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CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF DIFFERENT BACTERIA FROM BURN WOUND INFECTIONS

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ABSTRACT

Human skin integrity is important for protection against infections, maintenance of body fluid and thermoregulation. Burn injury compromises the skin barrier and allows bacteria to enter, hence delaying the healing of burn wounds. Therefore, this study is aimed to find out the bacteriological profile of post burn and evaluate the antimicrobial susceptibility pattern of the organisms isolated. A total of 25 samples from patients with burn wound infections were collected from Hayatabad Medical Complex, Peshawar. Data on age, gender and type of burn were also collected from the patients' clinical folder. The samples were plated on MacConkey and blood agar. Biochemical tests were carried out on the representative isolates on each plate and antibacterial susceptibility pattern was assessed using the Kirby-Bauer disc diffusion method. Out of the total 25 samples, 75% were collected from female patients, while 25% from male patients. Most of the patients 40% were within the in 21-30 years age group, followed by 20% in 31-40 years age group, 15% in 41-50 years age group, 10% in 1-10 years age group, 5% in 51-60, and with the 61-70 years age group having zero number of patients. Electricity was the cause of burn in 25% of people, flame was responsible for 45% of burn injuries and 30% of patients had been burned by scald. Out of the total 25 blood samples, 80% of the samples were culture positive and 20% of the samples were culture negative. Among positive samples, the

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predominant organisms isolated were *Staphylococcus. aureus* (55%) followed by *Pseudomonas* (35%), *Klebsiella* (10%). The results of antibiotic susceptibility testing showed that all the *Staphylococcus. aureus* isolates were methicillin resistant *Staphylococcus. aureus* (MRSA), while all other bacterial isolates exhibited resistance to multiple drugs. Methicillin Resistant *Staphylococcus aureus* (MRSA) and MDR bacteria are the most common isolates associated with burn wounds in Peshawar, Pakistan. Hence a careful selection of antibiotics to control the wound infection is required for proper management of burn wounds in order to help reduce resistance issue.

Keywords: MRSA, MDR, burn wounds, *S. aureus*, flame.

INTRODUCTION

Burn injuries among the most prevalent and devastating forms of trauma (Rezaei *et al.*, 2020) and are caused by heat, friction, radiation, cold, electric and chemical sources. However, the majority of them are caused by heat from fire, solids and liquids (Caksen, 2023). Although, energy transfer results in tissue destruction in all burn injuries, however, several causes can be linked to various pathophysiological responses. These causes severe tissue damage in addition to cutaneous necrosis (Jeschke *et al.*, 2020).

Aside from defining the aetiology of a burn injury, it is crucial to classify injury based on its severity, size and its depth (Jeschke *et al.*, 2020). Burns that damage the top most layer of the skin *i-e.*, epidermis are classified as superficial or first degree. These burns cause the skin to turn red and cause short-term discomfort (Abubakar *et al.*, 2019). Superficial partial thickness or second degree burns are weep, painful, require dressing and may scar but do not need surgery (Duggan, 2023). Deep partial thickness or second degree burns are less painful because the pain receptors are partially destroyed, drier and need surgery (Anis *et al.*, 2022). A full thickness or third degree burns penetrates the whole dermis due to the damage to the nerve endings. These burns require protection from infection and minor surgical management. Finally, a fourth degree burns entails harm to deeper tissues, including muscles and bones and is typically associated with the loss of the burnt area. The superficial burns usually heal without surgery, more serious burns require meticulous management, which may involve topical antimicrobial dressing or surgery (Singh *et al.*, 2021).

Importantly, burns are categorized as mild or minor and major or severe. A minor burn is one that covers 10% of the total body surface area (TBSA) with superficial burns being the most common. In contrast, major or severe burn is not clearly defined; some guidelines to classify burn injuries are: >10% TBSA in elder patients, > 20% TBSA in adults and > 30% TBSA in children (Jeschke *et al.*, 2020). Major burn injuries causes pathophysiologic alterations that affect nearly all organs from the time of the injury until the wounds heal. Pathophysiologic consequences, such as insulin resistance, pain, neuromuscular dysfunction and pruritus persists for years, especially in individuals with serious injuries.

