

Original Article



Bacterial and Antimicrobial Susceptibility Persistence Pattern and Prevalence of Sepsis Among Burn Patients

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Abstract

Background: Infection is a major cause of mortality and morbidity among burn patients. Clinical diagnosis of bacteremia and/or sepsis in burn patients could be symptomatic and/or asymptomatic as a result of immune deficiency secondary to thermal injury. Accordingly, this study sought to characterize the microbial landscape, antibiotic susceptibility profiles, and sepsis incidence in burn patients.

Methods: This analytical cross-sectional study was performed on pediatric patients hospitalized in Imam Khomeini Hospital, Urmia, Iran, from October 2021 to September 2022. The subjects included 515 burn patients (1–85 years old) exhibiting symptoms suggestive of sepsis, aligned with the latest American Heart Association guidelines. Their blood samples were cultured, and antimicrobial susceptibility testing was conducted by the Kirby-Bauer disc diffusion technique. The required data were collected by pre-tested questionnaires to identify potential septicemia-associated factors.

Results: The mean age (standard deviation) of burn patients was 27.7 ($\pm$ 19.62) with a minimum of 1 year and a maximum of 85 years. Approximately 66.9% of patients were male, and the remaining 33.1% were female. Wound cultures were taken from 24 patients, and 16 (66.7%) were positive. *Acinetobacter baumannii* (29.2%) was the most common microorganism in wound culture. Resistance to co-trimoxazole and cefotaxime was 66.7% and 54.2%, respectively. Blood cultures were taken from 39 cases, and 10 (25.6%) were positive. The rate of sepsis was 1.94%. The most common bacterium isolated from blood culture was *A. baumannii* (10.3%), and resistance to gentamicin and cotrimoxazole was 23.1% and 15.4%, respectively.

Conclusion: Among the Gram-negative bacteria, *A. baumannii* was resistant to most antibiotics in the blood and wound culture and was the predominant isolate. Resistance to cotrimoxazole, cefotaxime, and gentamicin had a high frequency. It was revealed that an increase in burning percent leads to sepsis and increases the mortality rate.

Keywords: Antibiotics, Sepsis, Burn, Resistance to antibiotics

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Background

Damage caused by thermal, chemical, electrical, or frictional trauma to the skin or other tissues results in burn injuries (1). The rate of burn-related fatalities, primarily in low- or middle-income developing countries, is around 180 000 individuals annually (2). Non-fatal burn injuries cause substantial morbidity, protracted hospitalization, disfigurement, and disability, frequently leading to stigmatization and rejection (3). Burn patients are highly susceptible to opportunistic infections (4). The burn site remains sterile for approximately 24 hours; thereafter, Gram-negative bacterial colonization is common (5). *Pseudomonas aeruginosa* (74%), *Escherichia coli* (35%), *Acinetobacter baumannii* (24%), coagulase-negative staphylococci (21%), and *Enterococcus* spp. (14%) are the most prevalent Gram-negative bacteria isolated in burn

units globally (6). However, the microbial colonization spectrum varies among burn units, fluctuating over time and location (7).

Thermal injury disrupts the skin's barrier function against microbes, allowing microorganisms to penetrate the body and cause infection (8). Up to 65% of burn patient deaths can be attributed to infection and sepsis (9). Sepsis is caused by the presence and dissemination of microorganisms in the bloodstream, eliciting various severity systemic responses (10). The frequency and severity of septic complications in critically ill burn patients necessitate effective diagnostic and therapeutic strategies (11). Clinical diagnosis of bacteremia and/or sepsis is challenging due to potential symptoms or asymptomatic presentation resulting from thermal injury-induced immune deficiency, malnutrition,

anemia (reperfusion impaired), and immunological barrier damage (12). Nonetheless, regular culturing and surveillance of potential microorganisms and their sensitivity/susceptibility patterns can facilitate early intervention and potentially reduce morbidity and mortality from septicemia in burn patients (11).

Prophylactic antibiotic administration is a common practice among burn patients; however, widespread multi-drug resistance (MDR) among bacterial pathogens has become a significant challenge (13, 14). This MDR pattern exhibits geographical and regional variations influenced by healthcare settings and prevailing practices (15). Inappropriate sepsis treatment worsens the condition, potentially leading to patient mortality and the emergence of novel MDR strains (16). This issue poses a severe global health threat with substantial economic and social repercussions (17). MDR strain-induced infections result in prolonged hospital stays and increased mortality risks and thus necessitate treatment with more expensive antibiotics (18). Therefore, this study aims to characterize the microbial landscape, antibiotic susceptibility profiles, and sepsis incidence in burn patients.

