*Journal of Advances in Medicine and Medical Research*

***32(24): 342-351, 2020; Article no.JAMMR.65287***
***ISSN: 2456-8899***
*(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)*

# Aerobic Bacteriology of Burn Wound Infections in Burn Patients and Their Antibiotic Susceptibility Pattern in a Tertiary Care Hospital in Kashmir Valley

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author AN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HB and SA managed the analyses of the study. Author SMK managed the literature searches. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JAMMR/2020/v32i2430789
Editor(s):
(1) Associate Prof. Maria Aparecida Visconti, Universidade de São Paulo, Brasil.
Reviewers:
(1) Ira Arundina, Universitas Airlangga, Indonesia.
(2) Harun Agca, Bursa Uludag University, Turkey.
(3) Diorge Jônatas Marmitt, University of Taquari Valley - Univates, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/65287

***Original Research Article***

***Received 25 October 2020***
***Accepted 30 December 2020***
***Published 31 December 2020***

## ABSTRACT

**Aims:** The burn wound represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin. Burn wound infections are an important cause of mortality, morbidity and prolonged hospitalization in burn patients and the causative agent is generally a multidrug resistant organism. The pattern of microbial flora infecting burn wound varies according to geographical pattern as well as with duration of hospital stay. The main aim of the study was to determine the bacteriological profile and antimicrobial susceptibility patterns of burn wound isolates.
**Study Design:** It was a prospective cross-sectional hospital-based study.
**Place and Duration of Study:** The present study was conducted in the Department of Microbiology, Government Medical College, Srinagar, from December 2019 to November 2020.
**Methodology:** Swabs were taken from burn wound of 351 patients and cultured aerobically. Samples were processed for identification and sensitivity. Bacteria isolated were identified using their morphological characteristics, Gram staining reaction and biochemical tests. The antimicrobial

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susceptibility testing was done using Kirby-Bauer disc diffusion method.
**Results:** A total of 351 samples were obtained for the study out of which, the most common isolate was Klebsiella pneumoniae – 154 (38.3%), followed by Pseudomonas aeruginosa - 133 (33.08%), Proteus sp – 42( 10.44%) , Acinetobacter sp- 26 (6.46%), Staphylococcus aureus - 26 (6.46%), Escherichia coli - 17 (4.22%), Enterococcus - 2 (0.49%), and Providencia sp- 2 (0.49%). Colistin was the most effective drug against Gram negative organisms while as linezolid was most effective against Gram positives.
**Conclusion:** The finding of the study will be helpful for identifying the common bacteria causing burn wound infection and also to take proper precautions to prevent the emergence of antibiotic-resistant bacteria.

*Keywords: Burn wounds; bacteriological profile; antibiotic susceptibility; klebsiella sp; pseudomonas sp.*

## 1. INTRODUCTION

Infection of the burn wound is the main cause of morbidity and mortality in patients who are admitted to hospital with major thermal injuries. Burns provide a suitable environment for bacterial multiplication and are more persistent richer sources of infection than surgical wounds, mainly because of the larger area involved and longer duration of patient stay in the hospital [1].

Unlike other types of injury, burn wounds induce metabolic and inflammatory alterations that predispose the patient to various complications. Infection is the most common cause of morbidity and mortality in this population, with almost 61% of deaths being caused by infection [2].

The burn patients have unique predisposition to different infections which are linked to impaired resistance from disruption of the skin's mechanical integrity and generalized immune suppression. The skin barrier is replaced by a protein rich, avascular environment that provides a favourable niche for microbial colonization and proliferation. Additionally, migration of immune cells is hampered, which contributes to septic process. [3,4,5,6].

In addition to the nature and extent of the thermal injury influencing infections, the type and quantity of microorganisms that colonize the burn wound appear to influence the risk of invasive wound infection. The pathogens that infect the wound are primarily gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) [7] and gram-negative bacteria such as *Acinetobacter baumannii-calcoaceticus* complex, *Pseudomonas aeruginosa*, and *Klebsiella* species. These latter pathogens are no table for their increasing resistance to a broad array of antimicrobial agents [8,9].

