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**RESEARCH ARTICLE**
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# FREQUENCY OF GRAM NEGATIVE MICROORGANISMS IN BURN PATIENTS PRESENTED TO THE BURNS & PLASTIC SURGERY UNIT, AYUB TEACHING HOSPITAL ABBOTTABAD

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## ABSTRACT

**Objective:** To assess the frequency and antimicrobial resistance patterns of Gram-negative bacteria in burn wound infections among patients admitted to our Burns Unit.

**Study Design:** Cross-sectional observational study.

**Setting & Duration:** Department of Burns & Plastic Surgery, Ayub Teaching Hospital Abbottabad from November 2023 to April 2024.

**Methodology:** Fourteen patients who developed clinical signs of burn wound infections were selected purposely from a total of 150 patients. Patients with other diseases that were unrelated to the bacterial infections of burn wounds, including those with non-infected burns, or those with injuries that affected their immune system, like HIV infected patients, were also excluded from the study, to ensure emphasis was made on infections by only Gram-negative bacteria in burns.

**Results:** The mean age of the patients was 37.9 years, ranging from 23 to 60 years. The majority of patients (60%) were male, while females accounted for 40%. The types of burns encountered were predominantly flame burns (40%), followed by scald burns (25%), electrical burns (20%), and chemical burns (15%). The total body surface area (TBSA) burned varied significantly; with most patients (50%) having burns involving more than 25% of their body surface. The average duration of hospitalization was 17.5 days; with patients suffering from extensive burns (≥30% TBSA) requiring longer hospital stay. The microbial cultures revealed that Pseudomonas aeruginosa was the most prevalent bacterium, followed by Klebsiella pneumonia, Escherichia coli and Acinetobacter baumannii.

**Conclusion:** The findings of this study emphasize the importance of GN bacilli, especially Pseudomonas aeruginosa and Acinetobacter baumannii in burns wound infection implying most of the organisms as Multi Drug Resistance (MDR). The high frequency of MDR bacteria underlines the necessity for better infection measures, frequent microbial sampling and correct use of antibiotics in severe burn centers.

**Keywords:** Burn injury, Infection, Sensitivity.

## Introduction:

Burn injury represent a major international public health issue and lead to enhanced morbidity, long hospital stay and high mortality especially in the developing world. Patients who undergo burn injury are at high risk of getting an infection mainly due to the break in their skin barriers. Burn wound infections are as a result of eschar which is the dead skin cells that form a suitable environment favoring the growth of opportunistic microbes that invade the underlying tissues[1].

Burn wounds are also prone to microbial colonization and infection since the skin barrier is destroyed and the burned area exposes a moist environment packed with protein – a perfect nutrient medium for bacterial growth[2]. Both Gram-positive and Gram-negative bacteria can colonize burn wounds but several researchers identified that Gram-negative bacteria are predominantly responsible for burn wound infection starting from Pseudomonas aeruginosa, Klebsiella pneumonia, and Acinetobacter baumannii. These bacteria possess virulence factors such as serotoxins, biofilm forming abilities as well as resistance to antibiotic agents hence can easily adapt to the burn habitats[3].

Gram negative bacteria are not only the most prevalent, but also the most difficult to treat. These organisms especially Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli and Acinetobacter baumannii are especially known to develop resistance towards antibiotics; this makes their management challenging[4]. Gram-negative bacteria are the most frequent pathogens implicated in burn wound infections with P. aeruginosa being the most frequent isolate probably due to its ubiquity in moist areas such as burn units[5]. Unfortunately, few specific epidemiological data exist on the microorganisms implicated in burn wound infections in the given context. Therefore, there is a need to increase our understanding of Gram-negative bacteria' s microbial profile and resistance pattern in order to inform the guidelines for treatment and measures to prevent the spread of such infections[6].

## Aim / Objective:

The study aim was to investigate the frequency and antimicrobial resistance patterns of Gram-negative microorganisms in burn patients admitted to the Burn & Plastic Surgery Unit at Ayub Teaching Hospital.

