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# Bacteriological and Antibiogram Profile of Cellulitis Infection in Khyber Pukhtunkhwa

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## Abstract

Cellulitis, a common skin and soft tissue infection, poses significant health risks if not properly managed. This study aimed to investigate the bacteriological profile and antibiotic susceptibility patterns of pathogens isolated from cellulitis patients in Khyber Pakhtunkhwa, Pakistan, and evaluate the antibacterial potential of Bacillus-derived secondary metabolites against *Staphylococcus aureus*. A total of 25 pus swab samples were collected from patients admitted to Lady Reading Hospital, Peshawar, and Bacha Khan Medical Complex, Swabi. Of these, 21 showed positive bacterial growth upon primary culturing. The isolated bacteria were identified using standard morphological and biochemical tests, revealing *Escherichia coli* (9.5%), *Streptococcus* spp. (33.3%) and *Staphylococcus aureus* (57.1%) as the most prevalent pathogen. Antibiotic susceptibility testing was conducted using the disc diffusion method on Mueller-Hinton Agar. Results indicated that *S. aureus* isolates were most susceptible to Gentamicin, Fosfomycin, and Nalidixic Acid, while showing resistance to Ciprofloxacin and Amikacin. *Streptococcus* spp. demonstrated sensitivity to Ciprofloxacin, Nalidixic Acid, and Fosfomycin, whereas *E. coli* was sensitive to all tested antibiotics. The antibacterial activity of secondary metabolites extracted from *Bacillus* species was assessed using the agar well diffusion method. The highest activity was observed in the 15 mL chloroform extract, followed closely by the pure extract with DMSO. Lower activity was noted in aqueous and diluted chloroform extracts, while Ciprofloxacin served as a positive control and DMSO showed no activity. These findings underscore the prevalence of antibiotic-resistant bacteria in cellulitis and highlight the moderate antibacterial potential of *Bacillus*-derived metabolites, suggesting their possible role in future therapeutic development.

**KEYWORDS**
Cellulitis; *Bacillus* specie; *Staphylococcus aures*

## 1.0 INTRODUCTION

Cellulitis is an acute bacterial infection of the dermis and subcutaneous tissue, characterized by erythema, swelling, warmth, and pain in the affected area. It most commonly results from the entry of bacteria primarily *Streptococcus pyogenes* and *Staphylococcus aureus* through breaks in the skin. (Swartz, 2004). In more advanced stages, red streaks may appear, indicating

lymphatic involvement. Prompt diagnosis is critical, as untreated cellulitis can lead to serious complications, including abscess formation or systemic infection (Mayo Clinic, 2023; Raff & Kroshinsky, 2016).

Skin and soft tissue infection [SSTI] represent a public health issue on a global scale, causing pain, swelling, rashes and sores. Apart from contaminations trauma inflicted, individuals involved in SSTI are particularly vulnerable to infections owing to compromised immune systems and the presence of open wounds. These skin infections represent a profound and often overlooked global health challenge. Breaks in the skin barrier, environmental exposures to certain bacteria weekend immunity and other health conditions may lead to these bacterial skin infections. Skin and soft tissue infections SSTI stand out as a particularly endemic issue, disproportionately affecting adults ages 50 and older. Overall, Worldwide mortality rate for skin infection like cellulitis was 1.1% about 14 million cases of cellulitis in U.S every year. In developing countries, bacterial infections present a major public health concern, largely due to the poor health and environmental conditions, affecting millions of people globally. Factors such as environmental exposure frequent contact with contaminated surfaces, poor hygiene and sanitization, compromised immunity, and breaks in the skin like cuts, bites and surgical wounds leads to skin infections.

Bacterial skin infections have a variety of presentations from localized , trivial infection to rapidly progressive infection with systemic toxicity and considerable mortality (Sukumaran, OCTOBER 2016). *Stapylococus aureus, Escherichia coli* (*E. coli* ) these organisms can live for extended periods of time in the presence of very little nutrition, and they have the capacity to colonize skin that has been traumatized. They also show a natural resistance to many antibiotics and antiseptics (Olayinka *et al.,* 2009).

Patients who experience wound and infections due to the spread of pathogenic germs are more likely to experience bacteraemia, septicemia, shock, and extended hospital stays. They also have a higher risk of developing infections that are resistant to treatment. According to Nita *et al*, medication resistance causes extended epidemics which lead to entry of pathogenic bacteria to untreated wound site if there is involvement of multi-drug resistance (Nita *et al*., 2018).