Risk factors for burn wound colonization or infection are the size of the burn wound, i.e., the percentage of total body surface area (TBSA) burnt and the duration of hospitalization [10,11]. Burn patients have to stay for long period in the hospital and many intravascular and other devices are put in them. Hence, they are at greater risk of acquiring hospital- acquired infection. Nosocomial infection rates in burn wounds have been reported from 77 to 90 infections/100. patients or an incident density of 32-48 infections/1000 patient-days [12].

Improvements in the care of patients who suffer burns, especially initial burn shock resuscitation, airway management, burn wound care, and infection control practices has resulted in remarkably improved survival rates [13,14].

Also, the worldwide emergence of antimicrobial resistance among bacterial pathogens, limits the available therapeutic options for effective treatment of infections [15,16]. Robust surveillance and update of antibiotic resistance pattern of microorganisms is therefore essential for infection control programs and accurate antibiotic treatment in the burnt patients.

The aim of the present study was to study the microbial profile of burn wound infections in burn patients, and to evaluate the antibiotic sensitivity of causative agents, in a tertiary care hospital in our region.

## 2. MATERIALS AND METHODS

### 2.1 Study Design

This prospective cross-sectional study was done in the bacteriology laboratory of the Department of Microbiology for a period of one year, from December 2019 to November 2020. The patients with pre-existing chronic diseases such as

diabetes mellitus and tuberculosis were excluded from the study. A thorough history was taken regarding the demographic data such as age, sex, occupation, address of the patient, and mode of burn.

### 2.2 Sample Collection, Isolation, Identification, and Antimicrobial Susceptibility Testing

For each patient, two swabs were collected from the burn wound on admission and every week thereafter until discharge or death of the patient.

The swabs were transported immediately to the microbiology laboratory immediately. One swab was used for Gram staining and the other swab was used for culture. The samples were inoculated onto chocolate agar, blood agar and MacConkey agar. The isolates were identified as per standard microbiological techniques [17].

Antimicrobial susceptibility testing of the bacterial isolates was done by disk diffusion technique (using Kirby Bauer's method) [18] as per Clinical and Laboratory Standards Institute (CLSI) [19] guidelines. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls.

## 3. RESULTS

A total of 351 samples were collected from wounds of burn patients. 304 swabs (86.6%) showed growth and 47 swabs (13.39%) were sterile after 24 h of incubation. One hundred and ninety-two patients (54.71%) were males, while one hundred and fifty-nine patients (45 .29%) were females. As shown in Table 1, among the patients from whom samples were collected, 41.02% (144) were of 21-40 years age group, 19.94% (70) were of 61-80 years age group, 19.08% (67) were of upto 20 years and 17.94 % (63) were of 61-80 years age group. Patients of more than 80 years were few in number which was 1.99% (07).

The flame burn was the predominant cause of burn among patients; 170 (48.43%) had flame burns, 115 (32.76%) had scald burns, and 35 (9.97%) had electrical burns while as 31 (8.83%) had acid /chemical burns as shown in Fig. 1.

**Table 1. Age and Sex distribution of burn patients with positive bacterial cultures**

| Age Group (years) | Male (n) | Females (n) | Total (n =, %) |
|---|---|---|---|
| 1-20 | 39 | 28 | 67 (19.08%) |
| 21-40 | 82 | 62 | 144(41.02% |
| 41-60 | 32 | 31 | 63(17.94%) |
| 61-80 | 36 | 34 | 70 (19.94%) |
| >80 | 03 | 04 | 07 (1.99%) |
| Total | 192(54.71%) | 159(45.29% | 351(100%) |