Methods

In accordance with the Declaration of Helsinki of 1975, as revised in 2013, and after the approval of the Ethics Committee of Urmia University of Medical Sciences (with the code IR.UMSU.REC.1398.525), this analytical cross-sectional study was performed in Imam Khomeini Hospital, Urmia, Iran, from October 2021 to September 2022. The subjects of the study included burn patients of both genders and all ages (1–85 years) representing symptoms suggestive of sepsis, in line with the latest American Heart Association guidelines. The inclusion criteria were the presence of two or more of the manifestations, such as a respiratory rate ≥ 22 breaths per minute, systolic blood pressure < 100 mmHg, and a decreased level of consciousness (Glasgow Coma Scale score < 15). Blood cultures were performed to substantiate the diagnosis.

Sample Size Determination and Sampling Technique

The single population proportion formula was employed to determine the sample size, yielding a calculated sample size of 515. A convenience sampling technique was adopted, and all consecutive eligible patients encountered during the study period were enrolled until the target sample size of 515 was achieved. Prior to data collection, the study’s objectives and the benefits of participation were meticulously explained to the participants. Participation was voluntary, and participants were informed of their right to withdraw from the study at any point during data collection. A questionnaire, developed based on known and potential factors to address the study’s objectives, was pretested for validity and reliability.

Data Collection Procedures

A pre-designed and structured questionnaire was employed to gather sociodemographic (age and gender) and clinical (wound types, site of wound, wound percent, hospitalization duration, and prognosis) data from the patients. Additionally, information regarding antibiotic resistance tests and the types of involved organisms was incorporated into the questionnaire. The questionnaire was initially drafted in English and subsequently translated into Persian and back-translated into English to ensure translation accuracy. Data collectors, comprising nurses and laboratory technicians, were recruited and trained on questionnaire administration, data collection techniques, interviewing skills, and specimen collection procedures. Subsequently, sociodemographic data, clinical information, and specimens were collected.

Wound Swab Sample Collection

Physicians evaluated patients with wounds for signs of infection, including pain, redness, swelling, warmth around the wound, purulent discharge, unpleasant odor, fever, and chills. For patients suspected of bacterial infection, wound beds were prepared for specimen collection by cleansing the surface to remove exudates and contaminants using a moistened sterile gauze and sterile normal saline solution, following Levine’s technique (19). Next, swabs were aseptically collected from the wound using sterile cotton swabs. The swabs were rotated with sufficient pressure to collect adequate specimens. After collection, the wound was cleaned with an antiseptic solution. Within 30 minutes of collection, wound specimens were transported to the microbiology laboratory. The swabs were placed in sterile test tubes containing 0.5 mL of sterile normal saline solution to maintain specimen viability during transport.

Laboratory Inoculation and Identification

Each wound sample was simultaneously inoculated onto a mannitol salt agar plate, blood agar plate, and MacConkey agar plate (Oxoid Ltd.) and incubated aerobically at 37 °C for 24 hours. If mixed colonies were observed on the agar plates, subculturing onto MacConkey and blood agar plates was performed, followed by incubation at 37 °C for 24–48 hours. Bacterial isolates were identified using standard microbiological methods, encompassing colonial morphology, Gram’s stain reaction, and biochemical tests (3). Biochemical tests were conducted on pure colonies from cultures for definitive identification of the isolates. Gram-negative bacteria were identified through a series of biochemical tests (Oxoid Ltd.), including Simmons citrate agar, indole production, urease test, lysine decarboxylase, oxidase test, Kligler iron agar, H₂S production, and motility test. Moreover, Gram-positive cocci were identified based on their Gram reaction, coagulase, catalase, and hemolytic

pattern (19).

