was taken. The isolations were identified either with commercial identification kits or by following standard bacteriologic procedures. The disk diffusion method was used to conduct the methicillin resistance test in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI Document M100-S20, Wayne, PA).

3. STATISTICAL ANALYSIS

With the aid of Microsoft Excel expressive statistics was used to analyse the data collected for the current study. The quantitative data that the study produced was analysed using various statistical tools to make a qualitative inference. P-values less than 0.05 were regarded in every instance as statistically significant. The incidence of burns among both the genders and various age groups were compared using regression analysis to determine whether there was any significance.

4. RESULTS

There was a total of 181 patients who participated in this study. The average age was 39 years (range: 11 months-82 years) and the average surface area burnt among patients in the study group was 29.6% (range: 5-75%). The average age of burn patients was observed 36 years in females while 42 years in males, respectively. The duration of hospital stay was 42 days on an average, with a range of 15 to 133 days. During the study period, a total of 24 types of bacterial strains and 5 fungal species were identified in patients of different ages, both male and female.

Meropenem/imipenem, amikacin, netilmicin, vancomycin, and cefaperazone-sulbactam were the most commonly used antibiotics tested by Vitek 2 to treat the patients during the study period. To guide antibiotic therapy for methicillin-resistant staphylococcal infections, vancomycin was employed; while for *Pseudomonas aeruginosa* infections, meropenem/imipenem or cefaperazone-sulbactam was used in combination with either amikacin or netilmicin. Treatment decisions were made for other microbes based on their susceptibility to antibiotics. Initially, aminoglycosides and meropenem/imipenem were employed as an empirical combination therapy for severe infections.

The most common causal organisms in infections with full-body, lower-body and upper-body burns were gram-negative bacteria. When it came to upper body burns, gram-positive bacterial infections were the least common. Only one lower body burn case involving gram-negative bacteria and fungi was documented in the present investigation.

Table 1: The bacteria isolated from burn wounds of various patients of burn ward SUM Hospital, Odisha India (Total no. of patients = 181, No. of male patients = 88 and No. of female patients = 93).

Sl. No.	Name of bacteria	Occurrence in Males		Occurrence in Females	
		Number	%	Number	%
1	*Acinetobacter baumannii*	55	62.50	54	58.06
2	*Burkholderia cepacia*	08	09.09	39	41.93
3	*Citrobacter freundii*	00	00.00	08	08.60
4	*Delftia acidovorans*	00	00.00	08	08.60
5	*Enterobacter cloacae complex*	17	19.31	20	21.50
6	*Enterococcus faecalis*	42	47.72	19	20.43
7	*Enterococcus faecium*	17	19.31	02	02.15
8	*Escherichia coli*	37	42.04	22	23.65
9	*Enterococcus gallinarum*	12	13.63	00	00.00
10	*Klebsiella pneumoniae*	59	67.04	79	84.94
11	*Proteus mirabilis*	00	00.00	20	21.50
12	*Pseudomonas aeruginosa*	78	88.63	79	84.94
13	*Serratia liquefaciens*	00	00.00	19	20.43
14	*Staphylococcus aureus (MRSA)*	22	25.00	37	39.78
15	*Staphylococcus epidermidis*	00	00.00	57	61.29
16	*Staphylococcus haemolyticus*	13	14.77	42	45.16
17	*Staphylococcus hominis*	00	00.00	42	45.16
18	*Sphingomonas paucimobilis*	17	19.31	00	00.00
19	*Streptococcus sanguinis*	05	05.68	00	00.00
20	*Sphingobacterium spiritivorum*	17	19.31	00	00.00

Table 2: The fungi isolated from burn wounds of various patients of burn ward SUM Hospital, Odisha India (Total no. of patients = 181, No. of male patients = 88 and No. of female patients = 93).

Sl. No.	Name of fungi	Occurrence in Males		Occurrence in Females	
		Number	%	Number	%
1	*Candida albicans*	13	14.72	21	22.58
2	*Candida auris*	17	19.31	**57**	**61.29**
3	*Candida duobushaemulonii*	00	00.00	02	02.15
4	*Candida krusei*	00	00.00	20	21.50
5	*Candida tropicalis*	**30**	**34.09**	**46**	**49.46**

The widespread bacterial strain *Pseudomonas aeruginosa* was predominant in male patients with (88.63%), followed by *Klebsiella pneumoniae* with 67.04%, *Acinetobacter baumannii* with (62.50%), while in females, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were found equally predominant (84.94%) followed by *Acinetobacter baumannii* with (58.06%), and *Escherichia coli* with (23.65%) On the other hand, the fungus *Candida auris* was found (61.29%) to be a major isolate followed by *Candida tropicalis* (49.46%) in female patients. The presence of fungus in male patients was relatively less as the *Candida tropicalis* was only 34.09% (Table 1 & 2).