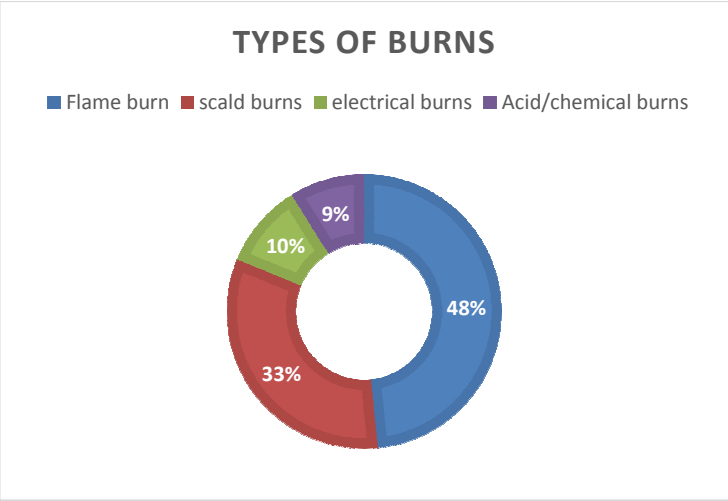


**Fig. 1. Distribution of patients as per type of burn**

Majority of burn patients were third degree (full-thickness) burns with 45% and second degree (partial-thickness) burn with 33.5%, while as first-degree burn (21.5 %) accounted for the least number of burns among total patients. Total body surface area (TBSA) affected ranged from 5% to 80%. The duration of hospitalization of burn patients ranged from 1 day to 30 days. Most of the patients who had third degree burns were admitted for a period of 16 to 25 days while as rest of the patients had a shorter hospital stay of ≤ 16 days.

Out of the 304 microbial growths, 67.10% (n=204) of isolates were monomicrobial whereas 32.22% (n=98) were polymicrobial.

A total of 402 bacterial isolates were recorded from 304 positive growths. Bacterial distribution was dominated by gram-negative bacteria, which accounted for 93.03%. of the cases, while the rest were gram-positive bacteria 6.96%. (Fig. 2).

*Klebsiella pneumoniae* (n=154; 38.3%) was the most frequent isolate followed by *Pseudomonas aeruginosa* (n=133; 33.30%), *Proteus sp* (n=42, 10.44%) *Acinetobacter sp* (n=26,6.46%) *Staphylococcus aureus* (n=26,6.46%), *Escherichia coli* (n=17,4.22%) and *Enterococcus* sp (n= 02, 0.49%) and *Providencia sp* (n=2,0.49%).

Antibiotic sensitive pattern of *Klebsiella pnuemoniae* (Fig. 3) revealed that most of these strains were resistant to Cefepime, Ceftriaxone and Imipenem.

Antimicrobial sensitivity of P. aeruginosa recovered from patient's samples was lower than other isolates. (Fig. 4) P. aeruginosa was found to be resistant to most of antimicrobials used except to colistin to which showed 100% sensitivity. The least sensitive antibiotics were Imipenem and ciprofloxacin (6.76%).

Fig. 5 and Fig. 6 depict the antibiotic sensitivity pattern of *Acinetobacter sp* and *Proteus sp* respectively, revealing high level of resistance against cephalosporins and carbapenems.