Antibiotic Susceptibility Testing

A suspension of pure colony from each confirmed culture isolate was prepared in sterile normal saline and incubated at 37 °C for at least 15 minutes. Then, the suspension was adjusted to 0.5 MacFarland standard. A sterile cotton tip applicator stick was used to uniformly distribute the suspension on Mueller-Hinton agar (Oxoid Ltd.). Next, the modified Kirby-Bauer disk diffusion technique was implemented for the antibiotic susceptibility pattern using different antibiotics, such as ciprofloxacin (5 µg), penicillin (10 IU), clarithromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), and erythromycin (15 µg). The other antibiotics included tetracycline (30 µg), ceftriaxone (30 µg), ampicillin (10 µg), chloramphenicol (30 µg), meropenem (10 µg), ceftazidime (30 µg), augmentin (20/10 µg), and piperacillin (100 µg; Oxoid Ltd.). After incubation at 37°C for 24 hours, the zones of inhibition were measured by a ruler. Finally, the results were measured, recorded, and classified as susceptible, intermediate, and resistant using the Clinical and Laboratory Standards Institute 2018 performance standards for antimicrobial susceptibility testing interpretation (20).

Data and Laboratory Quality Control

Several measures were taken for rigorous data quality control throughout the study. Prior to data collection, standardized data collection instruments were developed, and data collectors received comprehensive training. During data collection, the principal investigator closely monitored the data collection process to ensure adherence to protocols. A pretesting phase was conducted to assess the appropriateness and comprehensibility of the questionnaire. The collected data were carefully reviewed daily for consistency and accuracy. In the laboratory, quality assurance practices were strictly adhered to during the preanalytical, analytical, and post-analytical phases, as outlined in the Microbiology Laboratory of the Department of Military and Refugee Health’s standard operating procedures. Experienced and well-trained laboratory personnel performed the laboratory identification procedures. The performance of prepared media was verified by inoculating control strains of *S. aureus* (ATCC-25923) and *E. coli* (ATCC-25922). Additionally, 5% of the prepared media were incubated at 37°C for 24–48 hours to check for sterility. Reagents for Gram staining and biochemical tests were validated using standard strains of *S. aureus* and *E. coli*.

Statistical Analysis

Quantitative variables were reported as means ± standard deviations, and qualitative variables were presented as numbers (percentages) in the form of appropriate tables

and graphs. An independent t-test was used to compare quantitative variables between patients with positive and negative culture results, and the chi-square test was utilized to compare the distribution of qualitative variables between patients (if necessary, Fisher’s exact test). The data were then imported into Statistical Package for the Social Sciences (SPSS, version 20.0) software for analysis, and $P \leq 0.05$ was considered statistically significant.

Results

The baseline characteristics of patients included in this study are summarized in Table 1. Of 515 burn patients, 66.9% were male, while the remaining 33.1% were females, with a mean age of 27.7 ± 19.62 years (1–85 years old). The most common cause of burns was fire ($n = 474$, 92%). The mean duration of hospitalization was 4.65 ± 6.13 days. The total number of deaths that occurred during hospitalization at the burn unit was 10 patients (27.8%). Moreover, 85.7% of the examined patients were discharged from the hospital after recovery, 5.8% unfortunately died, and 8.5% were discharged from the hospital by personal consent (Table 1).

In this study, wound culture was performed for 24 patients. It was positive in 16 patients (66.7%) but negative in 8 patients (33.3%). Resistance to cotrimoxazole and cefotaxime had the highest frequency (66.7% and 54.2%, respectively), and no patient was reported to show resistance to nitrofurantoin, tetracycline, or meropenem (Figure 1).

Figure 2 displays the frequency distribution of reported cases based on the type of bacteria investigated in the patient’s wound culture samples. *Acinetobacter* microorganism was the most abundant, and no cases of *Pseudomonas*, *Klebsiella*, and *Staph saprophyticus* microorganisms were reported in the wound culture samples of the studied patients.

Table 1. Baseline and Demographic Features of Studied Patients

Variables	Total (515)
Mean age (1–71 years old)	27.71 ± 19.62
Gender	
Male	345 (66.9%)
Female	170 (33.1%)
Type of burn	
Fire	474 (92%)
Chemical	20 (3.9)
Electrical	21 (4.1)
Mean duration of hospitalization (day)	4.65 ± 6.13
Disease outcome	
Mortality	30 (5.8%)
Discharge after recovery	441 (85.7%)
Discharge by personal consent	88 (8.5%)
Burn percentage	10.6 ± 18.00

