

## Original Article

# Microbial species isolation and antimicrobial resistance patterns of burn wounds infections in a tertiary care hospital

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**Abstract:** Background: Antimicrobial resistance is a focus of global interest in the treatment of wound infections. The growing antimicrobial resistance, particularly in burn wound infections, poses a serious challenge, highlighting the need to understand pathogen distribution and drug susceptibility patterns. Objectives: This study aimed to identify the pathogens responsible for burn wound infections and determine their drug susceptibility pattern. This study was conducted from September 2018 to January 2020 and included 163 patients. Result: A total of 164 microorganisms were isolated from 129 burn wounds, yielding an isolation rate of 78.65%. A solitary agent was isolated in 80 (62.02%), whereas a mixed infection was detected in 49 (37.98%) patients. The most common microorganisms detected were Gram-negative bacteria (102 isolates, 62.19%), with *P. aeruginosa* being the most prevalent (39.21%). Gram-positive bacteria were detected in 55 patients (33.53%), with *S. aureus* being the most common (69.06%), while *Candida* spp were found in 4.26% of patients. For Gram-positive microorganisms, the highest resistance was reported against ampicillin (100%), followed by tetracycline (74.27%) and Imipenem (72.72%), and the highest susceptibility was reported against vancomycin and linezolid (98.19%). Gram-negative isolates showed resistance in 83.48% to ampicillin, followed by 76.14% to ceftriaxone and 72.47% to chloramphenicol, and showed the least resistance against colistin (8.25%). Multiple microorganisms exhibited a multidrug resistance (MDR) profile (79.61%). The overall MDR rate of gram-negative and gram-positive isolates were 80.40% and 78.19%, respectively. Conclusion: The findings provide valuable insight into the prevalence of microorganisms in burn wounds and suggest that antibiotic resistance patterns can be useful in guiding the development of effective treatment strategies.

**Keywords:** Burn wound infections, microbes, antimicrobial resistance, MDR, Pakistan

## Introduction

The skin is made up of the epidermis, dermis, and hypodermis, serve as the body's natural defense system, establishing an effective barrier against external risks and microbial infiltration. When this barrier deteriorates, particularly in burn injuries, patients become severely exposed to bacterial infection and colonization and systemic immunosuppression [1]. Burn wound infections are a significant cause of

morbidity and mortality globally, especially in regions where multidrug-resistant (MDR) microorganisms are frequently present [2]. In several low to middle class countries including Pakistan, the widespread and frequently inadequate use of antimicrobial agents contributes to the emergence and dissemination of antimicrobial resistance (AMR) [3].

Pakistan is presently experiencing a significant rise in antimicrobial misuse, leading to growing

resistance among prevalent infectious agents that include *P. aeruginosa*, *E. coli*, *S. aureus*, *Acinetobacter* spp, and *K. pneumonia* [4]. To mitigate the spreading of AMR, it is necessary to identify infections strains, trace transmission pattern, also to develop antimicrobial resistance/susceptibility patterns of prevalent strains to guide timely effective infection control. The World Health Organization (WHO) recognizes AMR as a critical global health concern, arguing for improved antimicrobial stewardship, awareness, and surveillance to ensure antibiotic effectiveness and public safety [5]. MDR microbes can persist for prolonged periods and proliferate on minimal nutrients, posing a serious concern to global public health by colonizing damaged skin. Previous studies have highlighted the increasing incidence of antimicrobial resistance (AMR) strains in burn wounds [2, 6, 7].

A burn refers to a tissue injury caused by electrical, thermal, radioactive or chemical sources, as described by the WHO [8]. The origins of wounds can be acute, such as post-traumatic wounds (such as burns or accidents), postsurgical wounds (surgical site infections) or chronic wounds resulting from conditions such as diabetes mellitus (diabetic foot ulcer) [9]. Burn patients frequently experience nosocomial infections (NOS), which with other factors such as pathogen types colonizing the burn sites, and sepsis, contribute to substantial morbidity and mortality [10]. Bacterial infections that occur in burns are frequently related to continual hospitalizations and various healthcare and therapeutic interventions. WHO estimates that burn injuries contribute to about 180,000 fatalities each year, with more than 11 million patients necessitating medical intervention for burn-related injuries globally [5]. Most of these injuries are reported in low- and middle-income nations, particularly in regions identified by the WHO as the South East Asian and African regions, which collectively represent two-thirds of burn injuries [5, 11]. Infection is a predominant cause of mortality in serious burn cases, with systemic sepsis and following multiple organ failure responsible for 40-50% of burn-related deaths [12]. Furthermore, mortality rates from burn injuries are reduced in high-income countries with advances in injury therapy and the management of related complications [13].

The microbial flora across various burn settings differs due to spatial and geographical location, affected by pre-existing conditions, types of antimicrobials used, and the microbial biodiversity of the burn unit. Antimicrobial resistance in burned individuals complicates conditions and raises healthcare expenses, challenging treatment and posing a significant threat to patient life. Identifying and cataloging the various microorganisms that inhabit burn wounds and their antimicrobial resistance/susceptibility profile is crucial to improve the surveillance, prevention, and control of burn wound infections. This study aims to identify the diverse spectrum of microbial species in burn wounds and also to investigate their resistance profiles to different antimicrobial agents.

