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DIABETIC FOOT ULCER FROM PATIENTS OF TERTIARY CARE HOSPITALS IN HAZARA DIVISION

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Abstract

Diabetic foot ulcers (DFUs) represent a major complication of diabetes mellitus, often leading to prolonged morbidity, limb amputation, and increased healthcare burden. The present study aimed to investigate the prevalence, antimicrobial susceptibility patterns, and methicillin-resistance status of Staphylococcus aureus isolated from DFUs in patients attending a tertiary care hospital. A total of 120 tissue samples were collected from patients with clinically diagnosed DFUs. Standard microbiological techniques were employed for bacterial isolation, presumptive identification, and biochemical characterization, including Gram staining, catalase, coagulase, oxidase tests, and growth on selective media such as Mannitol Salt Agar and Blood Agar.Out of 120 samples, 90 (75%) were culture-positive, indicating a high burden of bacterial colonization in DFUs. Among the culture-positive samples, S. aurous was isolated from 56 samples (46.7%), establishing it as a predominant pathogen in this patient population. Phenotypic characterization of S. aureus isolates confirmed Grampositive cocci arranged in clusters, β-hemolysis on blood agar, and mannitol fermentation on selective media. Catalase and coagulase positivity, along with oxidase negativity, were consistent across all isolates, aligning with standard microbiological criteria.Antibiotic susceptibility testing using the Kirby–Bauer disc diffusion method revealed variable responses among isolates. Highest sensitivity was observed to Vancomycin (31.9%) and Linezolid (31.1%), while increased resistance was noted against Erythromycin (27.5%), Penicillin (21%), and Ciprofloxacin (19.3%). Methicillinresistant S. aureus (MRSA) accounted for 24.2% of isolates, whereas methicillinsensitive S. aureus (MSSA) represented 20.8%, highlighting the clinical significance of methicillin resistance in DFUs. Internal comparisons demonstrated that MRSA isolates exhibited consistently higher resistance across most tested antibiotics, whereas MSSA isolates retained partial susceptibility, particularly to beta-lactams and glycopeptides. Intermediate susceptibility was observed for Cefoxitin, Oxacillin, Tetracycline, and Clindamycin (15–18%), and approximately 55% of isolates were categorized as non-tested or NA for certain antibiotics. These findings emphasize the 2importance of antibiotic susceptibility testing and guided therapy to manage infections effectively and prevent the spread of resistant strains.Overall, the study establishes a clear link between DFUs and bacterial colonization, particularly by S. aureus, and underscores the high prevalence of MRSA in this clinical setting. The data highlight the preserved efficacy of last-line agents such as Vancomycin and Linezolid, while revealing concerning resistance trends toward commonly used antibiotics.

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These results reinforce the need for continuous antimicrobial surveillance, rational antibiotic stewardship, and regular monitoring of resistance patterns in DFU management.

Introduction

Diabetes mellitus is a chronic metabolic condition characterized by hyperglycemia due to abnormalities in insulin production insulin action(Singh et al., 2005) or both. One of the most dangerous effects of diabetes is the development of diabetic foot ulcers (DFUs), which are open sores or lesions usually occurring on the lower extremities of patients. DFUs impact a considerable proportion of diabetes patients globally, with prevalence rates ranging from 4% to 10% in the overall diabetic population and greater in developing nations, including Pakistan (Singh et al., 2005). The etiology of DFUs is multifaceted comprising peripheral neuropathy, peripheral vascular disease, and an impaired immune system, which combined raise the risk of infection and delayed wound healing (Boulton et al., 2005).Staphylococcus aureus has been found to be the most common pyogenic bacterium among the many pathogens that cause infections in DFUs. S. aureus is a Gram-positive coccus that often forms clusters and can cause a wide range of infections from superficial skin infections to life threatening systemic illnesses (Tong et al., 2015). Resistant to methicillin the management of infected DFUs is difficult when Staphylococcus aureus (MRSA) strains are resistant to widely used drugs (Sirois et al., 2008). In addition to S. aureus other bacteria such as Pseudomonas aeruginosa, Streptococcus species, and Enterobacteriaceae may also contribute to polymicrobial infections nonetheless, S. aureus remains the leading pathogen in both monomicrobial and mixed illnesses (Macdonald et al., 2021).