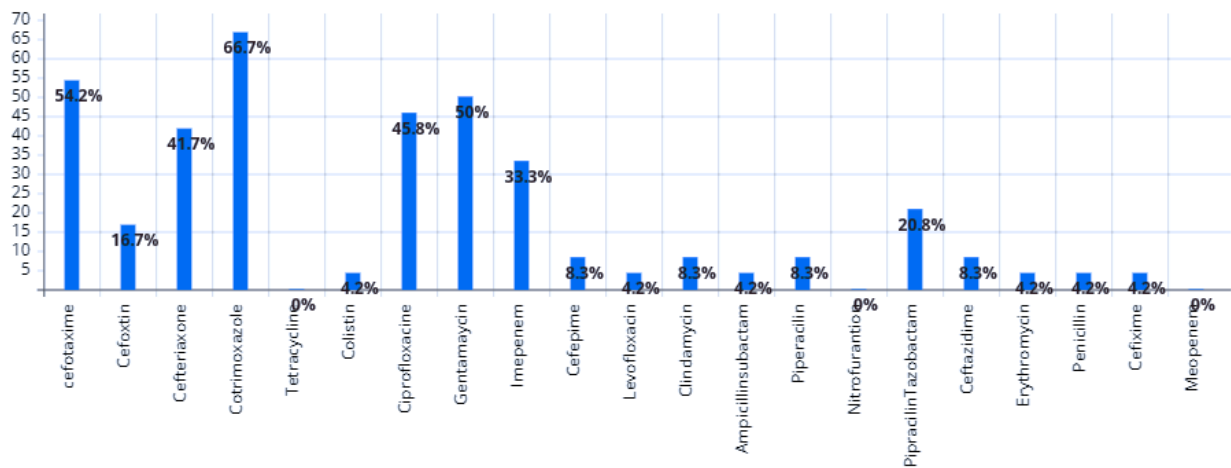


Figure 1. Frequency Distribution of Cases Resistant to All Types of Antibiotics Investigated in Wound Culture

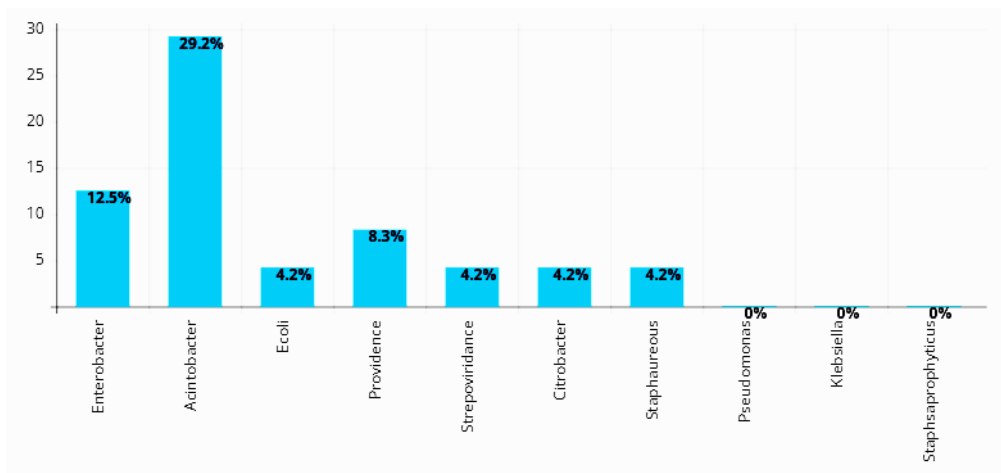


Figure 2. Frequency Distribution According to the Type of Microorganism in the Wound Culture of the Investigated Patients

Blood culture was performed in 39 patients. The result of the blood culture was positive in 10 patients (25.6%) but negative in 29 patients (74.4%). The frequency of sepsis was reported as 1.94%. Gentamicin resistance had the highest frequency. Resistance to cefixime, ceftazidime, and ampicillin/sulbactam was not reported in any of the blood culture samples of investigated patients (Figure 3). Figure 4 shows the frequency distribution of microorganisms in the blood cultures of the examined patients. In blood culture samples, *Acinetobacter* microorganism had the highest frequency (10.3%). No cases of *Enterobacter*, *Providencia*, *Strep-viridans*, or *Staphylococcus aureus* microorganisms were reported in the evaluated blood culture samples of these patients. Table 2 compares the demographic and clinical characteristics of studied patients based on blood culture results. The mean age of patients with negative blood culture (32.3 ± 16.95) was higher compared to patients with positive blood culture (30.40 ± 17.74). However, the observed difference was not statistically significant

