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IDENTIFICATION AND CHARACTERIZATION OF *BACTERIAL SPECIES* ISOLATED FROM CONTAMINATED BURN WOUNDS IN DISTRICT D.I.KHAN

Amina Abid[1], Nasreen Saif [2], Tauseef ul Hassan[3], Rahila Bano[4]

[1]Demonstrator in Pathology Department, Gomal Medical College, D.I. KHAN
[2]Demonstrator in Pathology Department, Gomal Medical College, D.I. KHAN
[3]Assistant Professor in Plastic Surgery Department, Mufti Mehmood Teaching Hospital, D.I. KHAN
[4]Associate professor in Pathology Department, Gomal Medical College, D.I. KHAN

Corresponding Author: Nasreen Saif
Demonstrator in Pathology Department, Gomal Medical College, D.I. KHAN
Email: nanikhan22@gmail.com

Abstract
A burn is an injury to the skin or other physiological tissues caused by several factors, including thermal injury, scalds, fire, electrical shock, and chemical exposure. The aims of the present study were to isolate, identify, and evaluate the sensitivity of bacteria isolated from burn-infected patients in District D.I. Khan to antibiotics. One hundred seven swab samples were collected from one hundred seven patients suffering from burn illnesses. Four unique bacterial isolates were identified based on colony morphology and biochemical testing, including oxidase, catalase, coagulase, indole, and motility: Pseudomonas aeruginosa, Klebsiella, Staphylococcus aureus, and Escherichia coli. The frequency distribution was as follows: Pseudomonas aeruginosa (71.43%), Klebsiella (14.29%), Staphylococcus aureus (10%), and Escherichia coli (4.3%). The predominant survey replies originated from persons aged 26-45 years, accounting for 45%, closely followed by respondents aged 11-25 years. Female infections comprised 65.42%, whereas male infections were 34.58%. A total of ten medicines were assessed, with levofloxacin and ciprofloxacin exhibiting enhanced efficacy. Moderate resistance to Amikacin, Chloramphenicol, and Gentamicin is observed. Imipenem, Amoxicillin, and Ceftriaxone were found to be less efficacious. Vigilance and diligence are crucial to avert infection in burn patients at District D.I. Khan. A comprehensive and meticulous study is necessary to avert or alleviate these disorders.

Keywords: Antibiotic susceptibility; Bacteria; Burn Victims; District D.I.Khan

INTRODUCTION
Burns are one of the most damaging types of injuries that can occur in people who have been subjected to significant thermal stress (WHO, 2010). Burns can be caused by intense heat or chemical exposure. An estimated fifty-one percent of all deaths are attributed to invasive infections that are caused by burns (Norbury *et al.,* 2016). For every year, the United States of America is home to approximately 500,000 individuals who are in need of medical treatment due to burns.
Approximately forty thousand of these individuals are need to be hospitalized in order to reduce the risk of contracting a bacterial infection (Forson *et al.,* 2017). The existence of virulence factors and the microscopic organisms that are present on the burn wound are directly related to one another, as evidenced by the observed correlation. For the time being, the burn wound will be sterilized by the

elevated temperature, according to the notion. The indigenous microorganisms that are present in normal skin flora as well as any illnesses that have occurred in the past have a rapid pace of development on their own. The pediatric burn unit has a total of 54% of patients who are infected with *Staphylococcus aureus* and 9% of patients who are contaminated with GAS (Group A Streptococcus) upon admission. It is through the examination of ordinary cultures that this information has evolved. To differentiate between contamination and colonization of the wound surface, it is crucial to notice that the two terms are not interchangeable (Nielson *et al.,* 2017; Cambiaso *et al.,* 2018). This is because the two concepts are not synonymous with one another. At this time, seventy-five percent of fatalities that occur in persons who have symptomatic burns that cover more than forty percent of the external surface area of their bodies are caused by septic shock and infection health problems. These two health problems are responsible for the majority of these fatalities.

When compared to other patients, the prevalence of nosocomial infections in burn patients is higher than in other patients (Al-Taie *et al.,* 2014). This is due to the severity of

the burn lesion, prolonged stays in the intensive care unit, invasive preventative and surgical interventions, and the combination of these factors. When it comes to the treatment of wounds and the promotion of the growth of beneficial bacteria within the wound, wound inoculation is a method that serves both of these intended aims. The onset of wound invasion often takes place between five and seven days following a severe burn in persons who have experienced significant burns.

*S. aureus*continues to be the most common source of infection in burn wounds (Al- Taie *et al.,* 2014), despite the fact that this is the state of affairs. Methicillin-resistant *Staphylococcus aureus* has emerged as the most prevalent bacteria in intensive care units over the course of the last several decades. This is mostly attributable to the growing utilization of broad-spectrum antibiotics. However, despite the fact that it is extremely rare for any of these bacteria to be asymptomatic when they colonize an injured person, they are also a source of infectious agents that can result in serious illness and even death on occasion (Hubab *et al.,* 2020).