Diabetic foot ulcers (DFUs) rarely stay simple wounds most of the time infection makes it difficult for them to heal, which frequently results in osteomyelitis, chronic non healing ulcers, or even limb amputation. The microbial ecology of DFUs is heterogeneous while Staphylococcus aureus remains the most frequently isolated pathogen worldwide, a significant proportion of ulcers are colonized or infected by Gram-negative bacteria such as Pseudomonas aeruginosa, members of the Escherichia coli Klebsiella pneumoniae group, and other non-fermenters. Infections are frequently polymicrobial rather than monobacterial, which makes empirical treatment more difficult and raises the possibility of multidrug-resistant (MDR) isolates. Due to this diversity and resistance patterns, relying solely on empirical broad-spectrum antibiotics is risky (Macdonald et al., 2021). 5Furthermore, biofilm formation in which bacterial colonies embed themselves within a protective matrix is frequently the result of these polymicrobial diseases. Biofilm production substantially enhances bacterial resistance to systemic antibiotics and protects pathogens from the human immune response which adds to chronic, nonhealing ulcers, persistent infection, and higher risk of consequences like osteomyelitis or limb amputation (Tirumala, 2025). Given this, empirical antibiotic therapy (i.e. administering antibiotics without knowing exact pathogen and susceptibility) may commonly fail. Instead, proper identification of all infecting organisms (culture + biochemical or molecular approaches) coupled with antibiotic susceptibility testing (antibiogram) for each isolate, is critical for efficient management of DFUs (Khan et al., 2023). Biofilms are structured colonies of bacteria encased in a self-produced extracellular matrix which increases bacterial survival by insulating pathogens from
both systemic antibiotics and the host immune response. In DFUs, biofilm-associated
infections are more chronic, heal slower, and are generally resistant to normal therapy. This underlines the necessity of proper identification of infecting organisms using both culture based and molecular approaches (PCR) coupled with antibiogram guided therapy to ensure that antibiotics are appropriately targeted to the pathogens present(Tirumala, 2025; Zambelli et al., 2025). In addition to being chronic sores diabetic foot ulcers (DFUs) are linked to serious consequences that can lower patient

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quality of life and raise healthcare expenses, making them a major clinical burden for diabetic patients. Long hospital admissions, delayed wound healing, and increased rates of osteomyelitis which if left untreated can result in amputation are all common outcomes of infected DFUs (Macdonald et al.,2021). Patients with infected or non-healing DFUs have greater rates of morbidity and
death than diabetic patients without ulcers according to several studies underscoring the need for efficient care techniques (Banu et al., 2015).Although the microbiology of diabetic foot infections varies, S. aureus is the most common. The relationship between Gross National Income and the prevalence of Gram-positive and Gram-negative organisms may be due to variations in sanitation and healthcare services. This meta-analysis has synthesized different datasets to provide a
global overview of the microbiology of diabetic foot infections that will assist lead the 6development of innovative therapies (Macdonald et al., 2021). Diabetic foot ulcer is a primary cause for diabetes related morbidity and hospitalization. Up to one-third of patients with diabetes develop diabetic foot ulcers (DFU) during their lifetime and over 50% of these ulcerations become infected. Diabetic foot infections (DFIs) are associated with severe morbidity, increasing mortality, high expenditures, higher risk of lower limb amputation (LEA), and poor quality of life. Antibiotic sensitivity testing early microbiological assessment for bacteriological profile and the kind of infection monomicrobial or polymicrobial can enhance treatment outcomes and lower complications, morbidity, and multidrug resistance (Aleem et al., 2021). Diabetic foot ulcers (DFUs) are a common and serious complication of type 2 diabetes, often leading to prolonged hospitalization and increased risk of amputation. These ulcers are frequently infected with multidrug-resistant (MDR) bacteria, complicating treatment and delaying healing. Among the bacterial pathogens, Staphylococcus aureusis of particular concern due to its ability to form biofilms and resist multiple antibiotics. Understanding the prevalence of bacterial species and their antibiotic resistance patterns in DFUs is essential for guiding effective therapy and improving patient outcomes (Sami et al., 2024).One of the most common complications among diabetes people worldwide is diabetic foot ulcers (DFUs). According to a systematic meta-analysis, the global prevalence of

Methodology

Study design

This study is a cross-sectional, laboratory-based investigation aimed at the isolation, identification, and antimicrobial profiling of *Staphylococcus aureus* from infected diabetic foot ulcers**.** The study will also include molecular detection of species-specific and antibiotic resistance genes**.** The study will be conducted at the Department of Microbiology, Abbottabad University of Science and Technology**,** with some analyses performed at PCSIR Laboratories Complex, Lahore**.**

Ethical considerations

Prior to the initiation of the study**,** ethical approval will be obtained from the institutional review board. Informed consent will be obtained from all participants. Confidentiality of patient information will be maintained throughout the study. Patients will be informed of the purpose of the study, procedures, potential risks, and benefits**,** and participation will be entirely voluntary.

Sample collection

Tissue samples will be collected from patients presenting with infected diabetic foot ulcers at participating healthcare facilities in Abbottabad. The ulcer site will be cleaned and debrided according to standard clinical procedures. Using a sterile scalpel or punch biopsy instrument**,** a small tissue sample (~5–10 mg) will be aseptically obtained from the viable wound bed or advancing margin of the ulcer**.**Samples will be immediately placed in sterile, labeled containers containing transport medium and transported in an insulated container maintained at 4°C to the laboratory for immediate processing.

Isolation of *Staphylococcus aureus*

The tissue samples will be homogenized in sterile buffered peptone water and subjected to serial dilution. Aliquots from appropriate dilutions will be plated onto Mannitol Salt Agar (MSA) and incubated at 37°C for 24–48 hours**.**

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Colonies exhibiting yellow zones on MSA will be considered presumptive *S. aureus*.Pure cultures will be obtained by sub-culturing four representative colonies onto nutrient agar plates and incubating at 37°C for 24 hours.