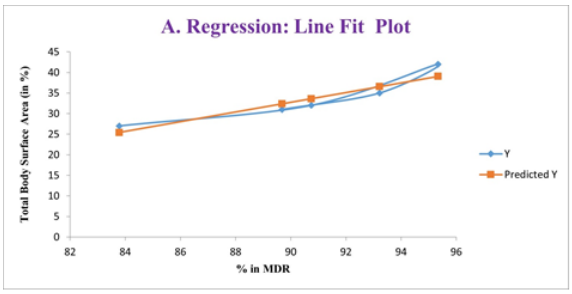
The widespread bacterial strain *Acinetobacter baumannii* (MDR) was found in male patients with 44.31% followed by *Klebsiella pneumoniae* (MDR) with 42.04%, both *Escherichia coli* and *Pseudomonas aeruginosa* found equally with 34.09% each; while in females, *Acinetobacter baumannii* (MDR) was predominant with 47.31% followed by *Pseudomonas aeruginosa* (MDR) with 45.16%, *Klebsiella pneumoniae* with 40.86% and least observed was Escherichia coli (MDR) with 19.35%. On the other hand, the fungus *Candida auris* was found 61.29% followed by *Candida tropicalis* 49.46% in female patients (analysed by Vitek 2).

Out of the total number of isolates for each bacterium, the MDR percentages were determined. Out of 24 types of bacterial species isolated, 4 were Multidrug-Resistant Organisms (MDRs) and only one was methicillin-resistant *Staphylococcus aureus* (MRSA). In case of Gram-positive isolates, MDR *Staphylococcus aureus* was 83.78% and in case of Gram-negative isolates *Acinetobacter baumannii* had the highest MDR isolates 91.62% respectively, followed by *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* at 79.25%,53.44%, and 82.90% (Table 3). The correlation between MDR (in %) and TBSA (Total body surface area) was validated using regression analysis of data (Graph 1 A & B).

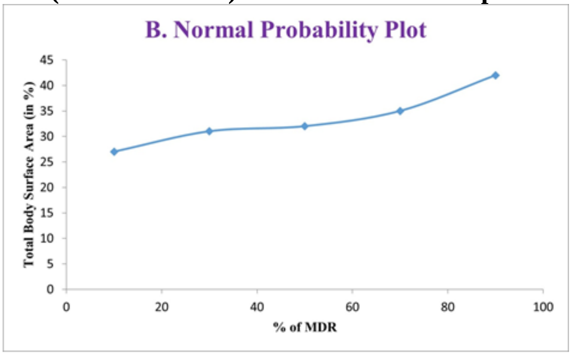
Table 3: Distribution (in %) of multi-drug resistance among burn wound isolates.

Sl. No.	Organism	MDR IN %	
		MALE	FEMALE
1	*Acinetobacter baumannii(n=39)*	44.31%	47.31%
2	*Escherichia coli(n=30)*	34.09%	19.35%
3	*Klebsiella pneumoniae(n=37)*	42.04%	40.86%
4	*Pseudomonas aeruginosa(n=30)*	34.09%	45.16%

A phylogenetic tree of five selected bacteria (both gram-negative and gram-positive bacteria), showed that *Acinetobacter baumannii* is the most closely related gram-negative bacteria identified in burn wounds of patients, followed by *Pseudomonas aeruginosa*. *Escherichia coli* and *Klebsiella pneumoniae* are also closely related phylogenetically (Figure 1). Gram-positive *Staphylococcus aureus* on the other hand, is more closely associated synergistically with *Pseudomonas aeruginosa*, a common burn wound bacterium.



Graph 2: The regression analysis of two variables (TBSA & MDR) relations. A: Line fit plot



Graph 2: The regression analysis of two variables (TBSA & MDR) relations. B: Normal probability plot

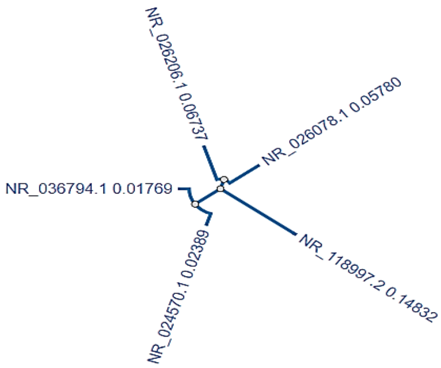


Figure 1: Phylogenetic tree of test bacteria (05) gram-negative (NR_026206.1: *Acinetobacter baumannii*, NR_024570.1: *Escherichia coli*, NR_036794.1: *Klebsiella pneumoniae* & NR_026078.1: *Pseudomonas aeruginosa)* and gram-positive bacteria (NR_118997.2: *Staphylococcus aureus*).