Contact with polluted external environmental surfaces, water, fomites, air, and the hands of healthcare professionals can all be potential vectors for the transmission of microorganisms to the skin surface of a patient (c *et al.,* 1997). There was a widespread belief that Streptococcus pyogenes was the principal cause of burn wound sepsis during the first half of the previous century. It has become clear that *S. aureus*and P. aeruginosa are the pathogens that are most frequently discovered in the majority of burn units during the course of time.

The process of wound invasion normally takes place within five to seven days for those who have sustained severe burns. This is the case in most cases. When a patient is admitted to the hospital, it is advised that a first wound culture be performed as a routine clinical practice. This is because the majority of early infections in burn patients are caused by bacteria that are located within the body of the patient (Al-Taie et al. 2014)

Methicillin resistance is possible. In the most recent decades, *S. aureus*has emerged as the most prevalent bacterium in intensive care units. This is mostly attributable to the widespread utilisation of antibiotics with a broad spectrum of activity. Despite the fact that colonisation with any of these bacteria in an injured people is frequently asymptomatic, they can also serve as a reservoir of pathogenic microorganisms that are capable of causing serious sickness and even death (Singh *et al*., 2017).

Regardless of whether or not microorganisms have been cultivated from the sick material, the presence of pus is a defining characteristic of an infection that has occurred in a wound. Instances in which the requirement of pus and clinical symptoms of infection as well as the absence of a wound culture were both satisfied have been judged to be indicative of infection (Meaume *et al*., 2012). They account for a sizeable fraction of the regularly taking place hospital-acquired infections and are a key factor in the development of sickness as well as death. The progression of such infections causes patients to experience a delay in their recovery, causes those patients to experience anxiety and concern, extends the length of their hospital stays, and significantly raises the cost of healthcare services (Cooper *et al*., 1996).

Even though there have been a lot of attempts made to address this problem, hospital wound infections

remain to be a significant problem in the field of medical care. Due to the frequent use of antibiotics in healthcare facilities such as hospitals and clinics, antibiotic resistance has grown widespread. This is a consequence of the widespread use of antibiotics. As a consequence of this, it is necessary to investigate the specific antibiotics and proper measures that ought to be employed in the treatment of wound infections. This should be done while taking into consideration the reason for the infection as well as the duration of time that antibiotic therapy is supplied.

The current advancements in medical technology and treatments, in particular antibiotics, are leading to an increase in the number of people who are extremely susceptible to illness. As a result of the emergence of antibiotic resistance that is transferable in pathogenic organisms this situation has become even more severe. Before the findings of cultures and antibiotic sensitivity tests were obtained, the objective of this study was to evaluate the antibiotics that were used to treat infected wounds caused by a variety of pathogens that were isolated from infected wounds. According to the available evidence, S. aureus possesses an innate resistance to this antibiotic. The presence of Staphylococcus aureus has been documented in a number of different regions, including Africa, Europe, and the United States. Consequently, cases were reported in hospital settings, notably at the community level, without any direct engagement with hospitals. They were documented in hospitals. One of the many resistance mechanisms that Staphylococcus aureus and Staphylococcus faecal is possess is a reduction in the permeability of the membrane. Other resistance mechanisms include the modification of porine proteins or the growth of a thick fat layer on the cell wal

In the present investigation, the burn unit of the Mufti Mahmood Teaching Hospital D.I.Khan, which has been operational since 2022, will serve as the location of the operations. This facility was responsible for treating between fifty and seventy patients with burn wounds on a monthly basis, according to observations. In accordance with the information provided by the physician, bacterial growth was found on burn wounds. One of the microorganisms that was found to be the most abundant was *Pseudomonas aeruginosa*.

Statement of problem

Bacterial infection continues to be a significant challenge in the treatment of individuals with burn injuries. Among individuals who have sustained extensive burns covering over 40% of their total body surface area (TBSA), a staggering 75% of fatalities are presently attributed to sepsis resulting from infection of the burn wounds or other infection-related consequences, as well as inhalation injury. Burns are a significant public health issue, with roughly 265,000 fatalities occurring annually just due to fire-related incidents.

Objectives of the study

The current study conducted with the following objectives

To isolate and identify the bacteria linked to burn wounds.

Examine the susceptibility of microorganisms to a selection of frequently administered antibiotics.

To determine the bacteriological profile of burn wound bacteria

Significance of the study

This was be the first thorough study ever conducted with the purpose of isolating and characterization of Some Bacteria Contemn in Burn Wounds in District D.I Khan who come from a variety of different backgrounds. An investigation into the genetic diversity of Bacteria Contemn in Burn Wounds were of great assistance in the management of this infection.