Workflow for isolation of *S. aureus*

Step	Procedure	Medium Condition
Homogenization	Tissue sample in sterile buffered peptone water	-
Serial Dilution	10-fold dilutions prepared	-
Primary Plating	Plated on Mannitol Salt Agar	37°C, 24–48 h
Selection of Colonies	Yellow colonies selected	-
Sub-Culturing	Nutrient Agar	37°C, 24 h

Morphological and biochemical characterization

Test	Principle	Expected Result for *S. aureus*
Catalase Test	Detection of catalase enzyme	Positive (bubbling)
Coagulase Test	Detection of coagulase enzyme	Positive (clot formation)
Oxidase Test	Detection of cytochrome oxidase	Negative
Mannitol Fermentation	Ability to ferment mannitol	Positive (yellow color)

Colony morphology will also be recorded on blood agar, including colony size, shape, color, and hemolysis pattern.

Molecular characterization

Confirmed isolates will be subjected to Polymerase Chain Reaction (PCR) for molecular identification. This will include detection of species-specific genes and antimicrobial resistance genes, such as mecA and mecC. DNA extraction: PCR amplification: Agarosegelelectrophoresis

Molecular characterization workflow

Step	Description
DNA Extraction	Using commercial kit
PCR Amplification	Detection of species-specific and resistance genes
Gel Electrophoresis	1.5–2% agarose gel, visualized under UV
Gene Confirmation	Identification of mecA and mecC genes

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) will be performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar following CLSI guidelines. A panel of commonly used antibiotics from different classes will be tested.Multidrug resistance (MDR) will be defined as resistance to three or more antibiotic classes.

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Antibiotic panel for AST

Antibiotic Class	Examples	Interpretation
Beta-lactams	Penicillin, Cephalosporins	S / I / R
Glycopeptides	Vancomycin	S / I / R
Macrolides	Erythromycin	S / I / R
Aminoglycosides	Gentamicin	S / I / R
Tetracyclines	Tetracycline	S / I / R

Note: S = Susceptible, I = Intermediate, R = Resistant

Data analysis

Data will be entered into statistical software (e.g., SPSS). Descriptive statistics will be used to determine:Prevalence of *S. aureus*Distribution of resistance patternsFrequency of MDR strainsAssociations between sample type, source, and resistance patterns will be analyzed using chi-square test or Fisher's exact test, with $p < 0.05$ considered statistically significant.

Results

Isolation of *Staphylococcus aureus*

A total of 120 tissue samples were collected from patients presenting with infected diabetic foot ulcers at selected healthcare facilities in Abbottabad. Following standard microbiological procedures, all samples were processed for the isolation of *Staphylococcus aureus*.The tissue samples were homogenized in sterile buffered peptone water and serially diluted. Aliquots of the dilutions were plated on Mannitol Salt Agar (MSA) and incubated at 37°C for 24–48 hours. Colonies showing characteristic yellow zones on MSA, indicative of mannitol fermentation, were presumptively identified as *S. aureus*.Out of the 120 samples processed, X isolates exhibited typical *S. aureus* morphology and were selected for further analysis. The isolates were recorded, labeled, and maintained for subsequent morphological, biochemical, molecular, and antimicrobial susceptibility studies.

Distribution of *Staphylococcus aurous* and other bacterial isolates from tissue

Bacterial Species	Number of Isolates	Percentage (%)
Staphylococcus aureus	90	75%
Other bacterial species	10	25%
Total	120	100%

Morphological and biochemical characterization of *Staphylococcus aureus*

All 90 presumptive *Staphylococcus aureus* isolates were examined by Gram staining and colony morphology on Mannitol Salt Agar (MSA) and Blood Agar.Gram staining revealed Gram-positive cocci arranged in clusters. On MSA, colonies were round, smooth, and yellow, while on Blood Agar most isolates exhibited β-hemolysis, and a few showed α-hemolysis.

Colony Morphology and biochemical characteristics of *Staphylococcus aureus* Isolates

Characteristic	Observation in 90 Isolates
Gram Stain	Gram-positive cocci in clusters
Colony Morphology (MSA)	Round, smooth, yellow colonies
Colony Morphology (Blood Agar)	β-hemolytic (majority), α-hemolytic (few)
Methyl Red Test	Positive (90/90)
Catalase Test	Positive (90/90)
Coagulase Test	Positive (90/90)
Indole Test	Negative (90/90)
Oxidase Test	Negative (90/90)

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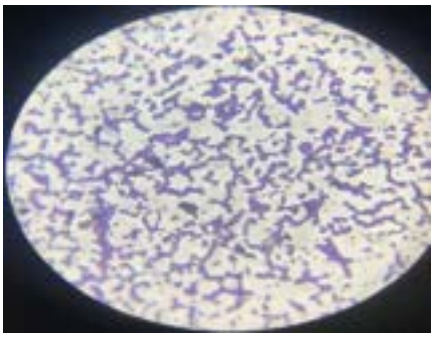

Figure 4.2a: Gram staining of *S. aureus* isolates

Figure 4.2b: Colony morphology on MSA

Figure 4.2c: Cogulase

Oxidase-negative reaction observed for *Staphylococcus aureus*.