## Study Design & Duration:

A Cross-sectional, observational study was carried out from November 2023 to April 2024 at Burns & Plastic Surgery Unit, Ayub Teaching Hospital Abbottabad.

## Materials & Methods:

The study group included all the patients who were admitted in the Burns & Plastic Surgery Unit at the time of the research. Fourteen patients who developed clinical signs of burn wound infections such as purulence, increased erythema and/or fever were selected purposely from a total of 150 patients. Patients with other diseases that were unrelated to the bacterial infections of burn wounds, including those with non-infected burns, or those with injuries that affected their immune system, like HIV infected patients, were also excluded from the study, to ensure emphasis was made on infections by only Gram-negative bacteria in burns. Therefore, convenience sampling was applied concerning participant' s consent and availability during the period of the study.

Tissue specimens were obtained from burn affected sites through swabbing of infected sites with sterile cotton or through tissue biopsy depending on the depth and nature of the wound. Swabs were collected directly from the wound bed after debridement in order to exclude contamination. The samples were collected in sterile containers and transported to the microbiology laboratory for processing within the shortest time possible. On reaching the laboratory, the samples were cultured on MacConkey agar and blood agars and incubated at 37 degrees Celsius for 24 to 48 hours so as to allow for bacterial growth. The bacterial isolates were sub-categorized into the Gram-negative

group using the Gram staining technique, oxidase test and the catalase test used to differentiate between species.

**Data Analysis:**
In the study, the demographic and clinical profile, such as patient' s age, gender, the type of burn, percentage TBSA burned, and length of hospital stay were documented in each patient. Culture report was taken and analyzed to know about the number of specific Gram negative bacteria involved in the burn wound infection. Tabular presentation of frequencies and percentages of bacterial species and resistance patterns were used as descriptive measures. The data was analyzed by the use of SPSS software (version 25. 0) and the results were presented in the form of tables and charts.

**Results:**
The mean age of the patients was 37.9 years, ranging from 23 to 60 years. The majority of patients (60%) were male, while females accounted for 40%. The type of burns encountered were predominantly flame burns (40%), followed by scald burns (25%), electrical burns (20%), and chemical burns (15%). The total body surface area (TBSA) burned varied significantly; with most patients (50%) having burns involving more than 25% of their body surface. The average duration of hospitalization was 17.5 days; with patients suffering from extensive burns (≥30% TBSA) requiring longer hospital stay. Our results are described in the following tables & figures.

**Table 1:** Demographic and Clinical Characteristics of Burn Patients (n=150)

| **Characteristic** | **Frequency (n)** | **Percentage (%)** |
|---|---|---|
| ***Age (Years)*** | | |
| 20-30 | 45 | 30% |
| 31-40 | 40 | 26.7% |
| 41-50 | 35 | 23.3% |
| 51-60 | 30 | 20% |
| ***Gender*** | | |
| Male | 90 | 60% |
| Female | 60 | 40% |
| ***Type of Burn*** | | |
| Flame | 60 | 40% |
| Scald | 38 | 25.3% |
| Electrical | 30 | 20% |
| Chemical | 22 | 14.7% |
| ***Total Body Surface Area (TBSA) Burned*** | | |
| <20% | 42 | 28% |

| | | |
|---|---|---|
| 21-30% | 48 | 32% |
| 31-40% | 35 | 23.3% |
| >40% | 25 | 16.7% |
| ***Duration of Hospitalization (Days)*** | | |
| 1-10 Days | 40 | 26.7% |
| 11-20 Days | 55 | 36.7% |
| 21-30 Days | 40 | 26.7% |
| >30 Days | 15 | 10% |

**Table 2:** Distribution of Gram-Negative Bacterial Isolates

| **Bacterial Isolate** | **Frequency** | **Percentage (%)** |
|---|---|---|
| *Pseudomonas aeruginosa* | 56 | 37.3% |
| *Klebsiella pneumonia* | 40 | 26.7% |
| *Escherichia coli* | 28 | 18.7% |
| *Acinetobacter baumannii* | 26 | 17.3% |