### Methodology

#### *Study site and samples collection*

This study was conducted at Mayo Hospital, Lahore (31.5204° N, 74.3587° E) using a cross-sectional design that was carried out from September 2018 to January 2020 to characterize the diversity of microorganisms and assess their antibiotic resistance profile. A total of 163 samples were collected aseptically from patients, comprising 112 males and 51 females, ages ranging from 7 to 58 years (median age, 32 years), admitted to the burn unit of Mayo Hospital, following informed written consent. The total burn surface area (TBSA) ranged from 1% to 68% (median TBSA, 13%). Samples were collected with sterilized swabs (Dynarex, USA) by hospital staff, properly labeled, representing the gender and age of patients. Collected samples were placed in the collection tube containing the non-nutritive semi-solid medium used for sample transport for microbiological analysis. The inclusion criteria were as follows: all patients exhibiting clinical indications of burn wound infections, including pus-filled discharge, edema, erythema, intense pain, or slow wound healing, were included in the study, irrespective of gender. Patients with either partial or full-thickness burns, who were hospitalized for a minimum of 48 hours, were expected to have adequate microbial colonization. The following categories were excluded: the patients who have undergone systemic or topical antibiotic therapy within the last 72 hours were excluded from participating.

Moreover, individuals with chronic diseases, including tuberculosis and diabetes, were also excluded from the study, and incomplete clinical data.

### *Laboratory procedures*

For identification of microorganisms, samples were inoculated on various types of media, including Luria Bertani agar, Nutrient agar, Mannitol Salt agar, MacConkey agar, blood agar, Salmonella-Shigella agar, and Sabouraud dextrose agar (Oxoid, UK) for 24-48 h at 37°C. Preliminary identification of the microbes was based on colony characteristics and microscopic examination. Purified bacterial colonies were obtained and further identified by Gram staining and standard biochemical assays using the Culti-Loops API 20E Rap ID system kit (Thermo Scientific, USA) as per the manufacturer's instructions. Moreover, *Candida* species were identified based on the *Candida* colony's medium characterization, and all isolates were verified by the API 20C AUX-strip (BioMérieux, France), according to the manufacturer's instructions. Colonies were stored at -86°C in stocks containing 2.5 M glycerol.

### *Antibiotic susceptibility profiling*

To perform an antimicrobial susceptibility test for the isolated bacterial and fungal strains, a standard disk diffusion test of the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) described by the Clinical and Laboratory Standards Institute (CLSI) was used [14]. Antibiotics disc (Oxoid, UK) with the following concentration: ceftriaxone (30 µg), streptomycin (30 µg), tetracycline (30 µg), gentamicin (10 µg), tigecycline (15 µg), chloramphenicol (30 µg), Imipenem (10 µg), ampicillin (20 µg), colistin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), linezolid (30 µg), and vancomycin (30 µg) were used to evaluate the susceptibility/resistance pattern of bacterial isolates. The bacterial strains isolated from burn wound infections were also tested for MDR. The following antifungal discs (Oxoid, UK), namely Nystatin (100 units), itraconazole (30 µg), Amphotericin B (50 µg), 5-Fluorocytosine (30 µg), and Fluconazole (25 µg) were used to assess the susceptibility/resistance pattern of fungal isolates. Briefly, antibiotic discs were placed on the plate surfaces with a minimum distance of 15 mm from the edges

to avoid overlapping inhibition zones. All samples were inoculated in the suitable culture medium and incubated at 37°C for 18 to 20 hours, as per their respective requirements. Subsequently, the zones of inhibition were measure following CLSI guidelines using *E. coli* ATCC25922, *S. aureus* ATCC25923 and *P. aeruginosa* ATCC27853 as internal quality control organisms.

### *Statistical analysis*

IBM SPSS software v23.0 (Armonk, New York, USA: IBM corporation) was used for data analysis. The data was primarily entered and interpreted using Microsoft Excel 2019 (USA, Microsoft), and descriptive statistics were used to evaluate the data. Categorical variables such as microbial isolates and antibiotic resistance patterns, were presented as frequencies (n) and percentages (%).

## Results

### *Wound samples and the patient's demographic characteristics*

**Table 1** describes the clinical and demographic characteristics across the 163 burn wound patients. Among these, 129 (79.14%) samples yielded microbial growth, and 34 (20.86%) were observed to be negative for microbial isolation. Male patients represented the majority of cases (73.68%) and revealed a higher rate of infection (97/112, 86.60%) than that of female patients (32/51, 62.75%). Age-wise, the highest rate of wound infections was detected in the 21-30-year cohort (31.90%), followed by the 10-20-year group (28.83%), and the lowest was observed in only 4.91% of patients being >50 years old. Across all age ranges, scald burns were more frequently observed compared to open fire burns and were correlated with elevated rates of infection, especially among younger adults. In terms of burn severity, the majority of infected individuals have a TBSA involvement of 20-29% (89/163, 54.60%), followed by 1-9% (36/163, 22.08%), 10-19% (21/163, 12.89%), and >30% (17/121, 10.43%).

### *Distribution of burn wound infections*

A total of 164 microorganisms were identified from 129 clinical samples found positive from a total of 163 burn patients over a five-month

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**Table 1.** Patient's demographics