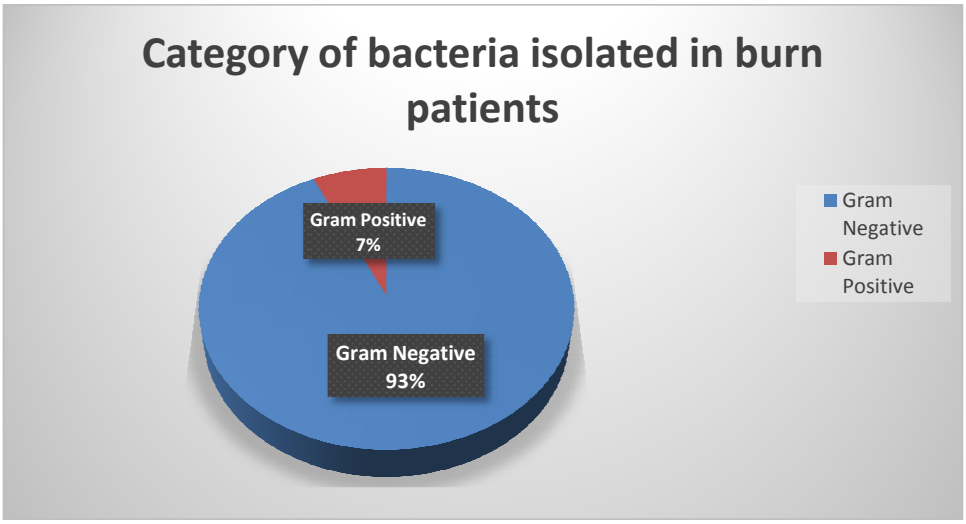


**Fig. 2. Distribution of bacterial isolates among burn patients**

**Table 2. Distribution and Frequency of organisms isolated from burn wounds**

| Bacterial Isolates | Number (%age) |
|---|---|
| *Klebsiella pneumoniae* | 154(38.3%) |
| *Pseudomonas aeruginosa* | 133(33.08%) |
| *Proteus sp* | 42(10.44%) |
| *Acinetobacter sp* | 26(6.46%) |
| *Staphylococcus aureus* | 26(6.46%) |
| *E. coli* | 17(4.22%) |
| *Enterococcus sp* | 02(.49%) |
| *Providencia sp* | 02(.49%) |