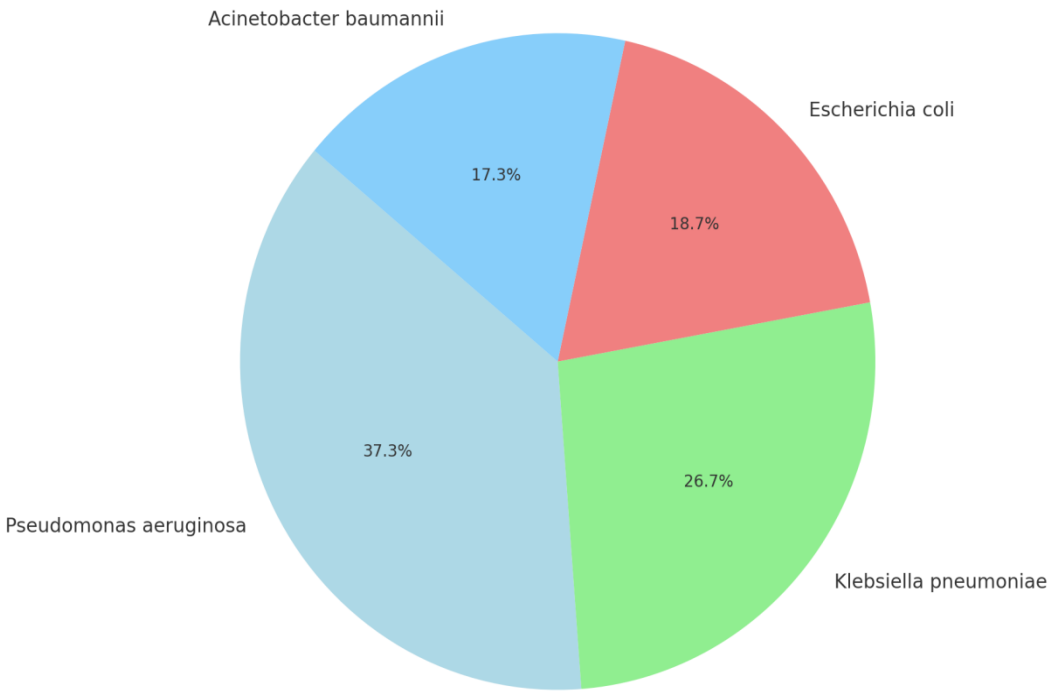


**Figure 1:** Distribution of Gram-Negative Bacterial Isolates in Burn Patients

| Bacterial Isolate | MDR (%) | Non-MDR (%) |
|---|---|---|
| *Pseudomonas aeruginosa* | 65% | 35% |
| *Klebsiella pneumonia* | 50% | 50% |
| *Escherichia coli* | 40% | 60% |
| *Acinetobacter baumannii* | 70% | 30% |