Limitations of the study:

This study is not representative of the entire province or country as it just focuses on gynecological patients in District D.I Khan. Furthermore, the purpose of this study does not involve investigating the impact of individual sensitivity.MATERIALS AND METHODS

The current study was carried out during the months of June 2024 and June 2025 in the district of Dera Ismail Khan, which is situated in the province of Khyber Pakhtunkhwa, Pakistan. The district

of D.I. Khan is the most southern district in Khyber Pakhtunkhwa (KP), and it is located approximately 300 kilometers away from Peshawar, the capital of the province to the south. The provinces of Punjab, Sindh, and Balochistan are all located on its border. According to the census completed in 2023, the total population of the district is greater than 1.8 million, with the number of people who speak Saraiki being the majority of the population. This figure has been steadily growing over the course of the years.

Sample Collection

At the burn unit of Mufti Mahmood Teaching Hospital D.I.Khan, a total of 107 burn patients from a variety of age groups and genders were screened for the purpose of this study. Between the months of May 2024 and July 2024, the screening took place. It is planned to collect wound swabs from each and every patient. When they are interacting directly with patients, healthcare staff were weared a protective gown and gloves that are disposable.

After the specimens have been transferred to a container that is both sterile and impermeable, they transported to the laboratory without any contamination. In order to culture the samples, nutritious agar, MacConkey agar, 5% Blood agar, and mannitol salt agar utilized. They are going to be incubated for a period of twenty-four hours at a temperature of 37 degrees Celsius and under aerobic conditions.

Isolation and Identification Bacteria from the burn wounds

Initially, the colonies were characterized by their phenotypic and cultural characteristics in order to complete the diagnosis. In order to determine the identity of the bacterial isolate, we will employ the conventional microbiological techniques, which
include analyzing the features of the culture. After that, the Gram stain isolates were diagnosed with the help of VITEK® 2 Compact Automated Systems, with ID-GN and ID- Gp cards being utilized in accordance with the instructions provided by the manufacturer.

Antimicrobial susceptibility test

Experiments on antimicrobial susceptibility was carried out using the Kirby-Bauer disk diffusion method, which was recommended by the Clinical and Laboratory Standards Institute. The Kirby-Bauer method is completely standardized, which ensures that the results will be accurate and reliable. Petri dishes with a diameter of either 100 or 150 millimeters were furnished with Mueller-Hinton agar, which serve as the medium for the experiment.

The depth of the agar in the plates were four millimeters. In order to be considered acceptable, the pH level of the agar must be between 7.2 and 7.4. With the goal of achieving a turbidity standard of 0.5 McFarland, which is equivalent to approximately 150 million cells per mL , the bacterial inoculum was produced by diluting a broth culture.

Statistical analysis

The descriptive statistics that are obtained with the assistance of Microsoft Excel were utilized in order to conduct an analysis of the data that was recorded in the current research. The statistical software SPSS (version 25) was used to code and enter the quantitative data that was generated from the investigation. Descriptive analysis was done to determine the frequency of different variables.

Table 1 Analysis of gender of the burn patients recruited (n=107)

Variables	Response	No. Patient%
Gender	Male	37 (34.58)
	Female	70 (65.42)

Table 1 of the study presented the Gender analysis of the burn patients. Among the study participants, the majority were female, accounting for 70 individuals or 65.42%, followed by males, at 37 individuals or 34.58%.

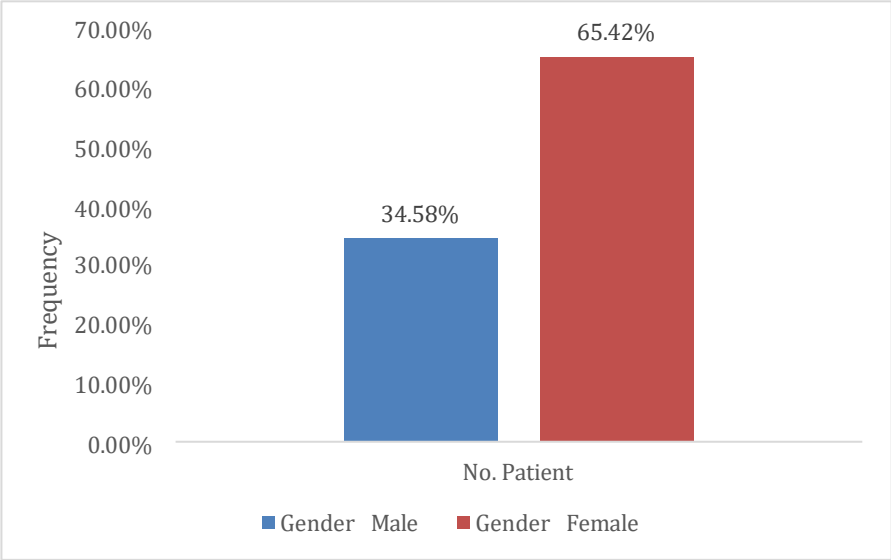

Figure 1 Graphical representation of gender

Table 2 Analysis of age of the burn patients recruited (n=107)

Variables	Response	No. Patient%
Age in years	1-10	10 (9.3)
	11-25	30 (28.04)
	26-45	45 (42.06)
	45-70	22 (20.56)

Table 2 of the study presented the analysis of the age of the people involved in the study. The data revealed that the majority of the participants in the survey were between the age ranges of 26-45 years, with 11-25 years following closely behind.