Infections of burn wounds are potentially serious complications that occurs immediately after an injury (Markiewicz-Gospodarek *et al.*, 2022). Infection of a wound is defined as "the invasion of the microorganism via tissues as a result of breakdown of local and systemic host defences" (Malone & Schultz, 2022). Major

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wound infection occurs when a wound exudes pus and may necessitate a subsequent procedure to ensure sufficient drainage (Bhattarai, 2021). In mild wound infections, pus or serous fluid is discharged without severe pain or systemic symptoms. Wound healing is the most prevalent and troublesome condition of wound healing (Sattar *et al.*, 2019).

The characteristics of a burn are determined by its depth. Superficial burns produce discomfort for two or three days followed by skin peeling for a few days (Ochoa & Lesher, 2023). People with more severe burns may show discomfort or complain of pressure rather than pain. Full thickness burns might be completely insensitive to light touch or penetration. Whereas, superficial burns are normally red, severe burns can be pink, black or white in color. Burns surrounding the mouth area and singed hair within the nose indicate that the airways have been burned. Wheezing, shortening of breath, stridor and hoarseness are some of the concerning symptoms. Up to 90% of adults and almost all children experience itching throughout the healing process. After an electrical injury, tingling and numbness might last for a long time. Burns might also cause emotional and psychological distress (Hasan *et al.*, 2019).

The current mortality rate for severe burns is 87% (Etchells *et al.*, 2020), with almost 11 million people seeking medical attention each year (Jyoti *et al.*, 2020). More than 95% of these occurs in low and middle income countries (Mehta *et al.*, 2022). Approximately 486,000 burn injuries are treated annually in the US Medical Institutions, with 40,000 requiring hospitalization and approximately 3275 fatalities (Young *et al.*, 2019).

Burn wound infection is troublesome because it slows down healing, promotes scarring and might lead to sepsis, bacteremia, organ failure or multiple organ dysfunction, in which organs from multiple systems are unable to maintain homeostasis on their own, necessitating immediate medical attention (Vinaik *et al.*, 2019). Burn injuries are diverse but they all involve liquefactive necrosis of the body's largest organ, the skin. The skin is among of the most vital immunological defense system of the human body (Qadir *et al.*, 2022). Therefore, infection is a major issue for people who recover from a burn injury. The usual beginning of this problem is bacterial colonization which has a substantial influence on morbidity, mortality and health care expenses (Williams & Lee, 2021; Markiewicz-Gospodarek *et al.*, 2022).

Although burn wound surfaces are sterile immediately after thermal damage, bacteria gradually colonize these wounds (Tyagi *et al.*, 2021). Microorganisms colonizes burn sites are derived from the patient's endogenous skin, respiratory and gastrointestinal tract flora (Ghafil, 2021). Additionally, some microorganisms may be transmitted to a patient's skin surface by contact with the contaminated external environmental surfaces, air, water, formite and the hands of health care workers (Poggio *et al.*, 2020).

In the early 20th century, *Streptococcus* pyogenes and *Staphylococci* were the most frequently identified cause of burn wound sepsis (Kaftandjieva *et al.*, 2019). *Pseudomonas aeruginosa* has emerged as a major member of the burn wound flora and is isolated from the burn injuries of 70% of the patients in the absence of topical therapy (Sharma & Sharma, 2021). Some of the other aerobic bacteria isolated

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frequently from burns wounds include *E.coli*, *Klebsiella* Spp., *Proteus*. Anaerobic bacteria such as *Bacterroides fragilis*, *Peptostreptococcus* and *Fusobacterium* are also some of the main causative agents of infections in burn wounds (Bhattarai, 2021). Methicillin resistant *Staphylococcus aureus* (MRSA) has recently been isolated from burn wounds with increasing resistance to vancomycin (Adhikari *et al.*, 2023).

Burn wound infections are treated in several ways. The bacterial and nutritive burden is reduced by surgical debridement and assiduous cleaning methods. Aggressive grafting prevents bacteria from colonizing and infecting wound surfaces. Topical antimicrobial agents minimize pathogen load in the wounds and surrounding regions, allowing skin grafts to proliferate and cover the wounds. Systemic antimicrobials are used to treat invasive infections and stop or slow the systemic spread of infections (Cambiaso-Daniel *et al.*, 2018).

Emerging antibiotic resistance trends in burn wound bacterial infections pose a serious therapeutic challenge for burn care practitioners (Gupta *et al.*, 2019). Keeping in mind that infective agents and their sensitivity to antibiotics might change over time, it is preferable to conduct frequent evaluations of the bacterial flora of the burn wounds (Saha *et al.*, 2020). Uncontrolled and rapidly spreading anti-microbial resistance among bacterial populations has posed the management and treatment of burn wound infections a serious challenge in both clinical and surgical practice (Prasathkumar & Sadhasivam, 2021). Patients with post burn wound infections face additional exposure to microbial populations circulating in a hospital set up, as the hospital environment is always charged with microbial pathogens (Yadav *et al.*, 2021).