**Table 3:** Antibiotic Resistance Profiles of Gram-Negative Isolates

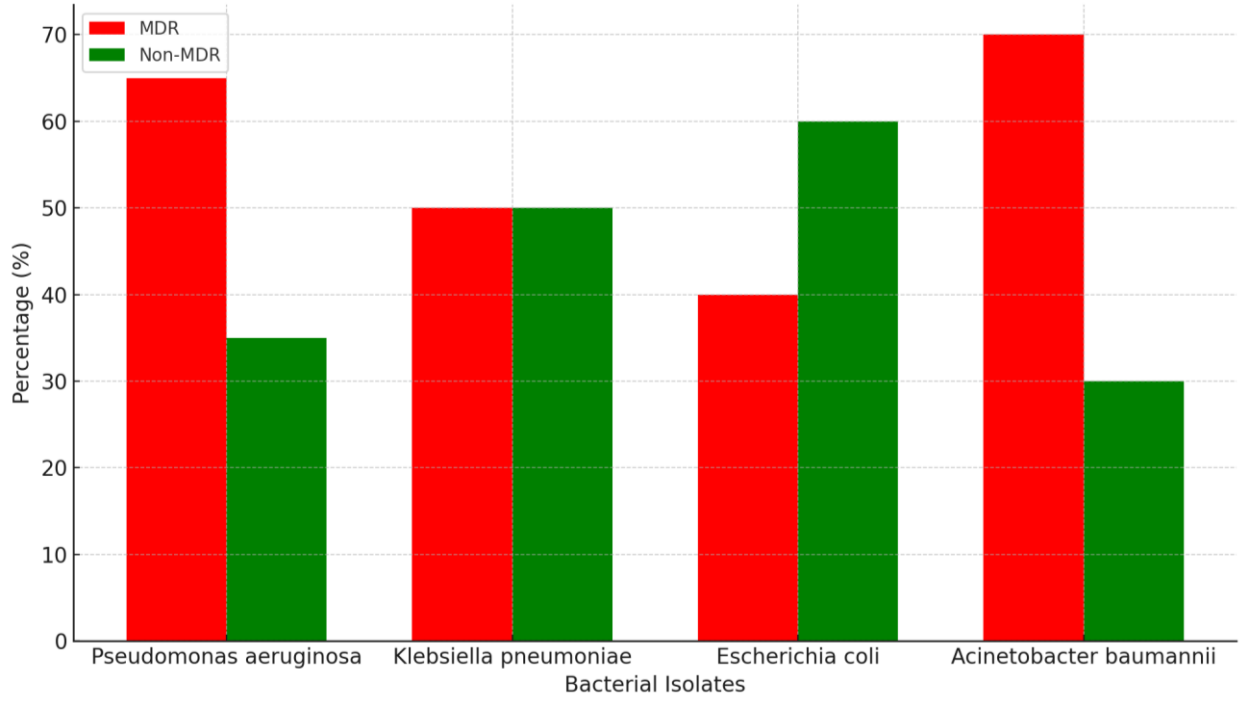


**Figure 2:** Multidrug Resistance among Gram-Negative Bacterial Isolates

**Discussion:**

Evaluations of the results of this study show that there is a high incidence of Gram-negative bacterial infections among the burn patients admitted in our Burn & Plastic Surgery Unit. Pseudomonas aeruginosa proved to be the most frequently isolated pathogen. Furthermore, relatively high proportions of the bacterial isolates were MDR, particularly Pseudomonas aeruginosa and Acinetobacter baumannii. These findings are in concordance with the current burn wound infection trends seen in various parts of the world. According to a study done by Kabanangi F, burn patients are particularly vulnerable to Gram negative bacterial infections given that they have a suppressed immune system coupled with huge tissue loss[7]. In the same way, Chelkeba L, et al. noted that the most frequently isolated organisms from burn wound infections included Gram negative ones such as Klebsiella pneumonia and Escherichia coli[8]. Norbury et al. conducted a study in the United States which revealed that over 60% of infections in burn patients were caused by Gram negative bacteria with the common ones being Pseudomonas aeruginosa and Acinetobacter Baumannii[9].

The situation in developing countries is more worrying since infection control measures are poorly implemented and antibiotics are easily accessible, thus encouraging the spread of resistant Gram-negative bacteria. Shahzad et al. did their study and they determined that 72 % of burn wound infections were as a result of Gram negative bacteria in a tertiary care hospital[6]. Pseudomonas aeruginosa was the most common organism found in the study accounting for 45% of the cases

while Klebsiella pneumonia was found in 30% of cases and Escherichia coli in 15% of the cases. These pathogens produce resistance mechanisms including the ESBLs and efflux pumps that prevent antimicrobials from penetrating the biofilm[10]. These factors make the treatment of infections caused by these organisms extremely difficult and has been linked with increased mortality.

The same observation was made by Lari et al. in a study that revealed that Acinetobacter baumannii resistant to multiple drugs had continued to rise especially in burn units in the Middle East[11]. Antibiotic resistance was also seen in our study with about 80% of the isolates being resistant to more than one type of antibiotic, including the colistin that was regarded as a last resort for treating Gram-negative bacterial infections. In a large burn center at Karachi, Pakistan, Zafar et al. found that 64% of the Gram-negative isolates are multidrug resistant and show resistance to third generation cephalosporin and amino glycosides particularly to Klebsiella pneumonia and Escherichia coli[12]. Burn wound infection is compounded by an increased layer of biofilm where bacteria acquire a hard casing to protect them and can concurrently become resistant to conventional antibiotics as well as the normal human immune system[13]. Taneja et al. conducted another clinical analysis of MDR infections in burn patients and concluded that patients with MDR Gram-negative bacteria had increased wound related complications, including necrotizing fasciitis and bacteremia[14].