($P=0.76$). In patients with positive culture results, the distribution of men and women was similar, while in patients with negative culture results, the number of male patients was more ($n=22, 75.9\%$). Nonetheless, the difference observed in terms of age distribution between the two groups was not significant ($P=0.23$). The mean age in patients with negative blood cultures (32.3 ± 16.95) was higher than that in patients with positive blood cultures (30.40 ± 17.74); however, the observed difference was not statistically significant ($P=0.76$). In patients with positive culture results, the distribution of men and women was the same, and in patients with negative culture results, more patients were male (75.9%) ($P=0.23$). The mean burn percentage in patients with positive culture results (54.3 ± 23.01) was significantly higher than that in the patients with negative culture results (22.24 ± 18.2), which was statistically significant ($P=0.001$). The mean length of hospitalization (days) was longer in patients with positive culture results (13.4 ± 10.6) compared to patients with

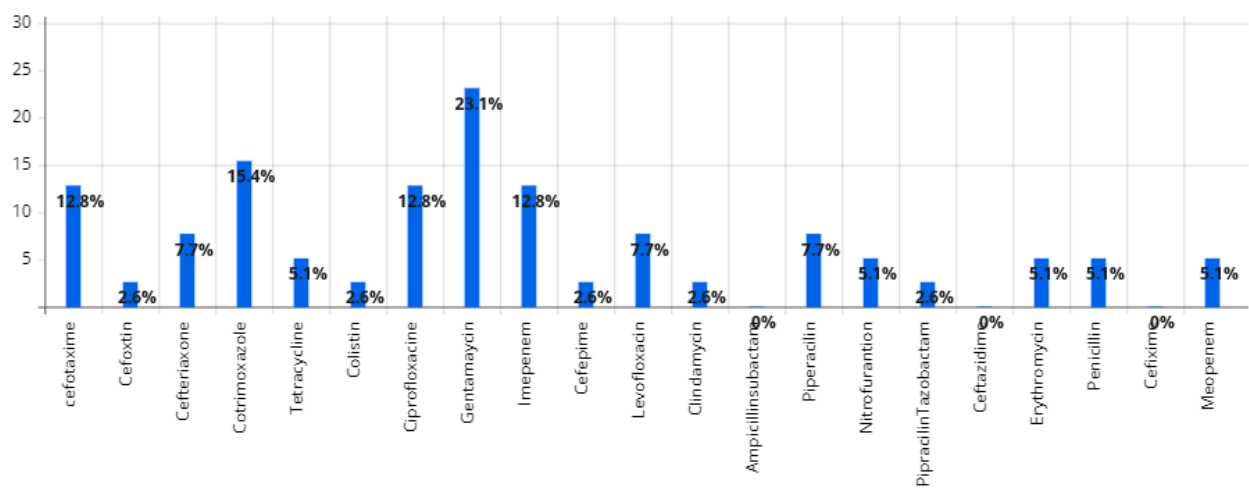


Figure 3. Distribution of the Frequency of Antibiotic Resistance Based on the Types of Antibiotics in the Blood Cultures of the Examined Patients

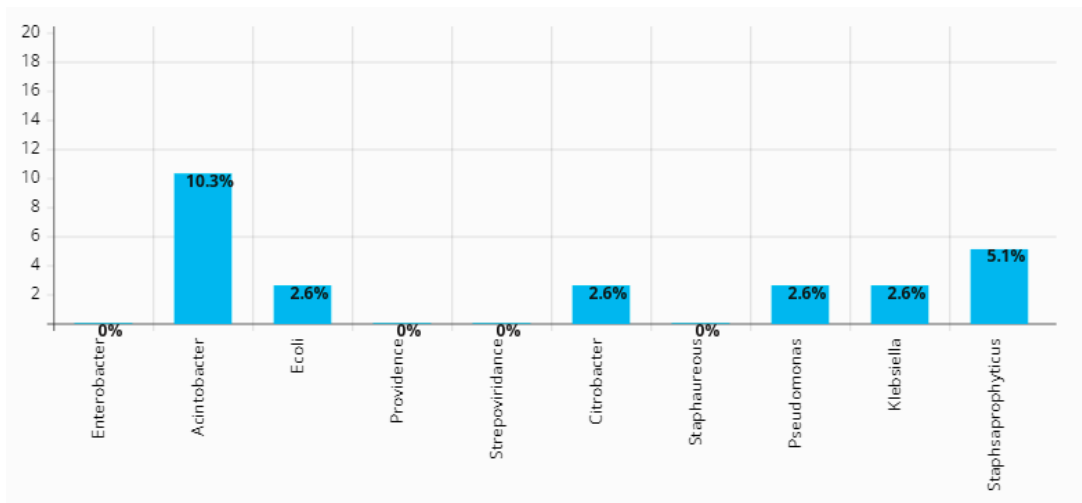


Figure 4. Frequency Distribution Based on the Type of Microorganism in the Blood Cultures of the Examined Patients