5. DISCUSSION

This investigation was conducted on a broad range of population that suffered burns due to any domestic / industrial accidents. Given high homogeneity among cases, our results demonstrated that the TBSA range and the hospitalization days were independent factors for the isolation of MDR. However, the severity of burns and the time elapsed after burns affected the various microbial isolates in various patterns.

5.1 MDR organisms and MRSA

According to several investigations, gram negative bacteria were the most often isolated generally from burn units, followed by gram positive bacteria [8], [2], [9], [10] . We found gram negative bacteria often in burn wound of admitted patients. However, *P. aeruginosa, K. pneumonae, Enterobacter spp., E. coli, and Acinetobacter spp*. were the most frequently isolated bacteria in our investigation. our finding is nearly in line with most previous published results [8], [2], [10], [11]. These bacteria can easily thrive in humid or sewage-filled conditions. Gram positive bacteria, in particular MRSA, are still a major cause of burn infections among MDR [11]. Compared to MRSA (88%), our data indicated a reduced frequency of isolation by *S. aureus* (14%). Patients with MRSA had a total body surface area greater than 30% and spent longer than two weeks in the hospital. This finding was analogous with that of another study, which tracked all hospitalized burn patients for a year and discovered that only more than 15% of *S. aureus* strains were methicillin resistant. Nevertheless, the study’s burn patients only had a median hospital stay of two weeks and a total body surface area of 8.5% (interquartile range: 5-12%) [10]. In addition to MDR, treating and caring for burn patients with MRSA infections has grown more difficult. The current study’s significance, healthy participant population as well as appropriate antibiotic treatment and infection control strategies may be the reason.

Because Gram negative bacteria can build biofilms that seem to increase their pathogenicity, they have become more and more resistant to antimicrobial drugs [12]. Our data displayed that in all types of ICU / burn CU increased during the first month after hospitalization. These infections happened in the first two weeks following admission, and the total body surface area in those cases varied from 60-95%. If molecular epidemiological surveillance was available, more data could be given. In contrast to *P. aeruginosa*, the occurrence of *A. baumannii* MDR isolates was, however, comparatively low, according to the study’s overall results [13], [14]. TBSA (total body surface area) / depth of burn wounds is supporting the growth of MDR in the wound (Table 3, Graph 1 A & B). We used UV light based sterilization of patient's cabin to reduce cross contaminations in this study. The cleaning staffs were trained about biosafety level for usage of UV lamp.

One key factor that emerged from this study is the strong correlation between burn severity and the prevalence of *Pseudomonas aeruginosa* infections. Patients with burns covering more than 30% of their TBSA had significantly higher rates of *P. aeruginosa* infections, which are notoriously difficult to treat due to their antibiotic resistance. This highlights the need for early and aggressive intervention to control infection in severe burn cases. In SUM, there are no particular recommendations for the first round of antibiotic treatment for burn patients’ infections. Prior to identifying bacteria, physicians used their experience to choose antibiotic medications. When antibiotics could not be applied to burn wounds, they were either applied topically or given systemically. Similar rules or tactics are used all around the world.

5.2 Infection, colonization and persistence

In the investigation, additional risk factors beyond endogenous and exogenous microbial colonization, such as burn severity and treatment duration, were identified as contributors to microbial infection and antibiotic resistance in burn patients. The diversity in patient profiles highlighted the significant influence of burn severity and time on microbial isolates. Multivariate analyses from previous studies have consistently identified the extent of burn injuries as a primary risk factor for microbial colonization and infection. Notably, patients with burns covering more than 20–35% of their total body surface area are at heightened risk of infection. The reliance on invasive devices for treatment further amplifies this risk, as these devices often serve as conduits for microbial [15]. In our study, the average total body surface area (TBSA) for severe burn cases was 42.3%, with infection rates increasing by 61% in patients with TBSA exceeding 43%. Patients with less than 35% TBSA had the highest prevalence of gram-positive bacterial isolates, but these proportions significantly declined (p = 0.05) as TBSA increased. While severe burns often lead to rapid mortality, potentially reducing the total microbial isolates, this was inconclusive in our data due to only one recorded death with TBSA > 65%. Regression analysis showed that for every 10% increase in TBSA, the likelihood of infections by multidrug-resistant organisms rose three to thirteen times. Additionally, third-degree burns were identified as a significant independent risk factor, nearly doubling the infection risk compared to other burn depths. [12], The application of multiple analyses showed a 43–69% reduction in MDR risk after adjusting for the total body surface area, potentially minimizing cross-contamination in extensive burns. This reduction may be attributed to early excision, debridement, grafting, the use of advanced dressings, and proper antibiotic stewardship alongside infection control measures. Time also plays a critical role, as gram-positive bacteria from the skin's indigenous flora commonly colonize burn wounds within the first 48 hours [16]. After that, within the second week, gram negative bacteria have taken the position of gram-positive bacteria [17]. After that, there is a further change in the flora toward yeasts, fungi, and bacteria resistant to antibiotics if the wound closure is postponed, the patient becomes infected, and broad-spectrum antibiotics are administered [18]. The use of stratification analysis to look for patterns in the main microbiological isolates according to the degree of burn and amount of time after burn was one of the study's main advantages.