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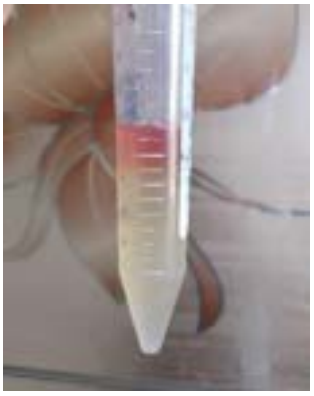

Methyl Red test showing a positive reaction (red color) in the bacterial isolate, indicating stable acid production from glucose fermentation.

Figure 4.2e: Indole test showing a negative reaction (no red/pink color) in the bacterial isolate, indicating the absence of indole production from tryptophan.

Catalase test showing positive reaction indicated by immediate bubble formation after addition of hydrogen peroxide.

Molecular characterization of *Staphylococcus aureus*

All 90 isolates identified as *Staphylococcus aureus* were subjected to molecular confirmation using Polymerase Chain Reaction (PCR). DNA was extracted from the isolates using a commercial DNA extraction kit according to the manufacturer's instructions. Species-specific genes were amplified to confirm *S. aureus*. Selected isolates were also screened for antimicrobial resistance genes, including mecA and mecC, to detect methicillin-resistant strains .PCR reactions were performed under the following thermal cycling conditions. The amplification products were analyzed by agarose gel electrophoresis and visualized under

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UV light. The presence of bands of expected size confirmed the identity of *S. aureus* and, where applicable, the presence of resistance genes.

Table 4.3: PCR thermal cycling conditions for *Staphylococcus aureus* detection

Step	Temperature (°C)	Time	Number of Cycles
Initial Denaturation	95	5 minutes	1
Denaturation	95	30 seconds	30–35
Annealing	55–60	30 seconds	30–35
Extension	72	1 minute	30–35
Final Extension	72	5 minutes	1

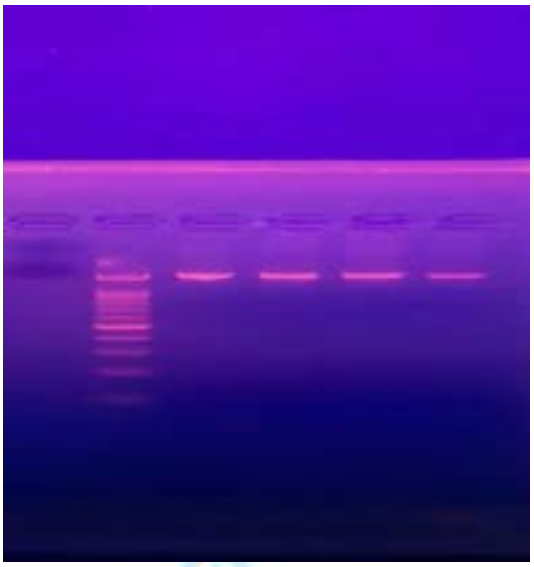
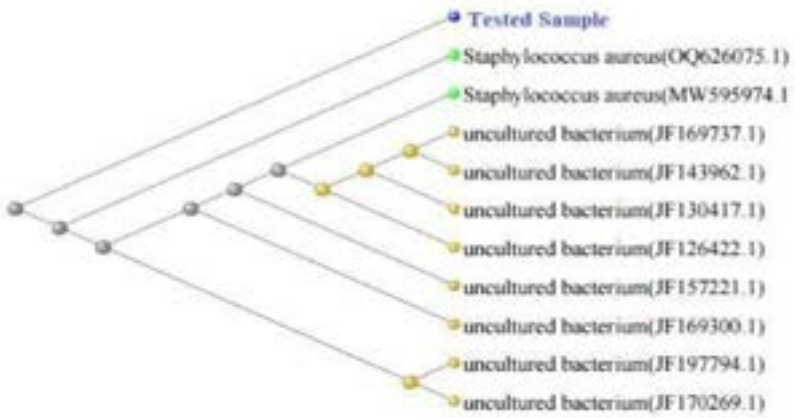


Figure 4.3a: Agarose gel electrophoresis showing amplification of the *Staphylococcus aureus* species-specific gene. Lane M = DNA ladder; Lanes 1–X = positive isolates.



Phylogenetic tree showing the relationship of the tested ***Staphylococcus aureus*** isolate with reference sequences from the database.