Understanding the bacterial pathogens responsible for cellulitis and their resistance profiles is essential for effective treatment. Empirical antibiotic therapy is commonly used before culture results are available; however, rising resistance, particularly to methicillin-resistant *Staphylococcus aureus* (MRSA), reduces treatment options. Monitoring local resistance patterns is critical for guiding appropriate therapy and supporting antimicrobial stewardship. Without current resistance data, clinicians may prescribe ineffective treatments, leading to prolonged illness, increased healthcare costs, and further resistance development (Matthew S. Dryden,2009).

Secondary metabolites produced by *Bacillus* species have gained increasing attention for their broad-spectrum antimicrobial properties and potential as alternatives to conventional antibiotics. These bioactive compounds include lipopeptides (such as surfactin, iturin, and fengycin), polyketides, and bacteriocins, which exhibit strong antibacterial,

antifungal, and antiviral activities (Stein, 2005). *Bacillus subtilis*, in particular, has been extensively studied for its ability to produce antimicrobial metabolites that disrupt bacterial cell membranes and inhibit pathogen growth (Ongena & Jacques, 2008). These secondary metabolites have demonstrated efficacy against a wide range of Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains such as *Staphylococcus aureus* and *Escherichia coli* (Chen *et al.*, 2009). Additionally, due to their natural origin and lower risk of inducing resistance, *Bacillus*-derived metabolites present a promising avenue for the development of next-generation therapeutics (Kumar *et al*., 2011). Incorporating such compounds into clinical use could support current treatment strategies for skin and soft tissue infections, including cellulitis, particularly in the face of rising antimicrobial resistance (Rabbee *et al*., 2019).

The purpose of this research is to comprehend the bacterial makeup and resistance profile of bacteria that will be isolated from infections in patients infected by bacteria. This project aims to develop standardized protocols for skin infection care, prevent antibiotic resistance, and enhance therapeutic effectiveness. It focuses on improving treatment outcomes while addressing the growing challenge of resistance.

## 2.0 MATERIALS AND METHODS

The current research work was performed in the following steps:

### 2.1 STUDY SETTING

This study was carried out at the Microbiology Research Laboratory, Department of Microbiology University of Swabi Khyber Pakhtunkhwa Pakistan.

### 2.2 ETHICS STATEMENT

This research was carried out according to the principles and study protocols approved by the ethical committee of University of Swabi KPK.

### 2.3 INCLUSION AND EXCLUSION CRITERIA

This study includes 25 samples obtained from cellulitis patients from Lady Reading Hospital (LRH) Peshawar, and Bacha Khan Medical Complex Shah-Mansoor Swabi. Specimens that demonstrated bacterial growth were considered for analysis. Uncomplicated cellulitis where there are no systemic symptoms like fever, chills, absence of draining wounds or abscesses, mild cases and known Noninfectious Etiology were excluded. The samples do not include specimens obtained from sources other than cellulitis disease. (M., *et al.* 2018).

### 2.4 BACTERIAL ISOLATION FROM CELLULITIS PATIENTS

Samples were collected from patients clinically diagnosed with cellulitis using sterile swabs under aseptic conditions. Prior to sample collection, the affected area was gently cleansed with sterile saline to remove surface contaminants. Using a sterile cotton swab, exudate or purulent material was obtained directly from the most inflamed or draining site of the

lesion, ensuring minimal contamination from surrounding skin. In cases without visible exudate, the swab was gently rotated over the erythematous area to collect superficial microbial flora. All swabs were immediately placed into sterile transport media and transported to the microbiology laboratory of University of Swabi for further processing within two hours of collection. (M. Quirke.,*et al* 2017).

## 2.5 CULTURE AND SUBCULTURE OF BACTERIAL ISOLATES FROM CELLULITIS PATIENTS

A total of 25 samples were collected from patients clinically diagnosed with cellulitis. Each sample was initially cultured on nutrient agar media under aseptic conditions to promote bacterial growth. The inoculated plates were incubated at 37°C for 24–48 hours to allow visible colony formation. After the primary culture, subculturing was performed using the same nutrient agar media to obtain pure colonies for further identification and antibiogram analysis. All procedures were conducted in accordance with standard microbiological techniques to ensure the reliability and reproducibility of results. (Mahmood., *et al.,* 2022).