| Demographic Characters | Infected No. (%) | Uninfected (%) | Total No. (%) |
|------------------------|------------------|----------------|---------------|
| Gender                 |                  |                |               |
| Male                   | 97 (86.60)       | 15 (13.40)     | 112 (73.68)   |
| Female                 | 32 (62.75)       | 19 (37.25)     | 51 (26.32)    |
| Total                  | 129 (79.14)      | 34 (20.86)     | 163 (100.00)  |
| Age in years           |                  |                |               |
| ≤10                    | 15 (11.62)       | 6 (17.64)      | 21 (12.88)    |
| Open fire burns        | 3 (42.85)        | 4 (47.15)      | 7 (33.33)     |
| Scald burns            | 11 (84.62)       | 2 (15.38)      | 13 (66.67)    |
| 10-20                  | 39 (30.23)       | 8 (23.52)      | 47 (28.83)    |
| Open fire burns        | 4 (44.44)        | 5 (55.56)      | 9 (19.15)     |
| Scald burns            | 35 (92.10)       | 3 (17.90)      | 38 (80.85)    |
| 21-30                  | 45 (34.88)       | 7 (20.58)      | 52 (31.90)    |
| Open fire burns        | 7 (58.33)        | 5 (41.67)      | 12 (23.08)    |
| Scald burns            | 38 (95.20)       | 2 (4.80)       | 40 (76.92)    |
| 31-40                  | 12 (9.30)        | 10 (29.41)     | 22 (9.02)     |
| Open fire burns        | 4 (50.00)        | 4 (50.00)      | 8 (36.36)     |
| Scald burns            | 8 (57.14)        | 6 (42.86)      | 14 (63.64)    |
| 41-50                  | 11 (8.52)        | 2 (5.88)       | 13 (13.49)    |
| Open fire burns        | 4 (80.00)        | 1 (20.00)      | 5 (38.46)     |
| Scald burns            | 7 (87.50)        | 1 (12.50)      | 8 (61.54)     |
| >50                    | 7 (5.42)         | 1 (3.44)       | 8 (4.91)      |
| Open fire burns        | 3 (75.00)        | 1 (25.00)      | 4 (50.00)     |
| Scald burns            | 4 (100.00)       | 0 (0.00)       | 4 (50.00)     |
| Total                  | 129 (79.14)      | 34 (20.86)     | 163 (100.00)  |
| TBSA (%)               |                  |                |               |
| 1-9                    |                  |                | 36 (22.08)    |
| 10-19                  |                  |                | 21 (12.89)    |
| 20-29                  |                  |                | 89 (54.60)    |
| >30                    |                  |                | 17 (10.43)    |

TBSA, Total burn surface area.

period. The total detection rate was 79.14%. Gram-negative bacteria (102 isolates, 62.19%) were the most prevalent, Gram-positive bacteria were detected in 55 of patients (33.53%), while 4.26% were *Candida* spp (Table 2). The occurrence of only one species infection was the most common case and was found in 80 samples, accounting for 62.02% of burn wound infections, and mixed infection by different species was found in 49 samples, accounting for 37.98% (Table 2). The microorganisms more commonly detected in co-infection were *S. aureus* and *P. aeruginosa* (39.29%), followed by *S. aureus* and *K. pneumoniae* (21.43%), *P. aeruginosa* and *K. pneumoniae* (10.71%), and *P. aeruginosa* and *P. mirabilis* (7.14%). Only one triple co-infection involving *S. aureus*, *P. aerugi-*

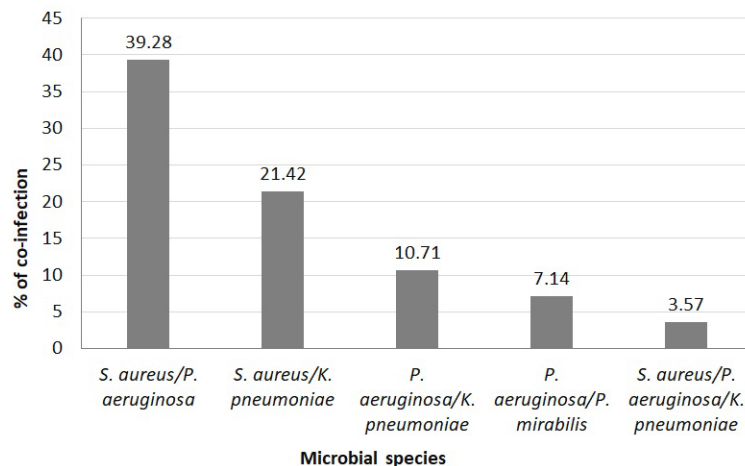
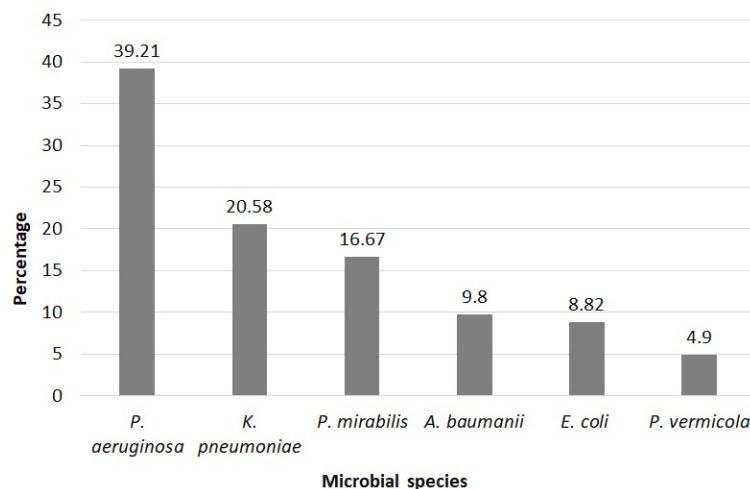
*nosa*, and *K. pneumoniae* (3.57%) was detected (Figure 1).

Among the gram-negative bacteria, *P. aeruginosa* was the most frequently isolated bacterial specie (39.21%,  $n = 40$ ), followed by *K. pneumoniae* (20.58%,  $n = 21$ ), *P. mirabilis* (16.67%,  $n = 17$ ) and *A. baumannii* (9.80%,  $n = 10$ ), *Escherichia coli* (8.82%,  $n = 09$ ) and *P. vermicola* (4.90%,  $n = 5$ ) colonizing burn wounds (Figure 2).

Among the gram-positive bacterial species, *S. aureus* (69.06%,  $n = 38$ ) was the most frequently detected microorganism, followed by *S. haemolyticus* (18.18%,  $n = 10$ ) and *S. epidermidis* (12.72%,  $n = 7$ ) (Figure 3).