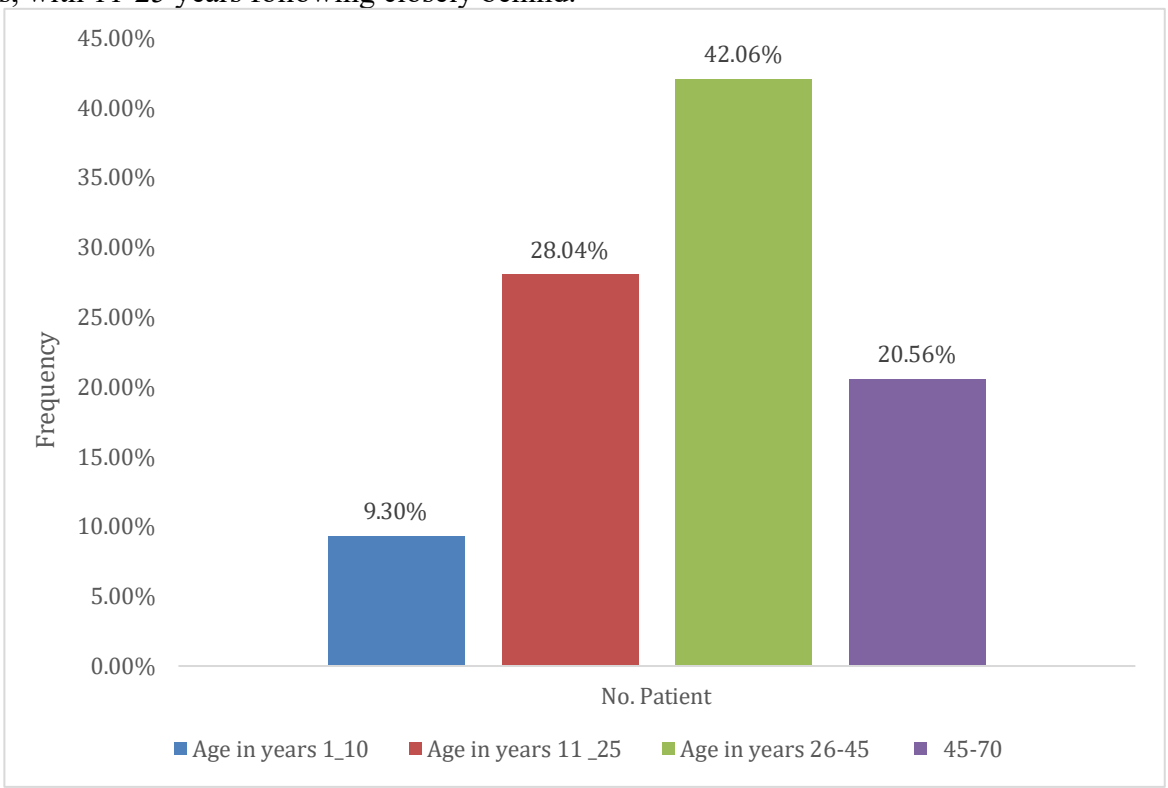

Figure 2 Graphical representation of age of the burn patients

Table 3 Analysis of Mechanism of Injury of the burn patients recruited (n=107)

Variables	Response	No. Patient%
Mechanism of Injury	Gas Flam	70(65.42)
	Scalds	20 (18.69)
	Electricity	17 (15.89)

Table 4.3 of the study presented the methodology of injury analysis for the burn patients. Among the burn patients, the most, namely 65.42%, were affected by Gas Flam. This was followed by Scalds and electricity.