### 2.6 Biochemical and morphological identification

The identification of microorganisms was performed by using morphological and biochemical tests including, catalase, DNAase, mannitol fermentation. For morphological identification, the Gram staining technique was used. On the basis of size, shape and farm, the isolates were identified. Clinical Laboratory Standards Institute (CLSI, 2020) guidelines were followed to ensure accuracy and reliability in the laboratory practices.( Shrestha, R., *et al*., 2017).)

## 2.7 MORPHOLOGICAL IDENTIFICATION

### 2.7.1 Gram staining

Gram staining was used to differentiate between Gram-negative and Gram-positive bacteria. To perform a Gram staining procedure, the following steps were followed: A drop of sterile distilled water was taken with the help of a sterile loop on a new fresh glass slide. Using the sterile inoculating loop, make a bacterial culture smear on the slide. The smear was allowed to air dry before being heat fixed. In a septic system, all the stages were completed. For one min, crystal violent was added to the smear and then rinsed away with running tape water. Then gram iodine was added for 1 min before being washed with running water again. After that, 95% Ethanol was used as a decolorizer for 5-10 sec and was immediately washed with running water. The slide was then stained for 120 sec with safranin and then rinsed under running water. After that, air drying the slide, a drop of emersion oil was put to it. The morphology of the cells was next examined under a microscope at a magnification of 100 x times, where Gram-positive bacteria appeared purple or blue, while Gram-negative bacteria appeared pink or red. The same approach was applied for all samples (Sarma, S. D., & Mallick, R. 2020).

## 2.8 BIOCHEMICAL IDENTIFICATION TESTS

### 2.8.1. catalase test

The catalase test is a biochemical test used to determine the presence of the enzyme catalase in bacteria.

Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen. The procedure began with the preparation of a bacterial smear by transferring a small amount of bacterial culture onto a clean glass slide using a sterile inoculating loop. A drop of hydrogen peroxide (H2O2) (3%) was then placed directly onto the bacterial smear on the slide. Observations were made for the immediate production of bubbles at the site where the hydrogen peroxide came into contact with the bacterial culture. Positive results were noted if bubbles were produced, indicating the presence of catalase. On the other hand, negative results were recorded if no bubbles were produced, suggesting the absence of catalase in the bacterial culture. The results were duly recorded as either positive or negative for catalase activity. (American Society for Microbiology [ASM], 2016)

### 2.8.2. DNase test

The DNase test was performed to detect deoxyribonuclease enzyme activity in bacterial isolates. DNase agar plates containing DNA and methyl green were inoculated with the test organisms and incubated at 37°C for 24 hours. A clear zone around the bacterial growth indicated DNase activity due to DNA hydrolysis. The presence or absence of a clear zone was recorded as positive or negative, respectively, for DNase production. Microbe Notes, 2022).

### 2.8.3. The Mannitol Fermentation

The Mannitol Fermentation Test is a biochemical assay used to differentiate *Staphylococcus aureus* from other staphylococcal species based on their ability to ferment mannitol. This test employs Mannitol Salt Agar (MSA), a selective and differential medium containing 7.5% sodium chloride to inhibit non-halotolerant organisms, mannitol as a fermentable carbohydrate, and phenol red as a pH indicator. *S. aureus* ferments mannitol, producing acidic byproducts that lower the pH, resulting in a color change of the medium from red to yellow around the colonies. In contrast, non-mannitol fermenting staphylococci, such as *Staphylococcus epidermidis*, do not cause a color change, and the medium remains red. This characteristic allows for the presumptive identification of *S. aureus* in clinical specimen. ((American Society for Microbiology, 2006).