MATERIALS AND METHODS

Study Area

This study was conducted in Peshawar Burn Center, Hayatabad Medical Complex, Peshawar.

Sample Size

A total of 25 samples from patients with burn wound infections were collected using swabs.

Sample Collection and Transportation

The burn wounds were first cleaned with normal saline. Then samples were taken from the infected areas using swab and brought to the Microbiology Laboratory of Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology, Peshawar for further processing. Data on age, gender and type of burn were also collected from the patients' clinical folder.

Isolation and culturing of bacteria

On arrival at the laboratory, the wound swabs were immediately cultured onto nutrient, blood and MacConkey agar, then incubated at 37°C for 24 hours. After 24 hours, the colonial morphology *i-e.*, color, shape and general appearance of the individual colony on each of the plates were examined (Wali *et al.*, 2022).

Identification of the isolated bacteria

The isolated bacterial pathogens were identified on the basis of morphology, gram staining and several biochemical tests according to standard microbiological techniques.

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Morphological Identification

The isolated colonies were then sub cultured on MacConkey, blood and Mannitol Salt agar and incubated at 37°C for 24 hours. After incubation, the plates were examined for morphological characteristics (Kabanangi *et al.*, 2021).

Gram Staining

The isolates were further identified via gram staining. For accurate results, 24 hours fresh cultured plates were used. A single and isolated bacterial colony was picked with the help of a sterilized loop for preparing smear on a sterile glass slide. The slide was first heat fixed. The slide was then stained with the crystal violet dye followed by treating with gram's iodine solution. Rapid decolorization was performed with the help of ethanol or acetone. It was then followed by counterstaining with safranin (Kabanangi *et al.*, 2021).

Biochemical Tests

Several biochemical tests including indole, citrate, triple sugar iron (TSI), oxidase and urease test was performed to identify the bacterial species isolated.

Indole Test

The isolated bacteria were first inoculated in peptone water and then incubated for 24 hours at 37°C. The xylene was added to the peptone water and mixed thoroughly to remove indole. After this, Kovac reagent was added to the tubes. The formation of a pink colored ring on the surface of the tube showed positive result (Mohammed *et al.*, 2023).

Citrate Test

The isolated bacterial colonies were gently inoculated on simmon citrate agar slants using a sterilized wire loop and incubated at 37°C for 24 hours. After incubation, the slants were observed for a color change from green to blue which showed positive results (Inya *et al.*, 2022).

Triple Sugar Iron (TSI) Test

TSI medium slants were prepared in a test tube. A single bacterial colony was picked via a sterilized wire loop and then streaked across the whole surface of the slant and then butt. The tubes were incubated for 24 hours at 37 °C. After 24 hours of incubation, the tubes were examined (Stanley, 2022).

Oxidase Test

Oxidase reagent was prepared by mixing oxidase in distilled water. Filter paper was soaked in it. A single isolated bacterial colony from 24 hours fresh culture was picked with the help of a loop and rubbed over the moist filter paper. The appearance of dark purple or blue color within 10 seconds showed positive result (Eze *et al.*, 2021).

Catalase Test

Few drops of hydrogen peroxide were added to the slide. Overnight fresh bacterial colony was placed on a slide using a sterile loop. The rapid formation of bubbles caused by the breakdown of H_2O_2 showed positive result (Bhalla *et al.*, 2018).

DNase Test

DNase media was prepared and isolated bacterial colonies were cultured on the test area and incubated for 24 hours at 37°C. After 24 hours of incubation, the plates were rinsed with 1 N HCl. Clear zones surrounding the bacterial colonies indicate positive result (Arjyal & Neupane, 2020).

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Antibiotic Susceptibility Testing

The confirmed bacterial isolates were tested for antibiotic Susceptibility by Kirby-Bauer disc diffusion method against frequently used antibiotics belonging to various groups (CLSI, 2022). Inoculums for susceptibility testing was prepared by suspending three to five well isolated colonies from nutrient agar plate into an eppendorf tube containing normal saline. The turbidity of the inoculum was adjusted by comparing it with 0.5 McFarland standard. Bacterial lawn was prepared by dipping a sterile cotton swab into the eppendorf tube. Excess fluid was squeezed against the wall of the eppendorf tube. The swab was streaked onto the Muller Hinton agar plate and left for five minutes to dry. The antibiotics were then aseptically placed on MHA plate and incubated for 24 hours at 37°C. After incubation, the zones of inhibition were measured with the help of scale (Al-Ezzy *et al.*, 2021; Al-Ezzy *et al.*, 2021). The antibiotics used for susceptibility testing are listed in Table 3.1.