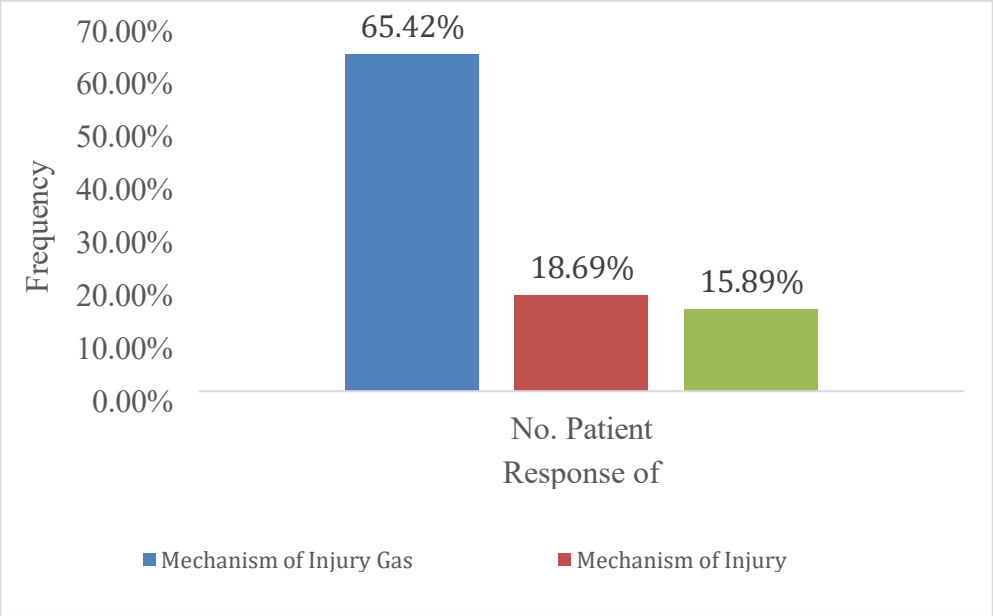

Figure 3 Graphical representation of age of the burn patients

Table 4 Category of culture and percentage of burn severity

Variables	Response	No.Patient%
Type of culture	Positive	70 (65.42)
	Negative	37 (34.58)

Table 4 presents the results of an investigation that looked at the cultural group as well as the proportion of burn severity. According to the findings of the investigation, 65.42 percent of the paint pigments exhibited favourable results, whereas 34.58 percent exhibited negative results.

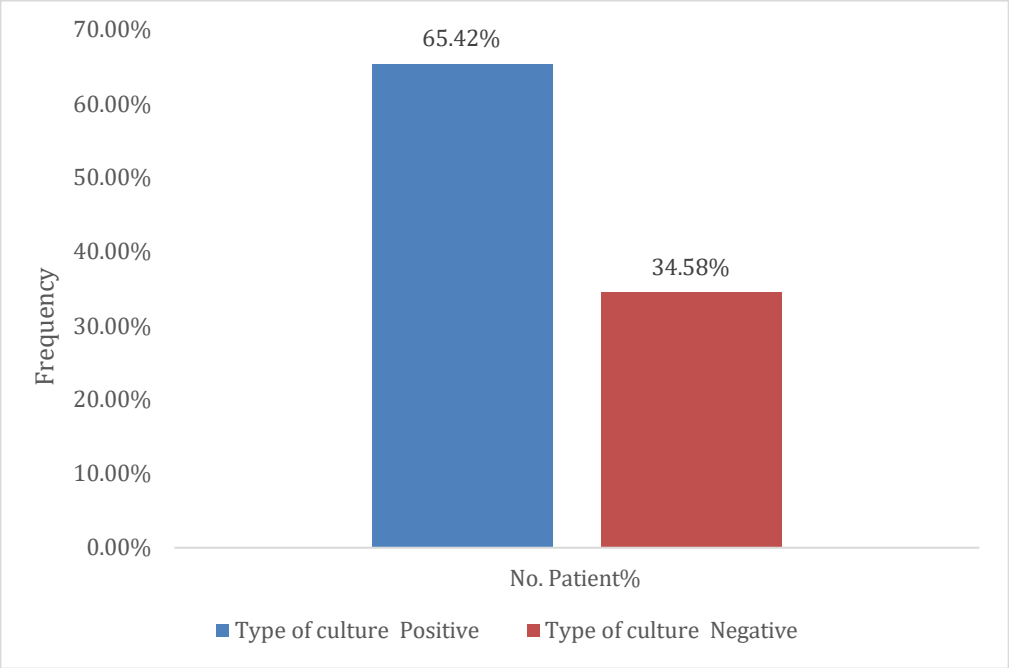

Figure 4 Graphical representation of category of culture burn

Table 5 Category of degree of burn severity

Variables	**Response**	**No.Patient%**
Degree of burns	1st Degree	20 (18.69)
	2nd Degree	70(65.42)
	3rd Degree	17 (15.89)

Analysis of burn severity was conducted in table 5 of the study. The findings indicated that the majority of burn victims have second degree burns, with first and third degree burns following closely behind.