**Table 2.** Microbial distribution among studied burn samples

| Characteristics                                               | Pathogens              | Isolates/<br>samples | Percentage<br>(%) |
|---------------------------------------------------------------|------------------------|----------------------|-------------------|
| Total no. of microbial strains identified $n = 164$           | Gram-positive bacteria | 55                   | 33.53             |
|                                                               | Gram-negative bacteria | 102                  | 62.19             |
|                                                               | Fungi                  | 07                   | 4.26              |
| Total no. of burn samples yielded microbial growth, $n = 129$ | Co-infection           | 49                   | 37.98             |
|                                                               | Monomicrobial          | 80                   | 62.02             |

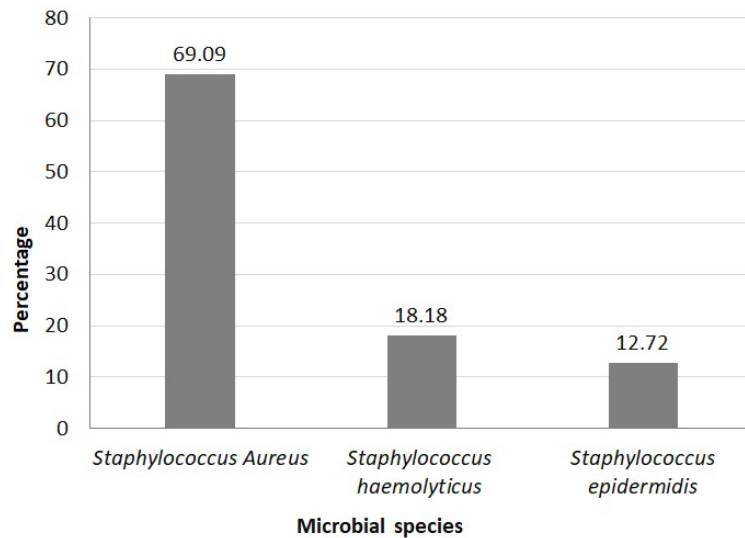

**Figure 1.** The percentage of co-infections found in burn wound samples comprising various microbial species.

**Figure 2.** Prevalence of gram-negative bacteria isolated from burn wounds.

Fungal infection was observed in a total of seven ( $n = 7$ ) burn wound samples, the most commonly detected fungi were *C. albicans* (57.14%,  $n = 4$ ), followed by *C. tropicalis* (28.57%,  $n = 2$ ) and *C. parapsilosis* (14.28%,  $n = 1$ ) (Figure 4).

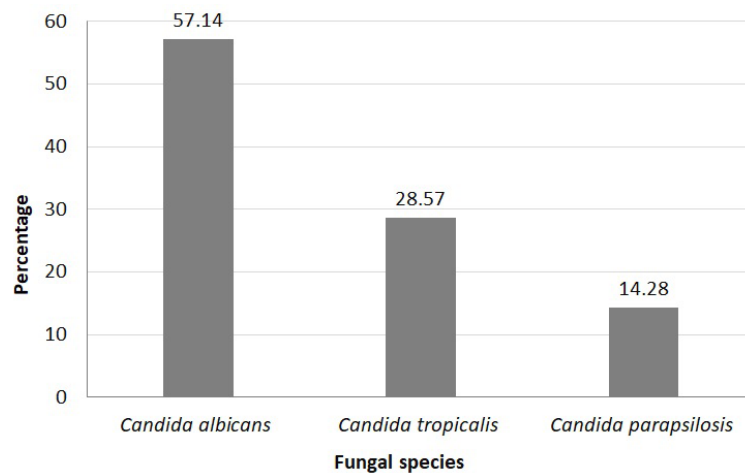
#### Antibiotic-resistant pattern of Gram-negative and Gram-positive bacteria

Gram-negative and gram-positive bacterial isolates were tested against a total of 14 commonly used antibiotics. The results showed that the microbes varied in their sensitivity to all antimicrobial agents used in this study. The antibiotic resistance patterns of the gram-negative and gram-positive isolates to commonly used antibiotics are shown in Tables 3 and 4, respectively. Overall, the gram-negative isolates obtained were found to be resistant to ampicillin (83.48%) while sensitive to colistin (91.75%). The predominant gram-negative isolate, *P. aeruginosa*, showed a high level of resistance to ceftriaxone and levofloxacin (82.5%), ampicillin and chloramphenicol (80.0%), and was found to be most susceptible to colistin (95.0%), ciprofloxacin, and streptomycin (77.5%). The second, predominantly gram-negative isolate, *K. pneumoniae*, showed high resistance to ampicillin (89.28%) and chloramphenicol (85.7%). It was highly sensitive to colistin (92.86%) and levofloxacin (71.43%) among the antibiotics used in this study. *P. mirabilis* also exhibited high resistance to ampicillin (82.3%), ceftriaxone, streptomycin, and tigecycline (70.6%). The resistance of *A. baumannii* determined in this study ranged from 30% (ciprofloxacin, levofloxacin, colistin, and streptomycin).

## Antimicrobial susceptibility profiles of microbial species



**Figure 3.** Prevalence of gram-positive bacteria isolated from burn wounds.



**Figure 4.** Prevalence of fungal species isolated from burn wounds.

cin) to 90% (chloramphenicol). *P. vermicola* isolates were susceptible to colistin (80%) and resistant to Imipenem, ampicillin, and ceftriaxone (80%). *E. coli* was also highly resistant to ampicillin (88.88%) and sensitive to colistin, amikacin, and Imipenem (22.22%) (**Table 3**).

The resistance rates of the gram-positive isolates *S. aureus*, *S. haemolyticus*, and *S. epidermidis* against ampicillin were 100% (**Table 4**). While the resistance pattern of these isolates to tetracycline and Imipenem was >70%. *S. aureus* and *S. haemolyticus* were 100% susceptible to vancomycin and linezolid, and *S. haemolyticus* was also 100% susceptible to Amikacin. Moreover, both strains were found to

be ≥80% sensitive to gentamicin, amikacin, and ciprofloxacin. *S. epidermidis* was 85.72% susceptible against multiple antibiotics such as vancomycin, linezolid, chloramphenicol, and gentamicin.