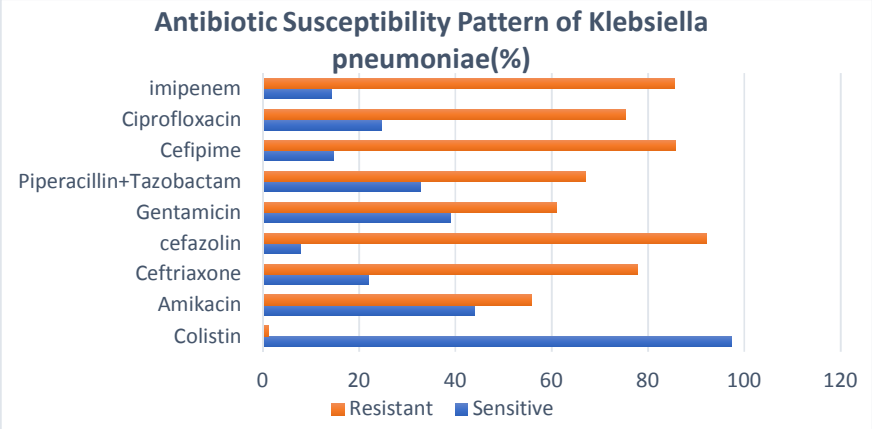


**Fig. 3. Antimicrobial susceptibility pattern of *Klebsiella pnuemoniae* (%)**

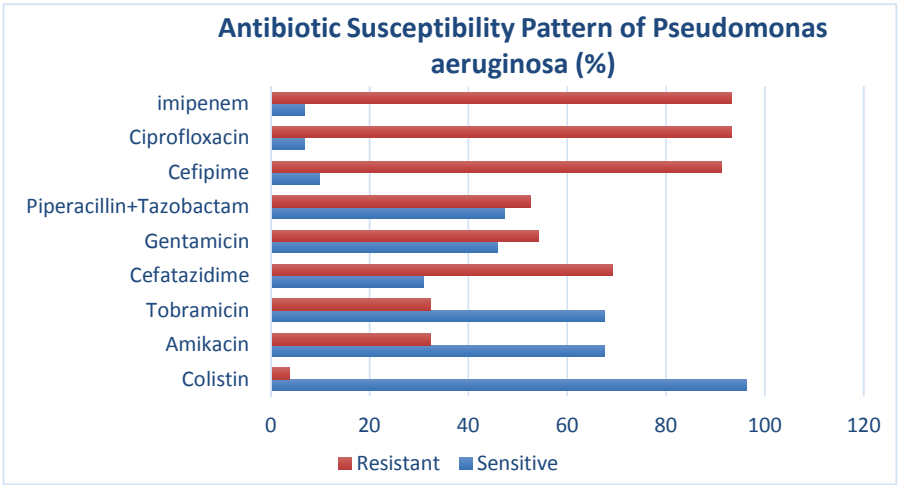


**Fig. 4. Antimicrobial susceptibility pattern of pseudomonas aeruginosa (%)**

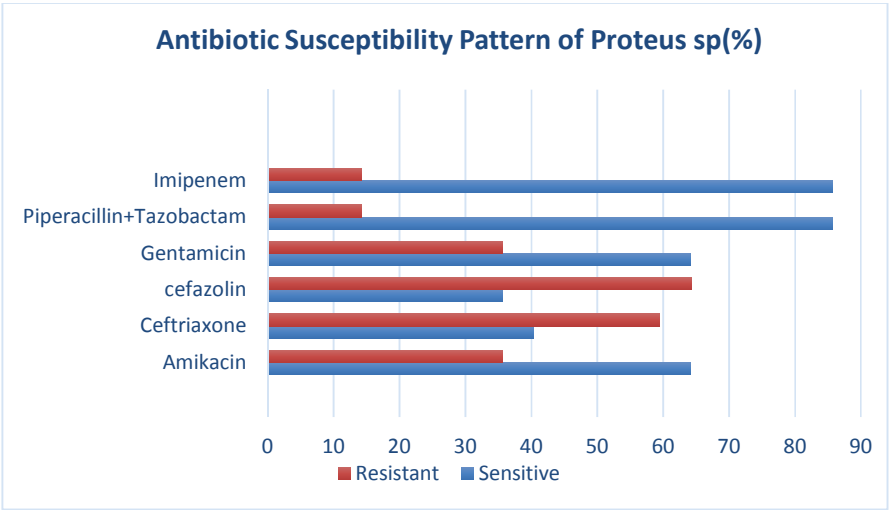


**Fig. 5. Antimicrobial susceptibility pattern of proteus sp (%)**

All the strains of Staphylococcus aureus (Fig. 6) were methicillin resistant and the most effective antibiotic against these strains was Linezolid (100%) followed by Tetracycline (96%), Clindamycin (48%) and Erythromycin (46%).

## 4. DISCUSSION

Infection of burn wound is the most common complication and remains a major cause of morbidity and mortality. According to the World Health Organization (WHO), burn injuries comprise over an estimated 180,000 annual deaths, representing a global public health problem [20]. Burn patients are at a high risk of infection as a result of the nature of the burn injury itself, the immunocompromising effects of burns, and intensive diagnostic and therapeutic procedures. Further, the need of a prolonged hospital stays raises the chances of nosocomial infections in an already compromised patient. Thus, the knowledge about the microbial flora and their current antibiotic susceptibility pattern is important for the treating doctor.

In our study, 351 samples were collected from wounds of burn patients out of which 304 swabs (86.6%) showed growth and 47 swabs (13.39%) were sterile after 24 h of incubation. Similar findings were seen by studies carried out by Srinavasan et al. [21] Dutta et al. [22] and Richcane et al. [23] who reported the isolation rate to be as high as 86.28%, 88.23% and 90.7% respectively.

In the current study males (54.7%) outnumbered the females (45.29%).

This result was in agreement with the finding reported by Ghaffar et al. [24] who found that burn wound infection in males was 189 (62.4%) and in females 114 (37.6%) Macedo and Santos [25] also found that burn wound infection in males 120 (59.1%) was more than burn wound infection in females 83 (40.9%). Similar results were seen by studies of Aali et al. [26], and Vostrugina et al. [27] who also showed a preponderance of infection in male patients. However, these findings were in contrast to results of some studies [28,29].