Table 1. List of various antibiotics selected from CLSI guidelines

S. No.	Antibiotics	Codes	Disc Potency
1.	Cefoxitin	FOX	30 µg
2.	Ciprofloxacin	CIP	5 µg
3.	Gentamicin	CN	30 µg
4.	Erythromycin	E	15 µg
5.	Clindamycin	CM	2 µg
6.	Linezolid	LZD	30 µg
7.	Vancomycin	VA	5 µg
8.	Fusidic acid	FD	10 µg
9.	Clarithromycin	CLR	15 µg
10.	Oxacillin	OX	5 µg
11.	Moxifloxacin	MXF	5 µg
12.	Ceftriaxone	CRO	30 µg
13.	Meropenem	MEM	10 µg
14.	Amikacin	AK	30 µg
15.	Ceftazidime	CAZ	30 µg
16.	Tigecycline	TGC	30 µg

RESULTS

Total culture-positive and culture-negative samples

Out of the total 25 samples, 20 (80 %) of the samples were culture positive and 5 (20 %) of the samples were culture negative as shown in Fig 1.

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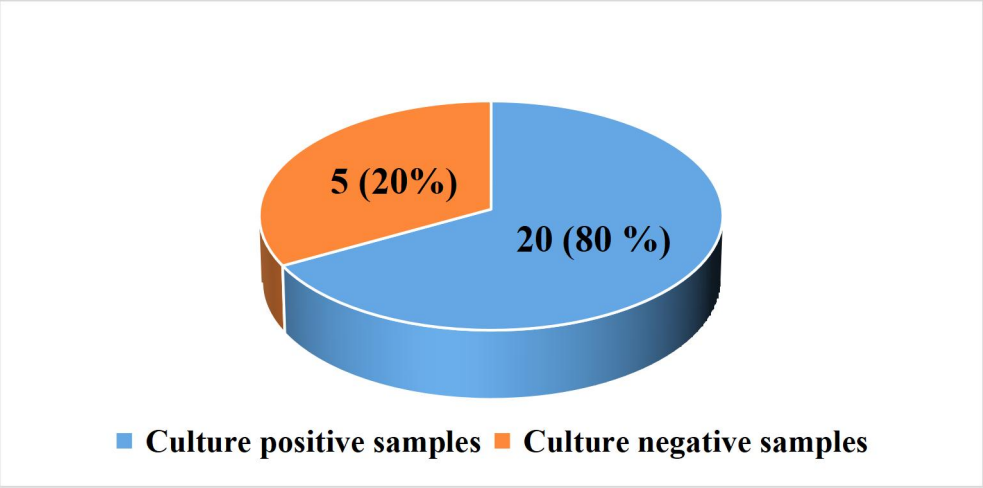

Fig. 1. Total culture-positive and culture-negative samples

Type of burn injuries in different burn patients

In about 25% individuals electricity was the main cause of burn, flame was responsible for 45% of individuals and 30% of patients had been burned by scalds as shown in (Table 2)

Table 2. Type of burn injury in recruited burn patients

Type of burn injury	Number of patients (%) n=20
Electricity	5 (25%)
Flame	9 (45%)
Scalds	6 (30%)

Morphological identification of bacteria from burn wound infection.

The collected samples when cultured on blood agar and macConkey agar formed various colonies as shown in (Table 3). *Staphylococcus spp* formed pale pink colonies on macConkey agar without crystal violet and golden like colonies on blood agar with zones of clear beta-hemolysis. *Pseudomonas spp* formed round, flat and colourless colonies on macConkey agar media and large colonies with undulated margins and beta hemolysis on blood agar. On the other hand *klebsiella spp* showed white grey colonies on blood agar with no hemolysis and pink-reddish mucoid colonies on macConkey agar media

Table 3. Identification of bacterial isolates from wounds of burnt patients

Predicted bacteria	Growth medium	Colonial characteristics	Microscopic characteristics
Staphylococcus spp	MacConkey agar	Flat Pale pink colonies	Gram positive
	Blood agar	Golden like colonies ,with zonesof clear beta-hemolysis	
Klebsiella spp	MacConkey agar	Pink-reddish mucoid colonies	Gram negative
	Blood agar	White grey colonies	