### Resistance pattern of fungi to antimicrobials

The resistance rates of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* to nystatin (NYC), and amphotericin B (AMB) were 0%. The resistance rates of *C. albicans*, *C. tropicalis* and *C. parapsilosis* to Itraconazole (ITC), 5-Fluorocytosine (5-FC), and Fluconazole (FLC) were 50% to 100% (**Table 5**). However, a relatively small sample size may not fully capture the exact patterns of antibiotic resistance and susceptibility profiles.

### Distribution of Multidrug-resistant isolates

MDR was observed in 125/157 (79.62%) of the bacterial isolates obtained in this study. A total of 82/102 (80.40%) of the gram-negative bacterial isolates showed MDR against 2 to 12 antimicrobial agents, and 43/55 (78.19%) of the gram-positive bacterial iso-

lates showed MDR against 2 to 12 antibiotics. The antibiotic susceptibility pattern assessed revealed that 20.38% of microbial strains exhibited a resistance against a single antimicrobial agent, 25.47% revealed resistance toward 2 antibiotics, 6.36% showed resistance against 3 antimicrobial agents, 5.73% revealed resistance to 4 and 10.82% showed resistance to 5 antimicrobial agents and more interestingly, 31.21% exhibited a resistance pattern against at least 6 antimicrobial agents. Among multi-resistant microorganisms, *S. aureus* is the one that most frequently presents a number of resistances equal to or greater than 6 (28.57%), followed by *P. aeruginosa* (20.41%), *P. mirabilis* (20.41%), *K. pneumonia* (14.28%),

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**Table 3.** AMR pattern of Gram (-) bacteria cultured from burn wounds

| S.N. | Antibiotics     | <i>P. aeruginosa</i><br>(n = 40) | <i>K. pneumoniae</i><br>(n = 21) | <i>P. mirabilis</i><br>(n = 17) | <i>A. baumannii</i><br>(n = 10) | <i>P. vermicola</i><br>(n = 5) | <i>E. coli</i><br>(n = 9) | Total<br>(n = 102) |
|------|-----------------|----------------------------------|----------------------------------|---------------------------------|---------------------------------|--------------------------------|---------------------------|--------------------|
| 1    | Ceftriaxone     | 33 (82.50%)                      | 20 (71.42%)                      | 12 (70.60%)                     | 7 (70.00%)                      | 4 (80.00%)                     | 7 (77.77%)                | 83 (76.14%)        |
| 2    | Streptomycin    | 9 (22.50%)                       | 9 (32.14%)                       | 12 (70.60%)                     | 3 (30.00%)                      | 2 (40.00%)                     | 3 (33.33%)                | 37 (33.94%)        |
| 3    | Tetracycline    | 13 (32.50%)                      | 10 (35.71%)                      | 7 (41.17%)                      | 4 (40.00%)                      | 2 (40.00%)                     | 5 (55.55%)                | 41 (37.61%)        |
| 4    | Gentamicin      | 13 (32.50%)                      | 9 (32.14%)                       | 6 (35.29%)                      | 5 (50.00%)                      | 4 (80.00%)                     | 4 (44.44%)                | 41 (37.61%)        |
| 5    | Tigecycline     | 18 (45.00%)                      | 12 (42.90%)                      | 12 (70.60%)                     | 4 (40.00%)                      | 2 (40.00%)                     | 4 (44.44%)                | 52 (47.70%)        |
| 6    | Chloramphenicol | 32 (80.00%)                      | 24 (85.70%)                      | 8 (47.05%)                      | 9 (90.00%)                      | 3 (60.00%)                     | 3 (33.33%)                | 79 (72.47%)        |
| 7    | Imipenem        | 18 (45.00%)                      | 9 (32.14%)                       | 6 (35.29%)                      | 4 (40.00%)                      | 4 (80.00%)                     | 2 (22.22%)                | 43 (39.44%)        |
| 8    | Ampicillin      | 32 (80.00%)                      | 25 (89.28%)                      | 14 (82.30%)                     | 8 (80.00%)                      | 4 (80.00%)                     | 8 (88.88%)                | 91 (83.48%)        |
| 9    | Colistin        | 2 (5.00%)                        | 2 (7.14%)                        | 1 (5.88%)                       | 1 (10.00%)                      | 1 (20.00%)                     | 2 (22.22%)                | 9 (8.25%)          |
| 10   | Amikacin        | 13 (32.50%)                      | 10 (35.71%)                      | 6 (35.29%)                      | 4 (40.00%)                      | 2 (40.00%)                     | 2 (22.22%)                | 37 (33.94%)        |
| 11   | Ciprofloxacin   | 9 (22.50%)                       | 11 (39.28%)                      | 6 (35.29%)                      | 3 (30.00%)                      | 3 (60.00%)                     | 5 (55.55%)                | 37 (33.94%)        |
| 12   | Levofloxacin    | 33 (82.50%)                      | 8 (28.57%)                       | 6 (35.29%)                      | 3 (30.00%)                      | 2 (40.00%)                     | 5 (55.55%)                | 57 (52.29%)        |

AMR, Antimicrobial resistance.