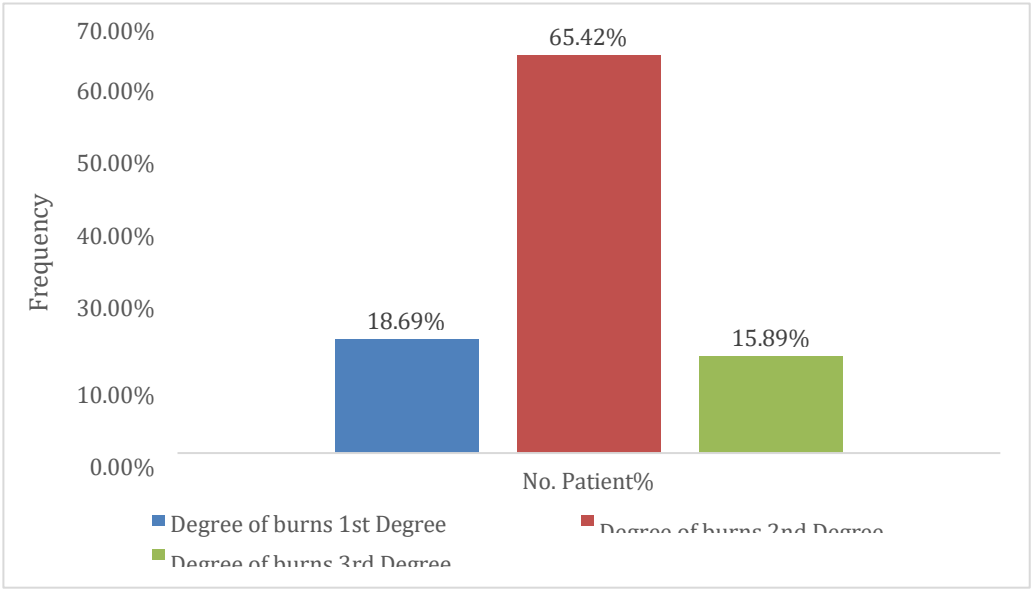

Figure 5 Graphical representation of Category of degree of burn severity

Table-6: Types of bacteria isolated from burn wounds

Name of bacterial strains	No of isolates	Percentage%
Pseudomonas aeruginosa	50	71.43
Klebsiella spp.	10	14.29
Staphylococcus aureus	7	10
E. coli	3	4.3
Total	70	100

The study table 6 provides a description of the names and total number of isolated bacterium colonies. The analysis revealed that 71.43% of the isolates were classified as *Pseudomonas aeruginosa,* followed by *Klebsiella spp, Staphylococcus aureus,* and *E. coli.*

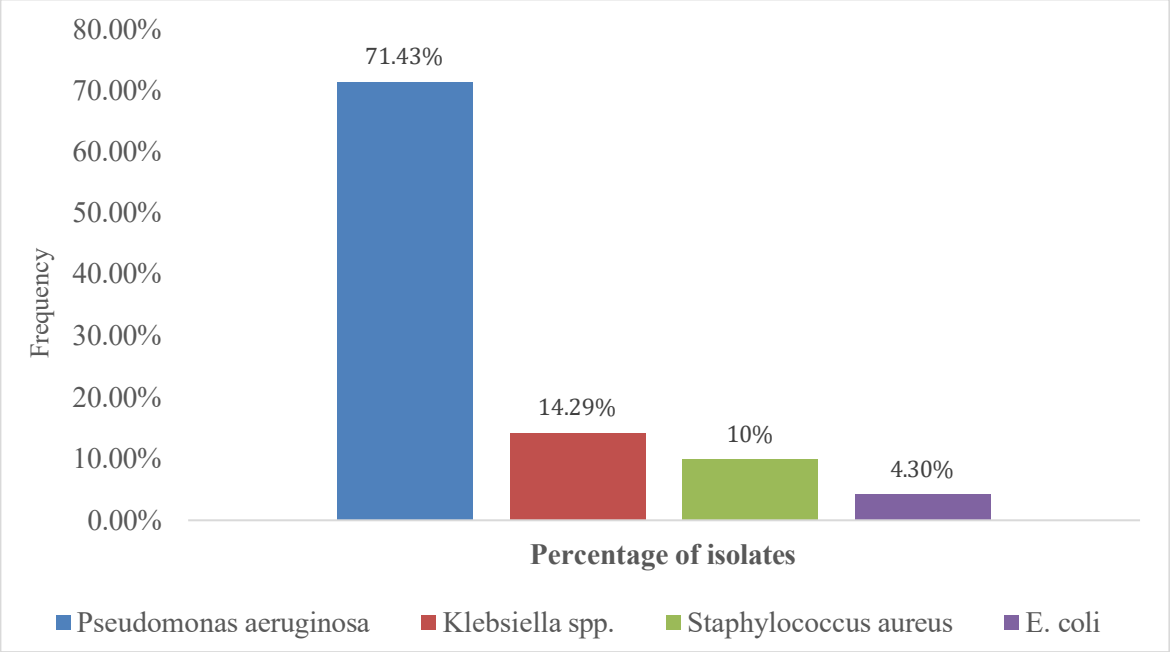

Figure 6 Types of bacteria isolated from burn wounds

Table 7 Prevalence and Antibiotic Resistance Profile

			Antibiotic Resistance Profile%									
Name of Isolates	No. Isolates	P %	AMP	CTX	KF	C	CIP	E	G	AN	TM	W
Pseudomonas aeruginosa	50	71.43	80	55.56	77.78	100	100	44.4	55.56	77.78	100	100
Klebsiella spp.	10	14.29	60	55	44.4	55	44.4	78	56	55	45	44.4
Staphylococcus aureus	7	10	40	50	44.4	43	40	44.5	60	22.2	67	45
E. coli	3	4.3	45	60	78	66	30	79	100	80	100	80

Ampicillin (AMP), Cefotaxime (CTX), Cephalothin (KF), Chloramphenicol(C), Ciprofloxacin (CIP) Erythromycin (E), Gentamycin (G), Nalidixic acid (NA), Tobramycin (TM), Trimethoprim (W)

Table 7 contains an analysis of the prevalence of antibiotic-resistant bacteria on isolated bacteria as

well as the antibiotic resistance profile of these bacteria. According to the most recent studies, the incidence of Pseudomonas aeruginosa was much higher than that of other isolates. When compared to other antibiotics, AMP indicated that the level of resistance to Ampicillin (AMP), Cefotaxime (CTX), Cephalothin (KF), and Chloramphenicol (C) was significantly higher.