Table 2. Comparison of Demographic and Clinical Characteristics Based on Blood Culture Results in the Examined Patients

Variables	Positive Culture Result	Negative Culture Result	P Value
Mean age (1-71 years old)	30.40 ± 17.74	32.3 ± 16.95	0.76
Gender			
Male	5 (50)	22 (75.9%)	0.23*
Female	5 (50)	7 (24.1%)	
Type of burn			
Fire	10 (100%)	24 (82.2%)	0.3*
Chemical	0 (0)	5 (17.2%)	
Electrical	0 (0)	0 (0)	
Mean duration of hospitalization (day)	13.4 ± 10.6	12.03 ± 9.77	0.71
Disease outcome			
Discharge after recovery	7 (70%)	26 (89.7%)	0.22*
Mortality	2 (20%)	3 (10.3%)	
Discharge by personal consent	1 (10%)	0 (0)	
Mean burn percent	54.3 ± 23.01	22.24 ± 18.2	0.001 >

Note. * Fisher exact test.

negative results; nonetheless, this difference was not statistically significant ($P=0.71$). The type of burn in all patients with positive culture results was fire (100%), and patients with negative culture results had 82.2% fire burn. The relationship between the culture result and the type of burn source was not statistically significant ($P=0.3$). The frequency of mortality in patients with positive culture results (20%) was higher than that in patients with negative culture results (10.3%). On the other hand, the frequency of discharge in patients with negative culture results (89.7%) was higher than that in patients with positive culture results (70%), which was not significant ($P=0.22$).

Discussion

Approximately 75% of burn patient fatalities are attributed to infections (21). Prolonged antibiotic use drives the emergence and selection of MDR bacteria, leading to treatment failure and exacerbated complications. Therefore, understanding the microbial flora and prevailing antibiotic susceptibility patterns is

crucial for clinicians managing burn sepsis.

A higher incidence of burn injuries was observed among males in our study. This finding aligns with the results of a study by Wardhana et al, implying that 63.9% of patients admitted to the RSCM center were male (7), attributing this to the more hazardous working conditions faced by men (22). Conversely, a study by Paudel (23) conducted at Bir Hospital in Nepal revealed a higher prevalence of burn injuries among females (55%) compared to males (45%). According to the World Health Organization (24) fact sheets, the increased risk for females is linked to open-fire cooking practices or inherently unsafe cook stoves that can ignite loose clothing. Additionally, open flames used for heating and lighting pose risks, and self-directed or interpersonal violence are also contributing factors. However, these data are only applicable to low- and low-middle-income countries. In high-income countries, males are more frequently affected by burn injuries. A systematic review by Smolle et al (25), which primarily included data from high-income countries, demonstrated an overall mean male:female ratio of 1.92:1.

The findings of the current study confirmed that burn injuries can occur at any age, with the youngest patient being one year old and the oldest being 85 years old. These findings conform to those of previous studies, indicating that burn risk encompasses all age groups (26). This can be attributed to the fact that children spend a significant portion of their time indoors, exposing them to potential burn hazards from stoves, heaters, and hot liquids. Therefore, children require increased supervision from family members. Further, workplaces should implement stricter safety measures to protect adults, particularly males. Moreover, elderly patients with compromised immune systems are more susceptible to infections and impaired healing. Furthermore, pulmonary and cardiovascular complications, as well as diabetes, in the elderly population contribute to poorer outcomes (27). The mean length of hospitalization in this study was 4.65 ± 6.13 days, and the overall mortality rate among the patients was 5.8%. This mortality rate is lower than those reported in previous Iranian studies (28) and other countries, particularly in Southeast Asia, as reported by the World Health Organization (29).

Our study identified fire as the predominant cause of burns in both patients with positive and negative culture results. Notably, 82.2% of burn injuries in patients with negative culture results were attributed to fire. These findings corroborate the results of previous research, highlighting that thermal sources (including fire, hot liquids, steam, and contact with hot surfaces) constitute the primary etiology of burn injuries, accounting for approximately 86% of burn cases necessitating burn center admission (1).