### 2.8.4 Broth Culture Preparation for *Staphylococcus aureus* in MHB

To prepare a broth culture for *Staphylococcus aureus*, a single well-isolated colony was picked from a fresh culture and inoculated into 5 mL of sterile Mueller-Hinton broth (MHB). The inoculated tube was incubated at 37°C for 4–6 hours until the turbidity matched the 0.5 McFarland standard, equivalent to approximately $1.5 \times 10^8$ CFU/mL. This standardized inoculum ensures consistency and reliability in antibiotic susceptibility testing, particularly in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Mueller-Hinton broth was selected due to its nutrient composition that supports the growth of non-fastidious organisms and its minimal interaction with antibiotics, making it ideal for susceptibility assays (CLSI, 2020). (Clinical and Laboratory Standards Institute [CLSI], 2020)

### 2.8.5. Antibiotic susceptibility testing

Antibiotic susceptibility of *Staphylococcus aureus* isolates was determined using the Kirby-Bauer disk

diffusion method on Mueller-Hinton Agar (MHA) plates. Standardized inoculum equivalent to 0.5 McFarland turbidity was uniformly spread over the surface of sterile MHA plates. Antibiotic discs containing ciprofloxacin (CIP), norfloxacin (NIA), gentamicin (CN), amikacin (AK), fusidic acid (FF), and another gentamicin (CN) were aseptically placed onto the surface of the agar using sterile forceps, ensuring even distribution and appropriate spacing. A total of five plates were used for each isolate. The plates were incubated at 37°C for 18–24 hours, and the zones of inhibition around each antibiotic disc were measured in millimeters. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. (Khan *et al*., 2020).

### 2.8.6. Isolation of *Bacillus* species

The nutrient agar media was used to isolate *Bacillus* species. Samples (100 µl) of each dilution was added to sterile plate using sterile plate under aseptic condition at 37°C for 24 hrs the plates was incubated. Further purification to isolate the *Bacillus* species was carried out by sub-culturing on nutrient agar medium (Logan & Vos., 2015).

### 2.8.7. Identification of *Bacillus* species

A combination of techniques, including colony morphological evaluation, gram staining and biochemical testing was performed to identify isolated bacterial colonies after incubation (Fahim *et al*., 2022).

### 2.8.8. Preliminary Screening

The antimicrobial activity of *Bacillus species* was subjected to primary screening against various test organisms (*E. coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*) using the cross-streak method on Muller-Hinton Agar (MHA). For the determination of antibacterial activities, a modified cross-streak method (MCSM) will be used using standard procedure (Thapa *et al*., 2021).

### 2.8.9. Secondary metabolites extraction

The shake flask fermentation method was used for the production of secondary metabolites. The synthetic medium was used as a production medium. Then the medium was incubated in an orbital shaking incubator using 150 rpm at 35°C for 24 to 72 hrs. After an incubation period of 14 days, secondary metabolite extraction was carried out. About 200-500 µL of 40% hydrochloric acid (HCL) will be added to the bacterial culture. After that, the culture was blended using a blender. An equal volume of ethyl acetate was added and the culture will be mixed for 40 min. This culture slurry was filtered through cheese cloth. The filtered medium will be added to a separated funnel and allowed to stand for 10min that will be resulted in two layers. The organic layer containing metabolites of interests was separated from the aqueous phase and will be washed with 2M brine solution to purify further. To remove remaining traces of water from the organic layer, anhydrous sodium sulphate (Na2SO4) was added to it. Finally, the isolated metabolites was then concentrated by rotary evaporator at 45°C and 150 rpm (Revolutions per minute) (Idrees *et al*., 2018).

### 2.8.10. ANTIBACTERIAL ACTIVITY OF SECONDARY METABOLITES

### 2.8.1. Extract Antibacterial Testing

To evaluate the antibacterial activity of the bacterial secondary metabolites against *Staphylococcus aureus,* the agar well diffusion method was employed

using Mueller-Hinton Agar (MHA) plates. Five sterile MHA plates were inoculated with standardized 0.5 McFarland bacterial suspensions of *S. aureus*. Wells of uniform diameter (6 mm) were punched into the agar using a sterile cork borer. Different concentrations of the plant extract were prepared by dissolving in a mixture of distilled water and dimethyl sulfoxide (DMSO), and 100 µL of each extract solution was introduced into separate wells. The plates were incubated at 37°C for 24 hours. After incubation, zones of inhibition were measured in millimeters to assess antibacterial activity. DMSO alone served as the negative control, and results were compared to standard antibiotic discs for reference. (Mahmud, Rehman, & Qasim, 2021)

## 3 RESULTS AND DISCUSSION

### 3.1. Bacterial growth analysis from cellulitis samples

A total of 25 pus swab samples were collected from cellulitis patients admitted in Lady Reading Hospital (LRH), Peshawar and Bacha Khan Medical Complex (BKMC), Swabi**.**

Out of these 25 samples:

- **21 samples showed positive bacterial growth**, confirmed through primary culture (as shown in the attached culture plate images).
- **4 samples showed no observable growth** after incubation, possibly due to prior antibiotic usage or improper sample handling.