**Table 4.** AMR pattern of Gram (+) bacteria cultured from burn wounds

| S.N. | Antibiotics     | <i>S. aureus</i><br>(n = 38) | <i>S. haemolyticus</i><br>(n = 10) | <i>S. epidermidis</i><br>(n = 7) | Total<br>(n = 55) |
|------|-----------------|------------------------------|------------------------------------|----------------------------------|-------------------|
| 1    | Ceftriaxone     | 10 (26.31%)                  | 4 (40.00%)                         | 4 (57.14%)                       | 18 (32.72%)       |
| 2    | Streptomycin    | 5 (13.15%)                   | 4 (40.00%)                         | 4 (57.14%)                       | 13 (23.63%)       |
| 3    | Tetracycline    | 29 (76.31%)                  | 7 (70.00%)                         | 5 (71.42%)                       | 41 (74.27%)       |
| 4    | Gentamicin      | 5 (13.15%)                   | 2 (20.00%)                         | 1 (14.28%)                       | 8 (14.54%)        |
| 5    | Tigecycline     | 12 (31.57%)                  | 5 (50.00%)                         | 2 (28.57%)                       | 19 (34.54%)       |
| 6    | Chloramphenicol | 12 (31.57%)                  | 3 (30.00%)                         | 1 (14.28%)                       | 16 (29.09%)       |
| 7    | Imipenem        | 27 (71.05%)                  | 8 (80.00%)                         | 5 (71.42%)                       | 40 (72.72%)       |
| 8    | Ampicillin      | 38 (100.00%)                 | 10 (100.00%)                       | 7 (100.00%)                      | 55 (100.00%)      |
| 9    | Linezolid       | 0 (0.00%)                    | 0 (0.00%)                          | 1 (14.28%)                       | 1 (1.81%)         |
| 10   | Amikacin        | 3 (7.89%)                    | 0 (0.00%)                          | 2 (28.57%)                       | 5 (9.09%)         |
| 11   | Ciprofloxacin   | 5 (13.15%)                   | 2 (20.00%)                         | 2 (28.57%)                       | 9 (16.36%)        |
| 12   | Vancomycin      | 0 (0.00%)                    | 0 (0.00%)                          | 1 (14.28%)                       | 1 (1.81%)         |

**Table 5.** Resistance rates of fungi isolated in this study to antifungals

| S.N. | Antifungal       | <i>C. albicans</i><br>(n = 4) | <i>C. tropicalis</i><br>(n = 2) | <i>C. parapsilosis</i><br>(n = 1) | Total<br>(n = 7) |
|------|------------------|-------------------------------|---------------------------------|-----------------------------------|------------------|
| 1    | Amphotericin B   | 0                             | 0                               | 0                                 | 0                |
| 2    | 5-Fluorocytosine | 3 (75.00%)                    | 1 (50.00%)                      | 1 (100.00%)                       | 0                |
| 3    | Itraconazole     | 3 (75.00%)                    | 1 (50.00%)                      | 1 (100.00%)                       | 5 (71.42%)       |
| 4    | Fluconazole      | 2 (50.00%)                    | 1 (50.00%)                      | 1 (100.00%)                       | 4 (57.14%)       |
| 5    | Nystatin         | 0                             | 0                               | 0                                 | 0                |

*E. coli* (10.20%), and *A. baumannii* (6.12%) (Table 6). *S. aureus* showed a resistance to 12 antimicrobial agents, whereas *P. mirabilis* exhibited a resistance to 11 antimicrobials.

### Discussion

Microbial infections are the most common and serious complication of burn injury and a

leading cause of morbidity and mortality. Understanding the prevalence and resistance profile of bacterial isolates causing burn wound infections is critical for effective treatment and antibiotic administration. In this study, *P. aeruginosa* (36.69%) and *S. aureus* (69.09%) were the predominant pathogens among the gram-negative and gram-positive bacteria isolated from the burn wounds, respectively. This is con-

## Antimicrobial susceptibility profiles of microbial species

**Table 6.** Multidrug resistance of gram-negative and gram-positive bacteria isolated from burn wounds

| No. of resistance               |            |            |           |           |            |            |           |           |           |           |           |            |
|---------------------------------|------------|------------|-----------|-----------|------------|------------|-----------|-----------|-----------|-----------|-----------|------------|
| Bacteria                        | R1 (%)     | R2 (%)     | R3 (%)    | R4 (%)    | R5 (%)     | R6 (%)     | R7 (%)    | R8 (%)    | R9 (%)    | R10 (%)   | R11 (%)   | R12 (%)    |
| <i>P. aeruginosa</i> (n = 40)   | 12 (37.50) | 7 (17.50)  | 2 (20.0)  | 3 (33.33) | 6 (35.29)  | 3 (15.78)  | 5 (35.71) | 1 (20.00) | 1 (33.33) | -         | -         | -          |
| <i>K. pneumonia</i> (n = 21)    | 3 (9.38)   | 6 (15.00)  | 1 (10.00) | 3 (33.33) | 1 (5.88)   | 5 (25.31)  | 1 (7.14)  | 1 (20.00) | -         | -         | -         | -          |
| <i>P. mirabilis</i> (n = 17)    | 2 (6.25)   | 2 (5.00)   | 1 (10.00) | 1 (11.11) | 1 (5.88)   | 3 (15.78)  | 2 (14.28) | 1 (20.00) | 1 (33.33) | 1 (25.00) | 2 (66.67) | -          |
| <i>A. baumannii</i> (n = 10)    | 2 (6.25)   | 4 (10.00)  | 1 (10.00) | -         | -          | 2 (10.52)  | 1 (7.14)  | -         | -         | -         | -         | -          |
| <i>E. coli</i> (n = 9)          | 1 (3.12)   | 1 (2.50)   | 1 (10.00) | 1 (11.11) | -          | 2 (10.52)  | 2 (14.28) | -         | -         | 1 (25.00) | -         | -          |
| <i>P. vermicola</i> (n = 5)     | -          | 2 (5.00)   | 1 (10.00) | -         | 2 (11.76)  | -          | -         | -         | -         | -         | -         | -          |
| <i>S. aureus</i> (n = 38)       | 8 (25.00)  | 12 (30.00) | 2 (20.00) | 1 (11.11) | 1 (5.88)   | 4 (21.05)  | 3 (21.42) | 2 (40.00) | 1 (33.33) | 2 (50.00) | 1 (33.33) | 1 (100.00) |
| <i>S. Haemolyticus</i> (n = 10) | 3 (9.38)   | 4 (10.00)  | -         | -         | 3 (17.64)  | -          | -         | -         | -         | -         | -         | -          |
| <i>S. epidermidis</i> (n = 7)   | 1 (3.12)   | 2 (5.00)   | 1 (10.00) | -         | 3 (17.64)  | -          | -         | -         | -         | -         | -         | -          |
|                                 | 32 (20.38) | 40 (25.47) | 10 (6.36) | 9 (5.73)  | 17 (10.82) | 19 (12.10) | 14 (8.91) | 5 (3.18)  | 3 (1.91)  | 4 (2.55)  | 3 (1.91)  | 1 (0.64)   |