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Pseudomonas spp	MacConkey agar	Round,flat and colourless colonies	Gram negative
	Blood agar	Large colonies with undulated margins and beta - hemolysis	

4.4. Biochemical identification

Several biochemical tests performed for the identification of the isolated gram positive and gram negative bacteria with their results are listed in (Table 4). Several bacteria were identified based on their reactions to various biochemical assays. *Staphylococcus aureus*, a Gram-positive bacterium, was positive for DNase test. *Pseudomonas*, a Gram-negative bacterium, exhibits positive reactions for catalase, citrate and oxidase tests. While *Klebsiella* was positive for indole and H_2S production.

Table 4. Several biochemical tests with their results

Biochemical tests							
Predicted bacteria	**Catalase**	**Coagulase**	**DNase**	**Oxidase**	**Indole**	**TSI**	**Citrate**
Staphylococcus aureus	+ve	+ve	+ ve	N/A	N/A	N/A	+ve
Pseudomonas	+ ve	-ve	N/A	+ ve	-ve	N/A	+ ve
Klebsiella	+ve	N/A	N/A	N/A	-ve	Acid/ Acid H_2S – ve	+ ve

+ve: Positive, -ve: Negative, H_2S: Hydrogensulphide

Isolation rate of bacterial pathogens

Out of total 20 positive samples prevalence of *staphylococcus spp* was 55%, followed by *pseudomonas spp* (35%) and low prevalence was obsereved in case of *klebsiella spp* (10%) as shown in (Table 5)

Table 5. Prevalence of different bacterial pathogens isolated from burn wound infection

S. No.	**Bacterial isolates**	**No: of isolates**	**Isolation rate**
1	*Staphylococcus spp*	11	55%
2	*Pseudomonas spp*	7	35%
3	*Klebsiella spp*	2	10%
	Total	20	100%

Prevalence of bacterial isolates in male and female burnt individuals

Among 20 positive individuals (25%) were male and (75%) female. High prevalence of *staphylococcus* spp was obsereved in females (35%) and in males (20%) as show in (Table 6), followed by *pseudomonas* (30%) in females. No prevalence was obsereved in case of *klebsiella* in male individual. However, in females the isolation rate of *klebsiella spp* was only (10%).

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Table 6. Isolation rate of different bacterial pathogens from male and female individulas

Bacterial isolates	Male patients (%)	Female patients (%)
Staphylococcus spp	4(20%)	7(35%)
Pseudomonas spp	1(5%)	6(30%)
Klebsiella spp	0	2(10%)
Total	5 (25%)	15 (75%)

Distribution of bacterial infection in burned individuals of various age groups

High prevalence (40%)of bacterial infection was observed in burned patients of age group 21-30, followed by age group 31-40 (20%), age group 41-50 (15%), 5% in age group in 51-60 and no prevalence was observed in patients of age group 61-70 as shown in (Table 6)

Table 7 Age-wise distribution of burn patients with bacterial infection

Age Group (Years)	Total No. of positive cases	Frequency of infection
1-10	2	10%
11-20	2	10%
21-30	8	40%
31-40	4	20%
41-50	3	15%
51-60	1	5%
61-70	0	0%
Total %	20	100%

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing showed that the *S. aureus* was resistant to Cefoxitin (FOX), Ciprofloxacin (CIP), Gentamicin (CN), Chloramphenicol (C), Clindamycin (CM) and Oxacillin (OX). Conversely, it showed sensitivity to Linezolid (LZD) and Vancomycin (VA). *Pseudomonas* exhibited resistance to most of the tested antibiotics, with sensitivity limited to Gentamicin (CN) and Amikacin (AK). *Acinetobacter* showed resistance to multiple antibiotics, but showed sensitivity to Meropenem (MEM) and Amikacin (AK). *Klebsiella* and *Proteus* also showed resistance to multiple antibiotics.