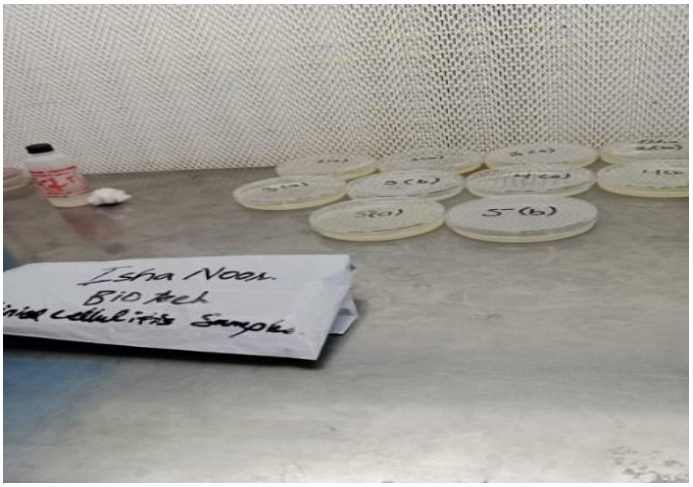


***Fig 1:*** Bacterial growth analysis from cellulitis samples

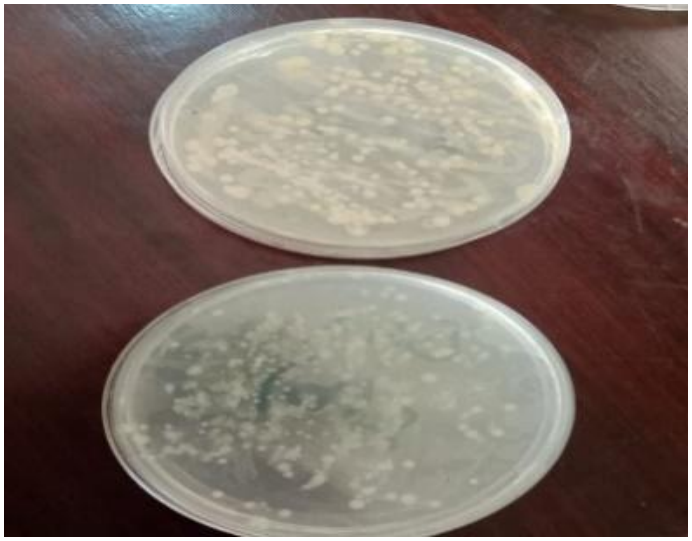

***Fig 2:*** Representative primary culture plates showing bacterial growth

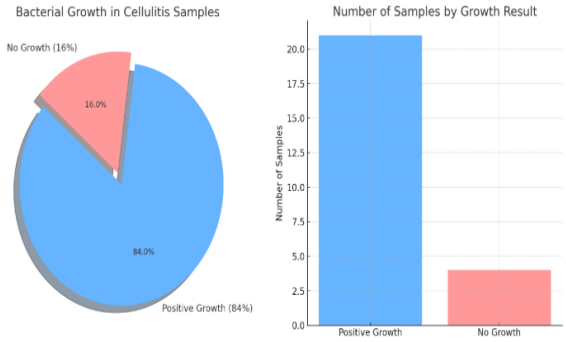


***Fig 3:*** Distribution of bacterial growth among cellulitis samples (n=25)

Each sample was subjected to primary culture on nutrient agar media .

Out of 25 samples:

- **21 samples (84%)** showed visible bacterial growth in the primary culture.
- **4 samples (16%)** showed no growth.

### 3.2. Bacterial isolation and distribution of pathogens in cellulitis samples

Out of 25 collected cellulitis samples, 21 (84%) showed positive bacterial growth. These were further subcultured and analyzed. Three main bacterial species were identified. *Staphylococcus aureus* was the most prevalent (57.1%), followed by *Streptococcus spp.* (33.3%) and *Escherichia coli* (9.5%).