R1-R12, resistant to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 classes of antimicrobials tested.

sistent with previous studies highlighting the dominance of these microbial pathogens in colonizing burn wounds [6, 15-19]. Vaez and Beigi [6] reported that *P. aeruginosa* (48.7%) and *S. aureus* (21.8%) were the most common pathogens isolated from burn wound infections. Another study conducted in Iran found that *P. aeruginosa* (57.3%) was the most common microorganism responsible for burn wound infections [15]. Tasnim et al. [20] also found that *P. aeruginosa* (57.3%) was the pathogen most commonly isolated for burn wound infections. In fact, it appears that the bacterial isolates *P. aeruginosa* and *S. aureus* produce various virulence factors, exhibit innate resistance to various drugs, and are known to be the leading cause of wound infections in hospital patients. *P. aeruginosa* is commonly found in hospital settings [21], which can often lead to the isolation of most organisms from burn wounds.

In the present study, a 78.65% of burn wounds had microbial growth. This proportion is higher than other epidemiological studies from Saudi Arabia (65.74%), China (49.2% and 49.3), and Iran (31%), and is less than previously reported from India (97.1%) in burn wound infections [6, 22-25]. The difference in prevalence may be due to different infection control strategies and general hygiene in the clinical settings. In this study, male patients were more likely to develop burn wound infections (86.60%) than females (62.75%), which is consistent with the previous studies [19, 22, 23].

Our findings showed that gram-negative bacteria dominated with a proportion of 62.19%, while gram-positive bacteria accounted for 33.53%. This prevalence is consistent with the findings reported by previous studies [6, 7, 25-27]. Related findings demonstrated that the gram-positive microorganisms are usually isolated in the early stages of hospitalization, while during longer hospitalizations due to nosocomial infection, gram-negative microorganisms predominate.

The prevalence of mono-microbial growth was the most frequent case (62.02%), while mixed bacterial species were detected in 37.98% of samples. Our findings were consistent with previous studies in which burn wound infections were mostly caused by a single microorganism [6, 26, 28]. Similarly, mono-microbial infections

were identified to be more prevalent than polymicrobial infections previously reported by Bessa et al. [9] and Ares et al. [29], with rates of 72.80% and 88.60%, respectively. However, our findings are different from the findings of Ahmed et al. [2], who reported a higher prevalence of polymicrobial species at 55% and 45% of monomicrobial infection. The microorganisms more commonly detected in mixed bacterial infections were *S. aureus* and *P. aeruginosa* (39.29%), in accordance with previously reported findings in wound infections [9, 26, 30]. Moreover, *P. aeruginosa* has been detected associated with *K. pneumoniae* (10.71%) and *P. mirabilis* (7.14%). Similarly, *S. aureus* has also been detected associated with *K. pneumoniae* (21.43%). A mixed infection of three distinct bacterial species, such as *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*, has been detected in 3.57% of patients, in accordance with Puca et al. [26] who reported 3.4% of mixed bacterial infections of *S. aureus*, *K. pneumoniae*, and *S. marcescens*. The occurrence of mixed bacterial infections makes microbial eradication more difficult. Moreover, chronic wounds with mixed microbial infection are likely to result in an environment that is favorable to horizontal gene transfer among microbial species.

Fungi represent the second most prevalent microbial agents in burn wound infections, which may appear as mono- or polymicrobial and opportunistic infections. These infections are frequently misinterpreted due to their similarities in characteristics with bacterial infections, further complicated by the absence of adequate mycology laboratories. Globally, 6.3% to 44% of all reported fungal infections have been documented in various burn centers [31]. In this study, we identified various species of *Candida* only, and *C. albicans* was the most predominant fungal species in burn wounds (57.14%), followed by *C. tropicalis* (28.57%) and *C. parapsilosis* (14.28%). Similarly, Macedo et al. [32] reported that *C. albicans* is the prevalent stain responsible for nosocomial fungal infections in burn wounds, with a fatality rate ranging from 38-50% due to persistent infections. In a case study involving 220 burn patients, 42% of the pathogens associated with burn wound infections were detected as *Candida* spp [33]. Chronic *Candida* infections are one of the leading causes of the majority of the complications and mortality among burn

patients [33]. The fungal colonization occurs more frequently after the 3<sup>rd</sup> and 4<sup>th</sup> weeks post-burn.

*Candida* infections possess various antifungal susceptibility patterns, thus exact identification of the specific *Candida* species and susceptibility testing for antifungal agents are essential. Our study showed that AMB and NYC showed effective overall potency with 100% of the *Candida* isolates exhibited susceptibility. These findings align with those of Maheronnaghsh et al. [34], who reported a susceptibility rate of 98.65% for NYC, and Ghoghghi et al. [35], who reported 99% susceptibility for AMB. Furthermore, previous studies by Khedri et al. [36] and Badiie et al. [37] found that all tested isolates were susceptible to AMB.