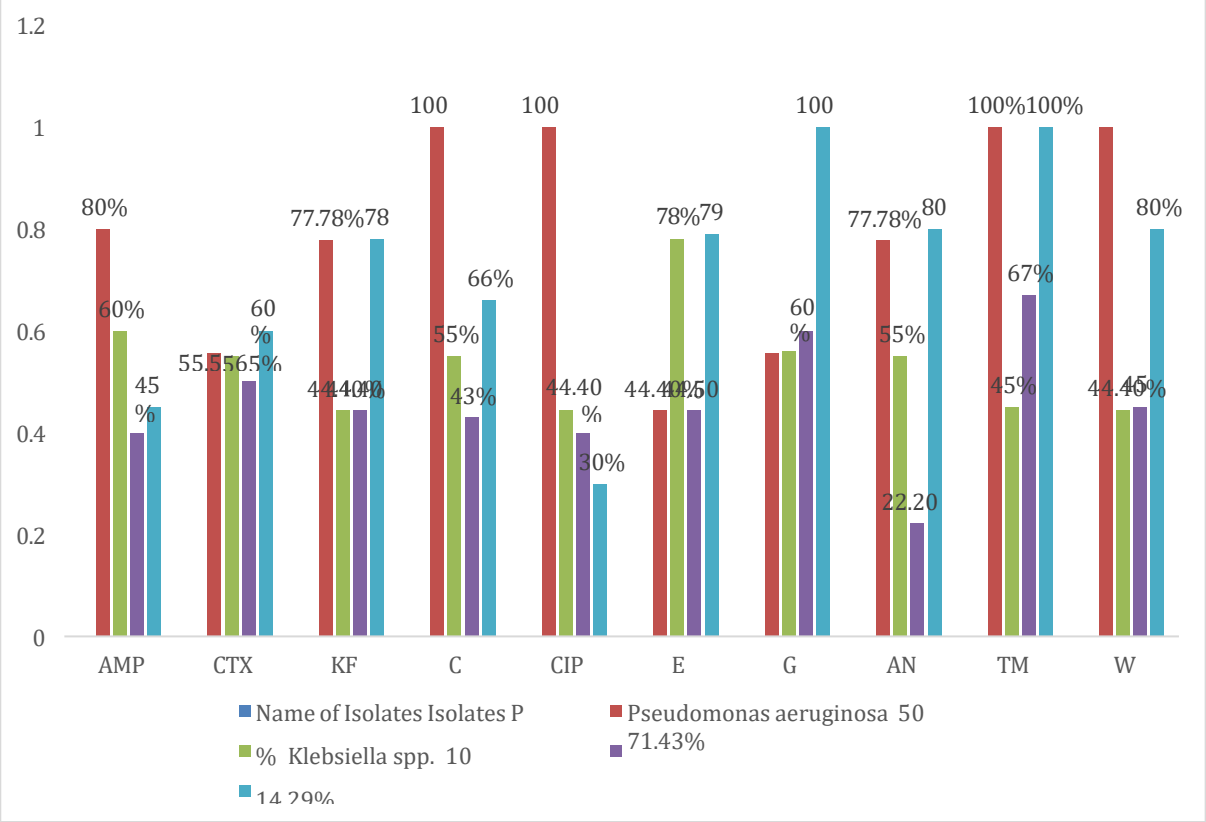

Figure 7 Prevalence and Antibiotic Resistance Profile

DISCUSSION

The phenomenon of burn infection is a significant public health concern in D.I Khan, Khyber Pakhtunkhwa. Burn injuries require body cleaning and some may result in lifetime complications or even death. The infection caused by the burn actively spreads bacteria from the native microbiota as well as the contaminated environment that is present within the damaged tissue (Opalekunde *et al*., 2014). There are a number of elements that contribute to the pathogenicity of an infection, including adhesion, nutrition, and the immune system of the patient. Antibiotics can be directly delivered as a topical preparation, either orally or through injections, to treat burn infections and prevent them from occurring in the first place. There was an infection incidence of eighty percent in individuals who had received burns, according to the findings of the current study (Mohammed, *et al*., 2013).