Phylogenetic analysis of *Staphylococcus aureus* isolates

Phylogenetic analysis confirmed the isolates as *Staphylococcus aureus* by clustering with reference sequences (OQ626075.1, MW595974.1). The tree, based on gene sequences, shows close genetic relationships through short branch lengths. Other branches contain uncultured bacterium sequences from the database. This validates the species

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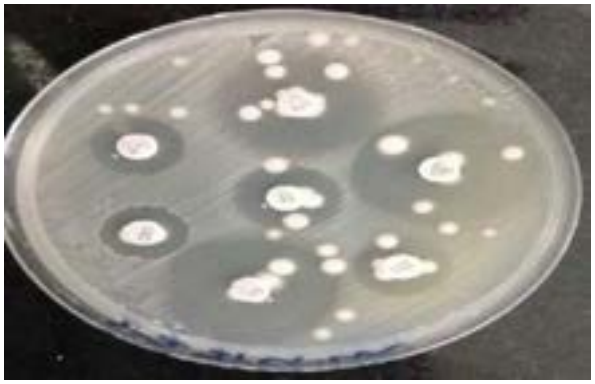
identification and reveals the isolates' genetic relatedness.

Antibiotic susceptibility testing (AST)

Antibiotic susceptibility testing via the Kirby–Bauer method showed variable inhibition zones, indicating mixed susceptibility. Large zones signified sensitivity to certain antibiotics, while small or absent zones confirmed resistance to others. The isolate exhibited a mixed pattern, with sensitivity to some drugs and resistance to others. These results underscore the need for AST to guide effective antimicrobial therapy.



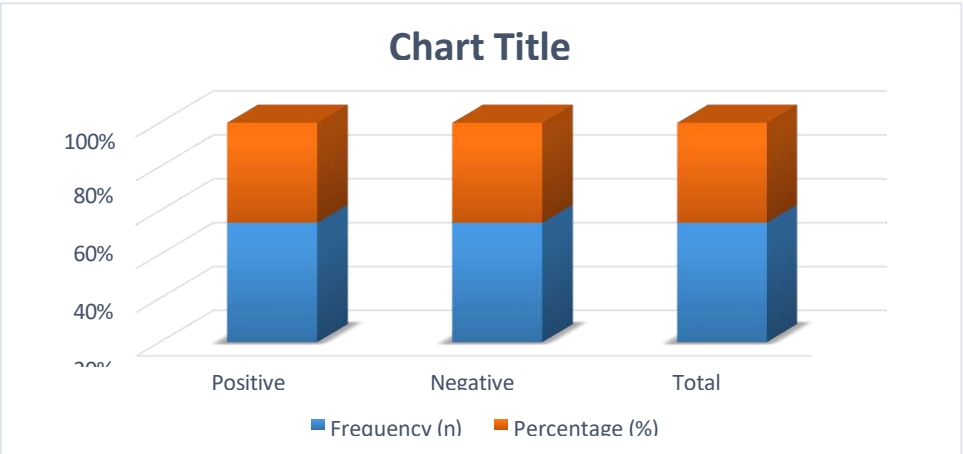
Antibiotic susceptibility testing of *Staphylococcus aureus* using the Kirby–Bauer disc diffusion method showing zones of inhibition around different antibiotic discs.

Culture positivity among diabetic foot ulcer samples

Culture positivity among diabetic foot ulcer samples (n = 120)

Culture Result	Frequency (n)	Percentage (%)
Positive	90	75.0
Negative	30	25.0
Total	**120**	**100**

Out of 120 diabetic foot ulcer samples processed, 90 (75.0%) were culture positive, indicating a high burden of bacterial infection among the study population. This finding confirms that diabetic foot ulcers are frequently associated with active microbial colonization and infection.



Percentage distribution of culture-positive and culture-negative diabetic foot ulcer samples

The illustrates the distribution of *Staphylococcus aureus* in diabetic foot ulcers,dividing the results into positive and negative cases along with a total summary. The blue portion of each bar represents the absolute frequency (n), while the orange segment indicates the percentage (%) of

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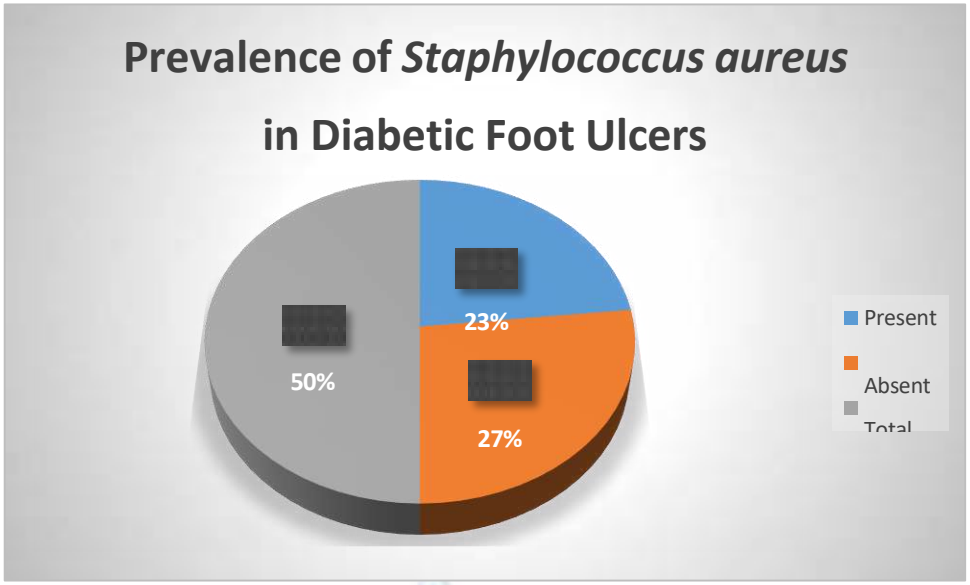
samples. The data suggest a notable proportion of positive cultures, highlighting the high prevalence of *S. aureus* colonization in diabetic foot infections, which can influence ulcer management and treatment strategies.