Table 8. Antibiotic susceptibility testing of the isolated bacteria

S. No.	Antibiotic Symbol	Antibiotic Name	*S. aureus*	*Pseudomonas*	*Acinetobacter*	*Klebsiella*	*Proteus*
1.	FOX	Cefoxitin	R	N/A	N/A	N/A	N/A
2.	CIP	Ciprofloxacin	R	R	R	R	S
3.	CN	Gentamicin	R	S	N/A	S	N/A
4.	E	Erythromycin	R	R	R	R	R
5.	CM	Clindamycin	R	R	N/A	R	N/A
6.	LZD	Linezolid	S	N/A	N/A	N/A	N/A
7.	VA	Vancomycin	R	N/A	R	R	N/A
8.	FD	Fusidic acid	R	N/A	N/A	R	N/A
9.	CLR	Clarithromycin	R	N/A	N/A	R	N/A
10.	OX	Oxacillin	R	N/A	N/A	R	N/A
11.	MXF	Moxifloxacin	R	R	R	R	R

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12.	CRO	Ceftriaxone	R	R	R	R	S
13.	MEM	Meropenem	R	R	S	S	S
14.	AK	Amikacin	R	S	S	S	R
15.	CAZ	Ceftazidime	N/A	R	R	R	R
16.	TGC	Tigecycline	N/A	N/A	S	R	N/A

R: Resistant, N/A: Not Applicable, S: Sensitive

DISCUSSION

Burn injuries are among the leading causes of injury and death worldwide, with about 11 million seeking medical care and over 265,000 deaths annually. Over 95% of these occur in low and middle income countries (Bailey *et al.*, 2019). Infections of burn wounds are potentially serious complications that occurs immediately after an injury (Markiewicz-Gospodarek *et al.*, 2022). Patients with post burn wound infections face additional exposure to microbial populations circulating in a hospital set up, as the hospital environment is always charged with microbial pathogens (Yadav *et al.*, 2021). Therefore, this study was designed to evaluate the microbial profile and antibiotic susceptibility of the isolated pathogens with respect to the source of burn wounds.

In the present study, very high culture positivity 20 (80%) was found in the total samples collected from burn patients. It is similar to other studies (Zhou *et al.*, 2019; Dudoignon *et al.*, 2019). Incidence of burn was higher in females because females mostly spend their time in kitchen where usually accidents happen. Similar observation was made by Baba *et al.* (2019). Highest incidence in our study was flame burn. Similar observation was made by Toppi *et al.* (2019), while in the study by Greenhalgh. (2019) scald was the most common cause of burns. This may be due to socioeconomic reasons in our society (Khazaei *et al.*, 2019). Torrente *et al.* (2021) observed maximum number of patients between 20 and 30 years age. This finding was consistent with the results of our study in which age group mostly affected by burn injury was between 21 and 30 years.

In the current study,both gram positive and gram negative isolates were identified. This result was in consistent with the results of Tchakal-Mesbahni *et al.* (2021). Jauhari *et al.* (2020) also reported high percentage of gram negative bacterial isolates from burn wound infections. Our findings were also supported by Hubab *et al.* (2020).

In the current study, *S. aureus* and *P. aeruginosa* were the frequently isolated bacterial pathogens. This finding was supported by Chaudhary *et al.* (2019). Our findings were further supported by Hampden-Martin *et al.* (2021) and Mater *et al.* (2020). Tchakal-Mesbahni *et al.* (2021) also reported highest isolation rate of *S. aureus* from burn wound infections. Similar to our study, Raouf *et al.* (2021) reported *P. aeruginosa* from burn wound infections. Similarly, Torabi *et al.* (2021) isolated *A. baumannii* from burn wounds.

Antibiotic susceptibility testing of the gram positive bacterial isolates *i-e.*, *S. aureus* showed that all the *S. aureus* isolates were methicillin resistant *S. aureus*, while the antibiotic susceptibility testing of gram negative bacterial isolates showed that all the isolates were MDR. Similar to our study, Egro *et al.* (2022) reported MRSA from burn wounds. Our results were also supported by Junaid *et al.* (2019). A study conducted by Tasnim *et al.* (2020) and Mahmoudi *et al.* (2019) supported our findings.

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Similarly, Sharahi *et al.* (2020) reported MDR gram negative bacterial isolates from burn wound infections.

CONCLUSION

Findings of this study suggest high incidence of burn in females because females mostly spend their time in kitchen where accidents happen. Our data showed high antimicrobial resistance rate of bacterial isolates. This study further suggests that methicillin resistant *S. aureus* and multi-drug resistant *P. aeruginosa* and *Klebsiella* are the most common isolates from burn wounds. Hence a careful selection of antibiotics to treat burn wound infection is required for proper management of these wounds in order to help reduce morbidity and mortality associated with multi-resistant bacteria.

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