Blood cultures were obtained from 39 patients, revealing positive results in only 10 cases (25.6%). *A. baumannii*

and *Staphylococcus saprophyticus* emerged as the most prevalent bacterial pathogens, accounting for 10.3% and 5.1% of infections, respectively. These findings are in line with observations from a tertiary care hospital in India (30, 31). The high prevalence of these pathogens stems from their ability to thrive in moist environments and persist within healthcare settings (32). Wound cultures, performed on 24 patients, yielded positive results in 16 cases (66.7%). *Acinetobacter* was the predominant pathogen (29.2%), followed by *Enterobacter* (12.5%) and *Providencia* (8.3%). The presence of *Acinetobacter* spp. as normal skin flora, its ease of transmission, and its ability to survive in hospital environments due to its MDR contribute to the increased incidence of nosocomial infections caused by this organism (33).

Contrarily, our findings revealed a significantly higher prevalence of antibiotic resistance among wound isolates, particularly for cotrimoxazole (66.7%), cefotaxime (54.2%), gentamicin (50%), and ciprofloxacin (45.8%) (34, 35). Similarly, blood culture analysis demonstrated elevated resistance rates for gentamicin (23.1%), ciprofloxacin (22.8%), cotrimoxazole (15.4%), cefotaxime (12.8%), and imipenem (12.8%). The observed high incidence of resistant isolates could be attributed to the indiscriminate use of antibiotics (11). Notably, the investigators were unable to identify any antibiotic use policies or infection control units within healthcare facilities in the country (36). While the prevalence of common bacterial pathogens, such as *S. aureus*, is similar across different burn centers, comparisons of antimicrobial susceptibility patterns are hindered by variations in prescription practices, potentially arising from the absence of standardized treatment guidelines (11). Nevertheless, in cases of positive blood cultures and signs of sepsis, antibiotic therapy should be tailored based on the observed susceptibility patterns (37).

The results of the present study confirmed a significant association between the extent of burns and the likelihood of positive blood cultures, with patients exhibiting more extensive burns having a higher risk of positive blood culture results. Furthermore, it was found that mortality rates are elevated among patients with positive blood cultures compared to those with negative blood cultures. These findings are in conformity with those of previous research conducted by Ladhani et al, reporting an increased susceptibility to infection and sepsis in patients with severe burns (38). The extent and severity of burns are established as the most critical prognostic factors for mortality in burn patients (39).

Limitations of the Study

The current study lacks detailed information regarding the timing of sample collection from each patient, which can be crucial for future investigations into the correlation between length of stay and microbial

colonization in burn patients. Further, the study's setting in a public hospital in a developing country limits the generalizability of the findings to healthcare facilities in developed nations, where infection control protocols may be more stringent. The overcrowded nature of the hospital and limited staffing pose challenges to effective infection control practices. Additionally, the majority of patients in this study presented with comorbidities (e.g., diabetes, chronic renal disease, and other conditions) that could influence susceptibility to pathogen infections. However, these comorbidities were not considered in the present study, as its primary objective was not to assess the association between infection and patient comorbidities. Therefore, prospective studies are warranted to control for all variables that could potentially impact the study's conclusion.

Conclusion

Aa a Gram-negative bacterium, *Acinetobacter* was the most common cause of infection in the blood and wound cultures of patients in this study. It was also found that the risk of bacteremia increases with increased severity of burn. Given that most of the isolated microorganisms are hospital-type and resistant to most of the existing antibiotics, it is essential to follow strict hygiene protocols to prevent hospital-acquired infections. These protocols include minimizing contact with patients, washing and disinfecting hands frequently, and properly isolating and disinfecting the environment. Data regarding the incidence of pathogens and their resistance patterns would benefit clinicians to prescribe appropriate antibiotics, articulating policies for empirical antimicrobial therapy to control the different types of infections.

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Authors' Contribution

Conceptualization: Rahim Nejadrahim, Matin Moradi.
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Supervision: Matin Moradi.
Writing–original draft: Rahim Nejadrahim.
Writing–review & editing: Ali Ranjbar.

Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

Ethical Approval

This study was performed in line with the principles of the

Declaration of Helsinki. Approval was granted by the Ethics Committee of Urmia University of Medical Sciences (No. IR.UMSU.REC.1398.525).

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