Burn wound infections represent a significant healthcare challenge due to their tendency for colonization and subsequent infection by MDR pathogens. The risk of MDR pathogens in burn patients increases with prolonged hospitalizations, more invasive procedures, and the long-term combined use of strong antibiotics [38]. The emergence of MDR strains among these pathogens complicates treatment plans and increases morbidity and mortality rates associated with burn injuries [39]. Gram-negative bacteria such as *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae*, as well as Gram-positive bacteria such as *S. aureus* species, are often involved in burn wound infections [2, 23, 40]. These bacteria show resistance to different classes of antibiotics, which limits treatment options and requires a more detailed examination of alternative therapeutic approaches. The emergence of MDR strains is attributed to several factors, such as the widespread use of antibiotics, prolonged wound exposure, burn severity, duration of hospitalization, ineffective infection control measures, and the genetic plasticity of bacteria, which favors the acquisition and spread of resistance genes. In the present study, the MDR profile of bacterial strains obtained was 79.62%, the MDR pattern was found to be higher in gram-negative bacterial strains (80.40%) compared to gram-positive bacterial strains (78.19%), and these findings are consistent with previously reported rates in wound infections [41]. Another study by Kabanangi et al. [42] reported 80.1% of bacterial strains obtained from burn wound infections in pediatric patients were MDR, with a

notable predominance of gram-negative strains such as *P. aeruginosa*, *Acinetobacter* spp, and *Klebsiella* spp.

This study identified 31.21% of microbial isolates exhibited resistance to at least six different antibiotics, particularly *S. aureus*, which is the one that most frequently detected strain, followed by *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *E. coli*, and *A. baumannii*. These microorganisms are characterized by genotypic multidrug resistance, and their ability to form polymicrobial biofilms complicates therapeutic approaches. The occurrence of multidrug-resistant bacterial strains, variability in biofilm composition, tolerance to antimicrobial agents, and the potential polymicrobial form of biofilms highlight the necessity for multi-target or combinational treatment strategies [7]. Among the MDR bacterial isolates, we identified some even resistant to 12 different antibiotics, such as *S. aureus*, which showed resistance to 12 antimicrobial agents, whereas *P. mirabilis* exhibited resistance to 11 antimicrobials.

Overall, the gram-negative bacterial strains showed the highest resistance to ampicillin (83.48%), while the most effective antimicrobial agent was colistin, with a susceptibility rate of 91.75%. The most prevalent gram-negative bacterial strain, *P. aeruginosa*, showed a high resistance rate against ceftriaxone and levofloxacin (82.5%), followed by 80.0% against ampicillin and chloramphenicol. Similarly, Ahmed et al. [2] found that *P. aeruginosa* was highly resistant to levofloxacin (83.3%). We found that the *P. aeruginosa* showed the lowest resistance to ciprofloxacin (22.5%), quite comparable to Ahmed et al. [2] reported resistant rate of 16.7%, Mama et al. [23] found a 100% susceptible rate, whereas other previous studies demonstrated 6.2-24% [43-45]. Surprisingly, Nikokar et al. [46] showed that *P. aeruginosa* was resistant to ciprofloxacin by 66.3%. Previous studies have shown that amikacin and imipenem were effective against all gram-negative species except *A. baumannii* and *P. aeruginosa*, respectively [26]. We found that *A. baumannii* and *P. aeruginosa* strains were 40% and 45% resistant to imipenem, and were also 40% and 32.5%, respectively, resistant to amikacin. On the contrary, Puca et al. [26] found that *A. baumannii* was resistant to amikacin in 70.6% of cases, while Guan et al. [7] found that it was resistant in 13.3%. Puca et al. [26] also

showed that *A. baumannii* was 100% susceptible to imipenem, instead Li et al. [25] found an 83.3% resistance rate.

The gram-positive microbes isolated in the present study showed 100% resistance to ampicillin, reflecting the global prevalence of  $\beta$ -lactam resistance in staphylococcal strains associated with  $\beta$ -lactamase production and the *mecA* gene. We found that linezolid, vancomycin, and amikacin are the most effective antimicrobial agents against gram-positive species. The efficacy of vancomycin and amikacin was also studied in previous studies [6]. Multiple studies have confirmed our findings that vancomycin is the most effective antimicrobial agent against gram-positive microorganisms [7, 10, 25, 47-49]. We also observed that *S. aureus*, *S. haemolyticus*, and *S. epidermidis* showed high sensitivity to gentamicin and ciprofloxacin (>84%). Hubab et al. [10] reported that *S. aureus* showed 11.5% resistance to gentamicin, and similarly Mohammadi et al. [50] reported 18% against the same antimicrobial agent. Gentamicin has been considered a crucial antimicrobial agent used in a combination approach to treat *S. aureus* infections [51]. However, our study had certain limitations. This study is limited by its single-center design, relatively small sample size, and lack of longitudinal follow-up, hence possibly affecting the generalizability of the findings. Furthermore, no information describing specific factors associated with bacterial resistance was collected during the study period. Prospective studies are required to use multicenter data, large patient's cohorts and advanced technologies to improve infection control strategies.

## Conclusion

Wound infections are often neglected, even though they may result in chronic disease. These findings may assist medical practitioners in selecting microbiology-based therapies for wound infections, hence reducing the development of MDR microbes. The prevalence of MDR microbes even to 12 distinct antimicrobial agents suggests the need for appropriate administration of antibiotics, avoiding empirical approaches, and preventing biofilm development. We found that the *S. aureus* and *P. aeruginosa* were most frequently isolated microbial pathogens. Gram-negative bacteria represent the majority of pathogens identified in burn

wounds. Gram-positive bacteria were highly susceptible to vancomycin, linezolid, and amikacin, whereas colistin was the most effective antimicrobial agent against gram-negative bacteria. AMB and NYC were the most effective antimicrobial drugs against fungal pathogens. The result of this study showed a high prevalence of MDR microbes among commonly isolated pathogens.

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## Disclosure of conflict of interest

None.

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