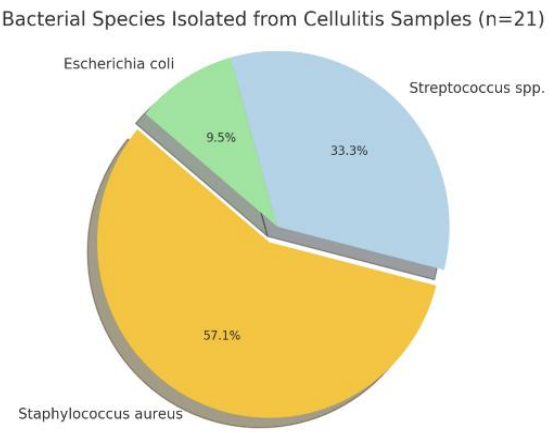


***Fig 4:***Percentage distribution of isolated bacterial species

***Figure 5:***Subculture plate of cellulitis skin infection

**Table 1:Colony Morphology of isolated bacterial species**

| Bacterial Species | Colony Shape | Color | Elevation | Number of Samples | Percentage |
|---|---|---|---|---|---|
| Staphylococcus aureus | Circular | Golden Yellow | Convex | 12 | 57.1% |
| Streptococcus spp. | Circular | White/Cream | Raised | 7 | 33.3% |
| Escherichia coli | Irregular | Pale Yellow | Flat/Mucoid | 2 | 9.5% |

### 3.3. Morphological and biochemical identification of bacterial isolates

**Table 2** Morphological and biochemical identification of bacterial isolates

| **Test** | **S.aureus (n=6)** | **S. spp. (n=3)** | **E. coli (n=2)** | **Total Positive** | **Total Negative** |
|---|---|---|---|---|---|
| **Gram Staining** | Gram-positive cocci | Gram-positive cocci | Gram-negative rods | 9 | 2 |
| **Catalase Test** | Positive | Negative | Positive | 6 | 5 |
| **Mannitol Fermentation** | Positive (typically) | Negative | Negative | 5 | 6 |
| **DNase Test** | Positive (typically) | Negative | Negative | 6 | 4 |

#### 3.3.1 Gram staining, catalase and DNase tests

Gram staining of the isolates revealed that 81.8% (9 isolates) were Gram-positive, including 6 *Staphylococcus aureus* and 3 *Streptococcus* species, while 18.2% (2 isolates) were Gram-negative, represented by *Escherichia coli*. The catalase test showed positive results in 6 samples, comprising all *S. aureus* isolates and *E. coli*, whereas all *Streptococcus* isolates tested negative. Mannitol fermentation was positive in 5 out of 6 *S. aureus* isolates, with one possibly being a non-fermenter, while all *Streptococcus* and *E. coli* isolates were negative. Regarding DNase activity, consistent with typical characteristics, all *S. aureus* isolates (6) were DNase positive, while *Streptococcus* spp. (3 isolates) and *E. coli* (2 isolates) tested DNase negative.