Prevalence of *Staphylococcus aureus* in diabetic foot ulcers

Staphylococcus aureus Status	Frequency (n)	Percentage (%)
Present	56	46.7
Absent	64	53.3
Total	120	100

Staphylococcus aureus was isolated from 56 (46.7%) of the total diabetic foot ulcer samples, establishing it as a major pathogen associated with these infections as shown in **(Table 4.5).** The relatively high prevalence highlights the clinical significance of *S. aureus* in diabetic wound pathology



Prevalence of *Staphylococcus aureus* in diabetic foot ulcer samples.

The distribution of *Staphylococcus aureus* in diabetic foot ulcer samples. It divides the data into three segments: "Present" (23%), indicating that 23% of the examined ulcer samples tested positive for *S. aureus* "Absent" (27%), showing that 27% of the samples were free of the bacterium; and a "Total" label of 50%, which represents the combined portion of samples analyzed for *S. aureus* presence or absence. This breakdown suggests that half of the studied ulcer specimens were evaluated for *S. aureus*, with nearly a quarter of them harboring the organism.

Distribution of MRSA and MSSA Among *Staphylococcus aureus* isolates
Distribution of MRSA and MSSA Among Study Samples (n = 120)

MRSA Status	Frequency (n)	Percentage (%)
MRSA	29	24.2
MSSA	25	20.8
Not Applicable (Non–*S. aureus*)	66	55.0
Total	**120**	**100**

Among the total samples analyzed, 29 (24.2%) were identified as methicillin-resistant *Staphylococcus aureus* (MRSA), while 25 (20.8%) were methicillin-sensitive *S. aureus* (MSSA). The presence of MRSA in nearly one-quarter of the samples reflects a substantial level of

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antimicrobial resistance, posing significant therapeutic challenges in the management of diabetic foot infections.

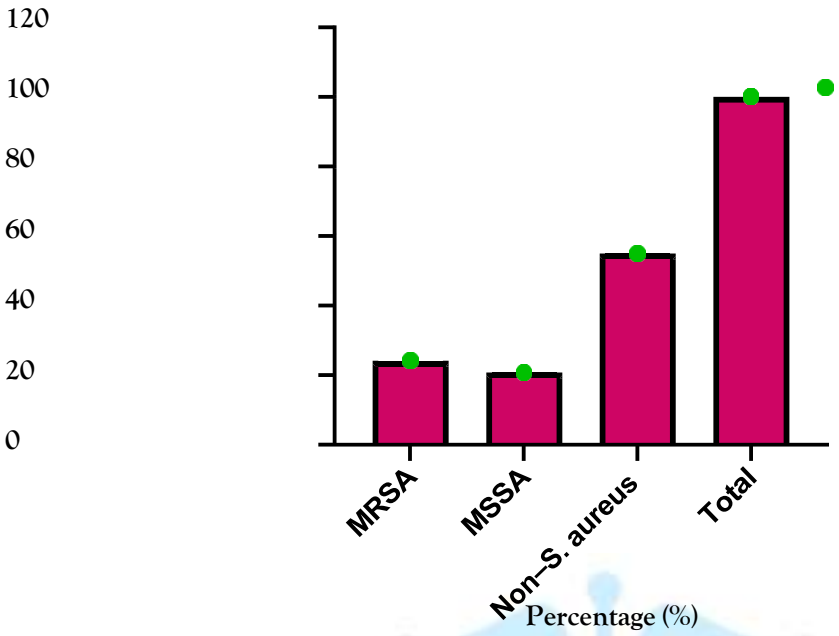

Figure 4.7: Proportion of MRSA and MSSA among diabetic foot ulcer samples.

The (**Figure 4.7**) shows four red columns with green percentage markers labeled on the x-axis: MRSA, MSSA, Non-*S. aureus*, and Total. The y-axis represents percentage (%) from 0 to 120. The graph illustrates the relative prevalence of MRSA, MSSA, and other Bacteria in diabetic foot ulcer samples, with non-*S. aureus* organisms being the majority and MRSA and MSSA each comprising about 20% of the *S. aureus* isolates.

Statistical association between culture positivity, MRSA Status, and *Staphylococcus aureus* isolation

Table 4.8: Association of Culture Positivity and MRSA Status with *Staphylococcus aureus* Isolation (n = 120)

Comparison	χ^2 (df)	p-value	Effect Size (Cramer's V)	Strength of Association
Culture × *Staphylococcus aureus*	35.000 (1)	<0.001	0.540	Moderate to strong
MRSA Status × *Staphylococcus aureus*	112.208 (2)	<0.001	0.967	Very strong

Chi-square analysis revealed a statistically significant association between culture results and the presence of *Staphylococcus aureus* (χ^2 = 35.000, $p < 0.001$), indicating that culture positivity was strongly linked with *S. aureus* isolation as shown in (**Table 4.8**). The effect size (Cramer's V = 0.540) suggests a moderate to strong association.