The bacterial isolates found in burn wounds are diverse in nature and either gram-positive or gram-negative. Microorganisms are phylogenetically linked to each other and as expected, *Acinetobacter baumannii, Pseudomonas aeruginoa, Escherichia coli* and *Klebsiella pneumoniae* are closely related gram-negative bacteria identified in burn wounds of patients. Surprisingly, *Staphylococcus aureus* which is a gram-positive bacterium was also found to be closely associated with *Pseudomonas aeruginosa.* Both *P. aeruginosa* and *S. aureus* are common burn wound bacteria and are widespread nosocomial pathogens, displaying high levels of multidrug resistance [19]. The synergistic relationship between the two bacteria could be due to the similarity in their growth pattern and virulence mechanisms. It has been observed that *S. aureus* usually leads *P. aeruginosa* in the earlier stages of disease; although, morbidity and mortality rates are increased during co-infection [20]. Both these bacterial agents are capable of biofilm formation which confers high levels of protection against host immune clearance and resistance to antimicrobial agents [21].

These findings revealed that *P. aeruginosa* and *S. aureus* exhibited increased survival against antibiotic treatment when grown together in planktonic co-cultures, with this effect becoming even more pronounced in the wound model. This enhanced antibiotic tolerance was linked to the combined influence of "host-derived" and "bacterium-derived" matrix components. Overall, the findings highlighted a potential mutual benefit for *P. aeruginosa* and *S. aureus* in co-infecting wounds, where the host-derived matrix plays a protective role comparable to the bacterial matrix in promoting antibiotic resistance [22].

The findings also highlighted the patterns of microbial isolation and key factors influencing isolation rates, despite some heterogeneity in sampling due to patient distribution across ICUs and burn wards. To ensure consistency, a multidisciplinary medical team was established, holding regular meetings to discuss treatment strategies. Future studies should explore the impact of surgical interventions and antibiotic stewardship on pathogenic microbial profiles and conduct molecular typing of isolates to deepen understanding.

6. CONCLUSION

We found that the burned body surface area and stay time in hospital are directly proportional to microbial infection. The primary factor influencing MDR was the size of the total body surface area burnt. The study showed that spread of microbial infection might be controlled to increase the survival rates of burn patients under well-equipped burn wards, advanced medical set-ups and dedicated medical staff. Also, this study paves the path for further research on poly-microbial infection and its effect on antibiotic resistance. If the molecular evidence of co-infection of different pathogens as P. aeruginosa and S. aureus can reveal their association, it could further give an insight to the mechanism of synergistic interaction among different bacteria in burn wounds.

Limitation of work

This study's relatively small sample size and focus on a single burn center may limit the generalizability of the findings, as regional variations in microbial resistance patterns were not captured. The patients admitted to SUM Hospital represented a random mix of industrial and domestic burn cases, including males and females across various age groups. Future research should adopt a multi-center or multi-regional approach to provide a more comprehensive understanding of burn infection etiology.

Risks factors

Several risk factors contribute to increased susceptibility to infection in burn patients. Larger burn sizes, particularly those covering more than 20% of the total body surface area (TBSA), increase the risk of infection. Elderly patients are more vulnerable due to weakened immune responses, while prolonged hospital stays raise the likelihood of hospital-acquired infections. Delayed admission, where patients initially rely on home remedies or primary care before reaching the hospital, can exacerbate infection risk. Additionally, high visitor numbers in burn wards can lead to cross-contamination and heightened infection risks.

Profit from the outcome

The outcomes of this study will aid healthcare professionals in designing targeted interventions to prevent infections in burn patients. Identifying microbial species enables optimized antibiotic use, reducing treatment costs, improving recovery times, and lowering mortality rates. Understanding microbial growth patterns further supports effective drug administration and wound management strategies.

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Author' contributions

SR was responsible for data analysis and drafting manuscripts. RK and KS was responsible for study conception and design, data analysis and interpretation, and SK was responsible for review, editing & drafting a manuscript.

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Conflict of Interests

The authors declare that they have no Conflict of interests.

Ethical Compliance

There was no direct involvement of any human or animal model in this study. Institute Ethical Committee approval number is Ref.no/ICE/IMS.SH/SOA/2024/892.

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