The reasons for this male preponderance can be related to socio-economic and cultural factors of earning the livelihood primarily by males and also to their adventurous nature and the greater desire to be active in comparison to their female counterparts.

Burn due to flame (48.43%) was the predominant cause among patients in our study. Similar results were recorded in other studies by Shahzad et al. [30] and De Macedo and Santos [25].

The patients belonging to 21-40 years age group (41.02%) were most affected. This finding may be because in these are the years of life, people have more exposure to working with fire, both household and occupational.

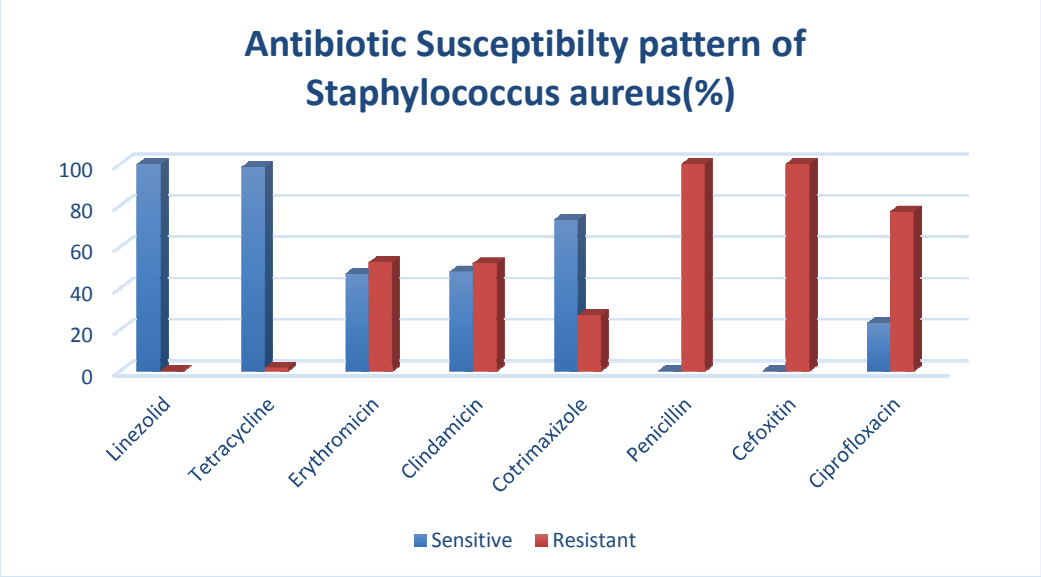


**Fig. 6. Antimicrobial susceptibility pattern of Staphylococcus aureus (%)**

In the present study *Klebsiella pneumoniae* (n=154; 38.3%) was the most frequent isolate followed by *Pseudomonas aeruginosa* (n=133; 33.30%), *Proteus sp* (n=42, 10.44%) *Acinetobacter sp* (n=26,6.46%) *Staphylococcus aureus* (n=26,6.46%), *Escherichia coli* (n=17,4.22%) and *Enterococcus* sp (n= 02, 0.49%) and *Providencia sp* (n=2,0.49%).

This high incidence of Gram-negative organisms in burn patients in our study may due to their ability to flourish and persist in a moist environment in hospitals [31]. Mundhada et al. [32] 5Srinivasan et al. [21] Kehinde et al. [33] and Mohammed et al. [34] also recorded that *Klebsiella* species was the most common isolate similar to our study.

In contrast, *P. aeruginosa* was the most common isolate from other studies conducted by Kaur *et* al. [35], Lakshmi et al. [36] Bhat et al. [37] and De Macedo and Santos [25].

*Klebsiella pneumoniae* is an opportunistic pathogen which causes serious infections like, urinary tract infection, pneumonia, burn infection, and soft tissue infections in compromised and hospitalized patients. It has number of virulence factors such as a capsule that enable this pathogen to colonize and provides phagocytosis resistance [38,39].