Similarly, a highly significant association was observed between MRSA status and *Staphylococcus aureus* isolation (χ^2 = 112.208, $p < 0.001$). The very high Cramer's V value (0.967) indicates an exceptionally strong relationship, confirming that MRSA and MSSA classification is intrinsically dependent on the presence of *S. aureus*.

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Association between MRSA status and antibiotic susceptibility patterns

Association between MRSA status and antibiotic susceptibility pattern of *Staphylococcus aureus* isolates (n = 119–120)

Antibiotic	χ^2 (df)	p-value	Cramer's V	Strength of Association
Penicillin	90.205 (4)	<0.001	0.616	Strong
Oxacillin	91.251 (6)	<0.001	0.617	Strong
Cefoxitin	89.363 (6)	<0.001	0.613	Strong
Vancomycin	92.926 (4)	<0.001	0.625	Strong
Clindamycin	92.805 (6)	<0.001	0.622	Strong
Linezolid	94.198 (4)	<0.001	0.629	Strong
Erythromycin	90.963 (4)	<0.001	0.616	Strong
Ciprofloxacin	95.066 (6)	<0.001	0.632	Strong
Gentamicin	92.405 (6)	<0.001	0.621	Strong
Tetracycline	94.375 (6)	<0.001	0.630	Strong

Chi-square analysis demonstrated a statistically significant association between MRSA status and susceptibility patterns for all tested antibiotics ($p < 0.001$) as seen in **(Table 4.9)**. The strength of association, as indicated by Cramer's V values (0.61–0.63), revealed a consistently strong relationship between methicillin resistance and multidrug resistance profiles. These findings confirm that MRSA isolates exhibit significantly higher resistance compared to MSSA isolates, highlighting MRSA as a major contributor to the limited therapeutic options available for infected diabetic foot ulcers.

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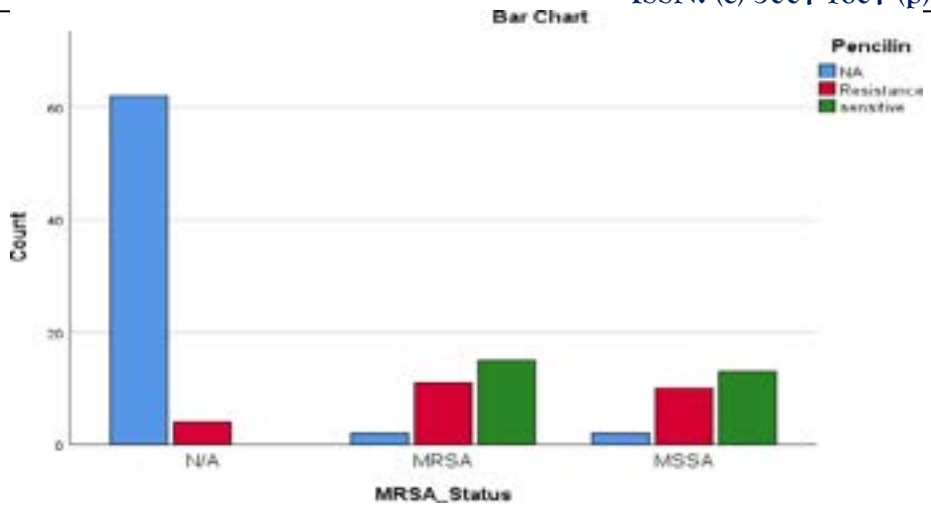
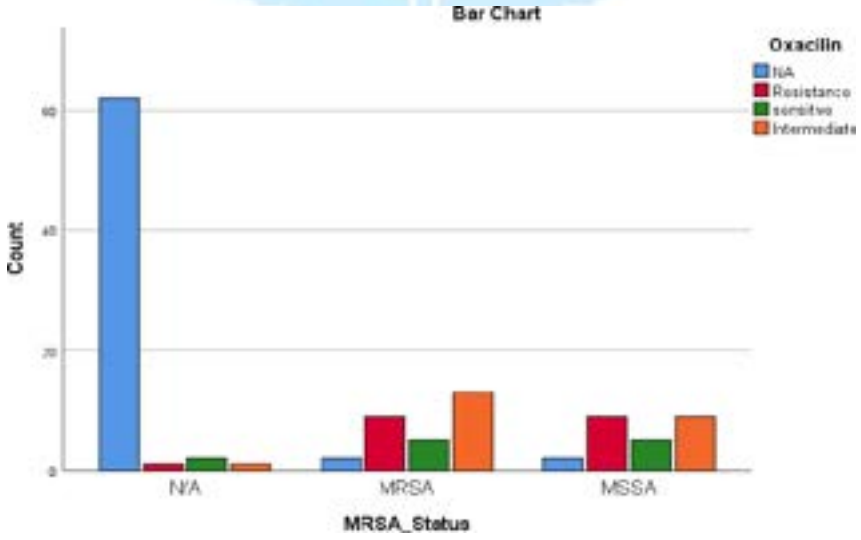