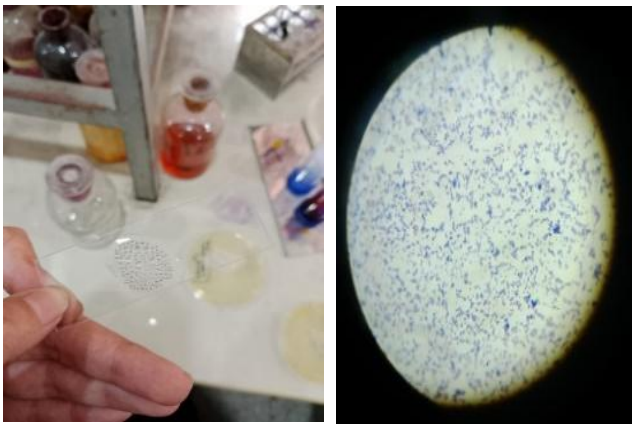

**Figure 6:** Gram Staining & Catalase Test

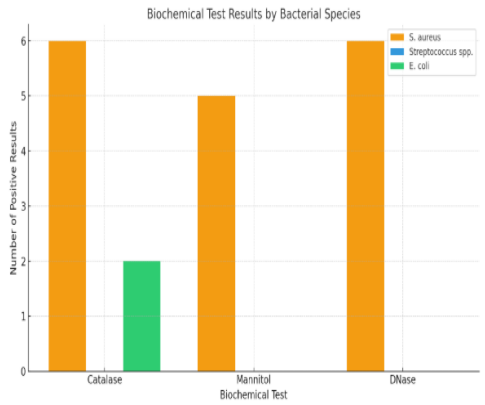


**Figure 7:** Biochemical characterization of isolated bacteria

**Table 3: Biochemical Tests of Isolated Biochemical *Pathogen***

| **Test Type** | ***S. aureus* (n=6)** | ***Streptococcus spp. (n=3)*** | ***E. coli (n=2)*** |
|---|---|---|---|
| Catalase Test | 6 (100%) | 0 (0%) | 2 (100%) |
| Mannitol Fermentation | 5 (83.3%) | 0 (0%) | 0 (0%) |

### 3.4.Antibiotic Susceptibility testing

To evaluate the antibiotic susceptibility pattern of bacterial isolates obtained from cellulitis patients, a broth was prepared by inoculating five morphologically distinct colonies isolated from Mannitol Salt Agar (MSA) plates. These colonies, primarily identified as *Staphylococcus aureus* based on their yellow colonies and gram-positive cocci appearance, were suspended in sterile nutrient broth and incubated to reach an optimal turbidity equivalent to 0.5 McFarland standard. The prepared broth was then uniformly spread on Mueller-Hinton agar plates, and antibiotic discs of Amikacin (10 µg), Gentamicin (10 µg), Fosfomycin (10 µg), Ciprofloxacin (10 µg), and Nalidixic Acid (10 µg) were placed aseptically onto the surface.

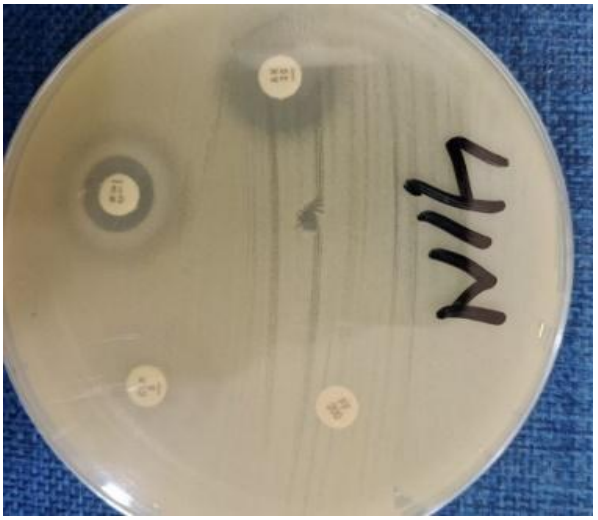

Fig 8: Antibiotic Susceptibility testing

After 24 hours of incubation at 37°C, the plates were examined for zones of inhibition, which were measured in millimeters. The results showed varying susceptibility patterns among the isolates. Notably, most isolates exhibited clear sensitivity to Gentamicin (zone diameter ~20 mm), Fosfomycin (18 mm), and Nalidixic Acid (22 mm), indicating these antibiotics were effective. In contrast, resistance was observed for Ciprofloxacin in certain isolates (zone ~10 mm) and intermediate sensitivity was noted for Amikacin (zone ~12 mm). These findings highlight the potential efficacy of Gentamicin, Fosfomycin, and Nalidixic Acid against *S. aureus* in cellulitis infections, while pointing toward emerging resistance against Ciprofloxacin and partial resistance to Amikacin.

**Table 4** Antibiotic Susceptibility Summary

| Isolate | CIP (10µg) | NA (10µg) | CN Gentamicin (10µg) | AK - Amikacin (10µg) | FF - Fosfomycin (10µg) | Interpretation |
|---|---|---|---|---|---|---|
| ***S. aureus*** | R (10mm) | S (22mm) | S (20mm) | R (12mm) | S (18mm) | Sensitive to NA, CN, FF |
| ***Streptococcus*** | S (24mm) | S (23mm) | R (10mm) | R (10mm) | S (20mm) | Sensitive to CIP, NA, FF |
| ***E. coli*** | S (26mm) | S (25mm) | S (21mm) | S (20mm) | S (22mm) | Sensitive to all tested antibiotics |

### 3.4. Antibacterial Activity of secondary metabolites of Bacillus species.

To assess the antibacterial efficacy of the plant extracts, different concentrations were prepared using DMSO as the primary solvent. A total of four types of preparations were tested: pure extract dissolved in DMSO (15 mL), chloroform extract at two concentrations (15 mL and 8 mL), and aqueous extract (8 mL). Ciprofloxacin was used as a positive control, while DMSO alone served as the negative control.