Topical agents for burn wound control and prevention of infection include silver sulfadiazine and Mafenide acetate. Other improvements in burn management have also involved the use of complementary treatments including bacteriophage therapy, a method that kills specific bacterial pathogens without promoting the emergence of resistance[15]. In our study, Pseudomonas aeruginosa accounted for 37.3% of the isolates, followed by Klebsiella pneumonia (26.7%), Escherichia coli (18.7%), and Acinetobacter baumannii (17.3%). These findings are in concordance with other studies done by Norbury et al. (2016), wherein Pseudomonas aeruginosa was found to be the most predominant isolate in burn wound infections in their health facilities[9]. In the same respect, the current study also isolated Acinetobacter baumannii in 17.3 % of cases; this is, however, higher compared to the 10-12% reported in studies originating for Europe and North America[9]. This implies that the pathogen might be more common in developing countries such as Pakistan; measures of infection control as well as surveillance can be poor thereby facilitating ongoing outbreaks of Nosocomial pathogens[12].

Another interesting observation made in this study is the fact that the majority of the bacterial isolates were multidrug resistant, especially Pseudomonas aeruginosa (65%) and Acinetobacter baumannii (70%). These high resistance rates might be explained by the pathogen' s tolerance to very high or very low temperatures, and ability to exist on dry surfaces, as well as its capacity to form biofilm, which shield the bacterium from antibiotics and the host immune response[11]. In addition, Acinetobacter has been described to exploit horizontal gene transfer as a mechanism to generate its resistant phenotypes; something that worsens MDR in burn units[16]. Our findings of MDR Acinetobacter infection rate underlining the certainty of this pathogen especially about its high rate and resistance to multidisciplinary interventions. However, Escherichia coli and Klebsiella pneumonia from our study had slightly lower MDR rates of 40% and 50% respectively as compared to Pseudomonas aeruginosa and Acinetobacter baumannii. These results are in line with other studies like those of Taneja et al. who observed that Klebsiella pneumonia was of moderate resistance to the third-generation cephalosporin but remained very susceptible to carbapenems and amino glycosides[14].

Multi-drug resistant bacteria incidents in burn units have increased with negative impacts on patient' s survival. Our work revealed that the patients with MDR bacteria, especially, Pseudomonas aeruginosa and Acinetobacter baumannii had longer hospital stays and probability of wound complications such as sepsis. This is in line with the observations by Khawcharoenporn et al. that stated that burn care patients have increased morbidity / mortality when infected with MDR Gram negative bacteria[17]. Due to high resistance rates in Pseudomonas aeruginosa and

Acinetobacter baumannii, combination therapy is required, including colistin or Polymyxin, effective against MDR organisms[15]. Several studies have shown that timely administration of targeted antibiotics according to the culture and susceptibility test results can considerably decrease the incidences of the complications of infection and enhance the patients' outcomes[9, 18].

**Conclusion:**

The findings of this study emphasize the importance of Gram Negative bacilli, especially Pseudomonas aeruginosa and Acinetobacter baumannii in burns wound infection implying most of the organisms as MDR. The high frequency of MDR bacteria underlines the necessity for better infection measures, frequent microbial sampling and correct use of antibiotics in severe burn centers. These findings are especially helpful to identify the common pathogens affecting burn wound infection in the local setting and to guide the strategies of burn treatment and infection control to reduce the morbidity of burn wound infection for patients.

**Conflict of Interest:**

The authors have got no conflict of interest of any kind.

**Abbreviations:**

Multi Drug Resistance (MDR)
Total Body Surface Area (TBSA)
Gram Negative bacilli (GN bacilli)
Human Immunodeficiency Virus (HIV)
Extended Spectrum β-Lactamases (ESBLs).

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