The high frequency of this bacteria can also be associated with the increasing level of resistance of *Klebsiella pnuemoniae* to most antibiotics. *Pseudomonas aeruginosa* was the second most commonly isolated organism in our study. This pathogen is well adapted to the hospital environments due to biofilm formation that provides long survival advantages for the pathogen, and effectively prevent eradication by the host immune system or antimicrobial drug treatment. [40]. In the present study, an isolation rate of *P. mirabilis* was 10.44%, which is similar to the study of Bhat et al. (12.4%) [37] but slightly higher than other studies done by Mehta et al. [41] (2.3%) and Mohammed et al. [34].

Antibiotic sensitivity patterns revealed that many of the isolates were resistant to commonly used antibiotics like cephalosporin group, quinolones etc. which are being indiscriminately used on empirical basis for prolonged duration of time.

Colistin was the most effective antibiotic showing 100% efficacy against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Amikacin, a second-generation aminoglycoside was effective against *Klebsiella*, *Pseudomonas, E. coli* and in our study. Same results were seen by other authors also [42,43].

In our study methicillin resistant Staphylococcus aureus was the main gram-positive isolated constituting 6.46% of the isolates. This is in contrast to many studies from India, wherein the authors have found Staphylococcus aureus to be a major cause of infection in burn patients [44,45,46,47].

The isolates of *S. aureus* were sensitive to linezolid (100%), tetracycline (96%%), Clindamycin (48%) and Erythromycin (46%). Mehta et al. [41] and Saxena et al. [48] also recorded similar findings in their study.

Despite the recent advances in burn wound management, microbial infections are an important complication and leading cause of morbidity and mortality among burn patients.

Most of the times such infections are the reason for a prolonged hospital stay which is a burden not only to the patient but also to healthcare facility as well. Predominant risk factors for burn wound infection are the size of burn wound, i.e., the percentage of total body surface area (TBSA) burnt and the duration of hospitalization. Burn wound itself provides a conducive environment for the microorganisms to colonize, which eventually leads to infection.

The results of the antimicrobial resistance pattern in our study are a serious cause for concern because the predominant bacterial isolates were highly resistant to the commonly available antimicrobial agents. Infection control must be prioritized in burn units to potentially halt or reverse the rapid evolution of antibiotic resistance [49]. Selection and cross-transmission of antibiotic-resistant pathogens occurs readily in inpatient burn units in the developing world. Basic hygiene standards are often not observed uniformly in inpatient burn units in resource-limited settings, creating conditions favouring the cross-transmission of drug-resistant bacteria. More research is needed on strategies to reduce colonization and infection with multidrug-resistant organisms in contexts where early excision and grafting are not routine, space constraints are great, and access to disposable materials is limited [49].

The increased incidence of multi drug resistant (MDR) organisms in our study underlines the

need for careful microbiological surveillance and *in vitro* testing before the start of antibiotic therapy. Once MDR strains become established in the hospital environment, they can persist for months. Also, increasing resistance to the commonly available antimicrobial agents leads to over reliance on colistin and polymyxin B, which is the last resort for many patients.

## 5. CONCLUSION

Strict antibiotic policy is imperative in prevention and treatment of MDR isolates in burn units and, thus, reduction of overall infection-related morbidity and mortality. Close follow up of patients and repeat isolations are necessary for appropriate change in antibiotics. The overcrowding in burns ward is an important cause of cross-infection and must be avoided in order to control a hospital-acquired infection. Aggressive infection control measures should be applied to limit the emergence and spread of multidrug-resistant pathogens.

## CONSENT AND ETHICAL APPROVAL

This study was approved by the institutional ethical committee. After ethical clearance, swabs were collected from a total of 351 patients admitted to burn unit. A written informed consent was taken from each patient before collecting the swab.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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