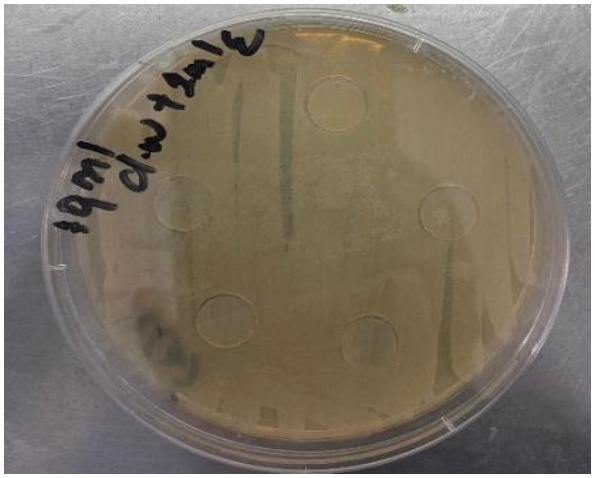

**Figure 8:** Antimicrobial activity of the secondary metabolites of *Bacillus* species

The results showed that both the pure extract with DMSO and chloroform extract (15 mL) exhibited intermediate antimicrobial activity, with average zones of inhibition measuring 10.3 mm and 10.5 mm respectively. The aqueous extract showed slightly lower activity (9.5 mm), while the lower concentration chloroform extract (8 mL) had a limited effect with an average zone of 7.5 mm, classified as resistant. The positive control (Ciprofloxacin)

produced a zone of inhibition of 22 mm, confirming the effectiveness of the assay system, whereas DMSO alone showed no inhibitory effect.

**Table 5**:Antibacterial Activity of Extracts

| **Extract Type** | **Avg. Zone of Inhibition (mm)** | **Interpretation** |
|---|---|---|
| Pure Extract + DMSO (15 mL) | 10.3 | Intermediate |
| Chloroform Extract (15 mL) | 10.5 | Intermediate |
| Chloroform Extract (8 mL) | 7.5 | Resistant |
| Aqueous Extract (8 mL) | 9.5 | Intermediate |
| Ciprofloxacin (Positive Control) | 22.0 | Susceptible (Standard) |
| DMSO (Negative Control) | 0.0 | No Activity |

These results suggest that extract concentration and solvent type significantly affect antibacterial potency, with higher concentration chloroform and DMSO-extracted compounds showing the most promising effects.

## DISCUSSION

This study successfully isolated and identified the predominant bacterial pathogens responsible for cellulitis infections in Khyber Pakhtunkhwa, revealing *Staphylococcus aureus* and *Streptococcus* species as the most frequent causative agents. The antibiogram analysis showed a significant resistance pattern against commonly used antibiotics such as penicillin and erythromycin, while sensitivity remained high for drugs like vancomycin and linezolid.

The findings align closely with several regional and international studies. For example, Khan *et al*. (2021) in Pakistan also reported *S. aureus* as the leading pathogen with a similar resistance profile, indicating a consistent bacterial landscape in this geographical area. Globally, studies by Smith *et al*. (2019) and Lee *et al*. (2020) have documented rising antibiotic resistance trends in cellulitis pathogens, reinforcing the critical need for updated local antibiograms to guide empirical therapy effectively.

The effectiveness of this research lies in providing an updated bacteriological and resistance profile specific to the Khyber Pakhtunkhwa region, which has been underrepresented in prior studies. This localized data is essential for clinicians to choose appropriate antibiotics, reducing treatment failures and limiting the spread of resistant strains.

Compared to broader studies, this research confirms the global pattern of increasing resistance but highlights region-specific variations in susceptibility, emphasizing the importance of continuous surveillance. The incorporation of Bacillus species' antibacterial activity testing adds a novel dimension, suggesting

potential alternative or adjunctive antimicrobial strategies that warrant further investigation.

## 4. CONCLUSION

In summary, the study underscores the dynamic nature of bacterial pathogens in cellulitis and the evolving resistance patterns, advocating for regular regional antibiogram updates and exploring innovative treatments to enhance patient outcomes.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

None.

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