In 2004, Mooney and colleagues carried out a study with the purpose of examining the microbial connection with burn wounds (Rao, *et al*., 2013; Shriyan, *et al*., 2010)). In line with the conclusions of the inquiry, these findings are consistent. In the year 2004, Manjula and her colleagues were successful in isolating a wide variety of bacterial isolates from illnesses that happened as a result of thermal injury. There were infections caused by

S. aureus, epidermis, and *Klebsiella* that were contained within the isolates (Mohammad, *et al*., 2013). During the course of our examination, we discovered the presence of eight distinct types of bacteria. These species include pseudomonas aeruginosa, *Staphylococcus aureus*, *klebseilla*, and *E. coli*. A detailed and trustworthy representation of the outcomes of our investigation is provided by the results that have been presented.

The outcomes of this investigation were succinct, as we were able to successfully identify eight different bacteria. These bacteria are as follows: pseudomonas aeruginosa, *Staphylococcus aureus*,

klebseilla, and *E. coli*. The combination of *pseudomonas auregenousa*, *Klebsiella*, *Staphylococcus aureus*, *E. coli*, and Epistema was shown to be the most common cause of burn infections, according to a study that was carried out by Devi and colleagues in the year 2011. There is a correlation between the findings of the current study and those of previous researchers. Additionally, it is consistent with the studies that Barrett and his colleagues conducted in the year 2002.

According to the findings of the current inquiry, the prevalence of *pseudomonas auregenousa* was 72.43 percent, whereas *Klebsiella* was 14.29 percent, *Staphylococcus aureus* was 10 percent, and *E. coli* was 4.33 percent. Both the findings stated in reference (Devi et l., 2011) and the results that were achieved are congruent with one another. A number of factors may be responsible for the increased prevalence of Pseudomonas aeruginosa, *Staphylococcus aureus*, and Klebsella. These factors include their capacity to live in extremely unfavorable environmental conditions, their ability to be resistant to a number of medications, and their pervasive presence in the air.

There is also a correlation between the findings of this current investigation with the findings of reference (Nazeer *et al*., 2014). It is consistent with the findings that were reported in reference (Ahmed *et al*., 2014) that the lowest frequencies that we observed in

E. coli were 6.97% that we observed. The fact that children between the ages of one and fifteen are at a great risk of experiencing burn injuries has been demonstrated by a substantial amount of research According to the results of our research, the age group that had the largest proportion of burn injuries was age group 26-45 years, followed by the age group of 11-25 years, which had a percentage of thirty percent. There is a correlation between the findings that have been reported here and the research that Chees Brough and his colleagues (Bayram *et al*., 2013)have conducted. There is a lack of awareness about the consequences of fire, and parents are negligent, which are both factors that contribute to the high rate. QUECH's study provides evidence that supports our results.

Children between the ages of one and fifteen years old had a significantly higher prevalence of burn infections, according to Komolafe *et al*. (2003). Prior research carried out by Zetola and colleagues, as well as research carried out by Church *et al*. in 2006, is in agreement with the findings of our study. A number of factors may be responsible for the heightened infection rate, including the prevalence of untreated wounds and the increased exposure of adults to contaminated settings outside. Not only was that, but the prevalence of infections in females 70%, which was significantly higher than the figure for males, which was 30% (Magnet *et al*., 2013).

According to these studies, hands are more likely to sustain burn injuries, namely an infection that accounts for the largest percentage. The hands accounted for 16% of the detections, with the legs coming in a close second. 12.3% chest, 9% belly, 4% face, 2% arm fit, and 1% back round out the percentages. For the same reason, the publications of the QECH point to the same proportion (Nayak *et al*., 2014). The term "antibiotic resistance" refers to the ability of microorganisms to demonstrate resistance to antibiotics that they were previously susceptible to producing. It is common practice to provide a wide variety of medications to burn patients, which leads to a high level of resistance among the bacteria that are associated with burns. In order to determine the effectiveness of antibiotics against major bacterial strains, around ten antibiotics of varied generations and modes of action were test. Among the bacteria that have higher levels of antibiotic resistance, Pseudomonas, *Staphylococcus aureus*, and *Klebseilla* are known. The conclusions of the current study are in direct opposition to those of other researchers.

CONCLUSION

The present study determined that a burn is an injury to the skin or other bodily tissues induced by different agents such as gas flames, scalds, and electrical currents. A wide range of microorganisms, including Pseudomonas Aeruginosa, *Klebsiella*, *Staphylococcus aureus*, and *E. coli*, were extensively isolated from these wounds. For the present study, 10 antibiotics were evaluated against these bacteria. It was shown that levofloxacin and ciprofloxacin were more efficacious than the remaining antibiotics. Therefore, careful antibiotic selection is crucial for the efficient treatment of burn site infections to reduce morbidity and death associated with multi-drug resistant organisms.

In the treatment of burn patients, bacteriological blood cultures, wound swab cultures, and antibiotic

susceptibility profiles of isolates must be performed for each patient under ideal conditions. Nevertheless, this is impractical for resource-limited countries; hence, regular monitoring of burn unit isolates should be performed to detect dominant circulating bacteria. This aids clinicians in choosing appropriate antimicrobial agents when empirical treatment is critical in urgent situations, a common procedure in burn units.

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