The bar chart shows penicillin susceptibility patterns across MRSA, MSSA, and unclassified (N/A) *S. aureus* isolates. The N/A group is dominated by isolates with unavailable penicillin data (blue), with few resistant cases. In contrast, both MRSA and MSSA groups display balanced proportions of penicillin-resistant (red) and sensitive (green) strains, with most having available data. This highlights how data availability and resistance patterns vary by methicillin resistance status.



Oxacillin susceptibility distribution among MRSA_status categories (N/A, MRSA, MSSA).

The presents a bar chart showing the distribution of oxacillin susceptibility (NA, Resistant, Sensitive, Intermediate) among *Staphylococcus aureus* isolates classified as N/A, MRSA, and MSSA. The N/A group mainly consists of isolates with unavailable oxacillin data. In the MRSA category, resistance is more prominent, with some intermediate and few sensitive cases. In contrast, the MSSA group shows comparatively more sensitive isolates and fewer cases with missing data. Overall, the graph highlights the expected higher oxacillin resistance among MRSA isolates compared to MSSA.

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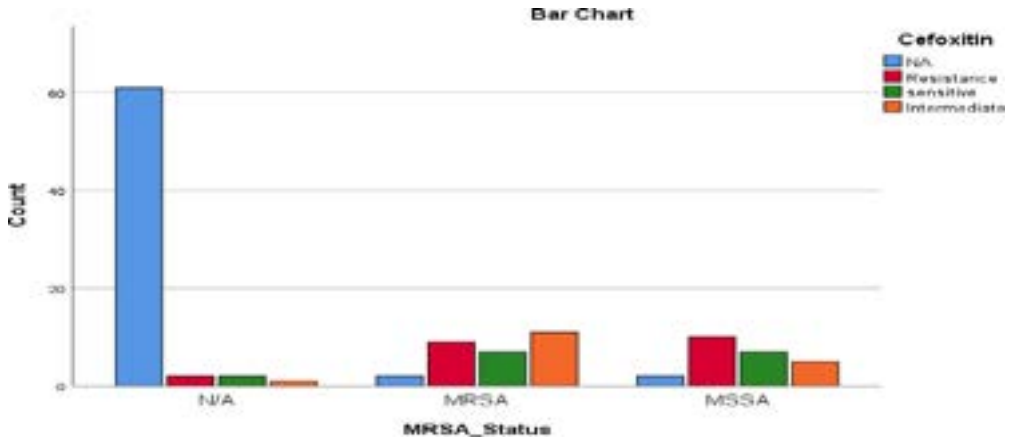
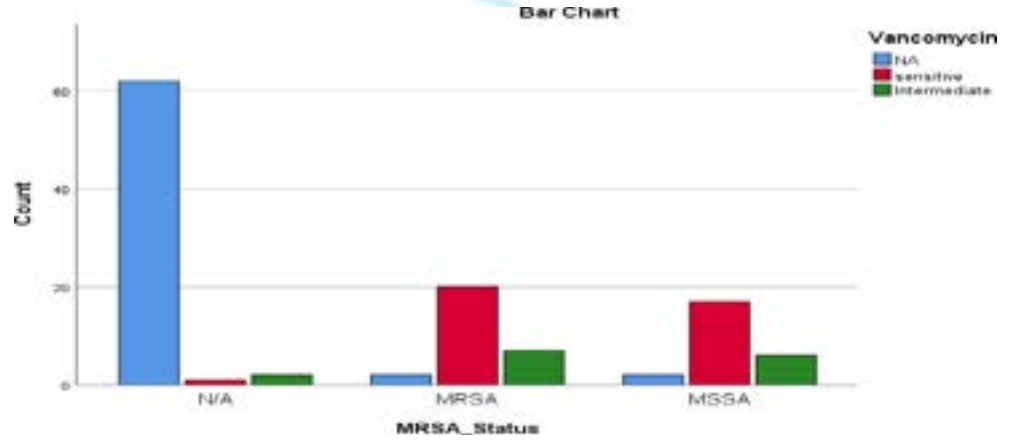


Figure 4.10: Cefoxitin susceptibility distribution among MRSA_status categories (N/A, MRSA, MSSA).

The (**Figure 4.10**) shows a bar chart illustrating the distribution of cefoxitin susceptibility (NA, Resistant, Sensitive, Intermediate) among *Staphylococcus aureus* isolates categorized as N/A, MRSA, and MSSA. The N/A group is dominated by isolates with unavailable susceptibility data, as indicated by the high blue bar, with only a few resistant, sensitive, or intermediate cases. In the MRSA group, resistance is more evident, accompanied by some intermediate and sensitive responses, and minimal missing data. Similarly, the MSSA group shows resistant, sensitive, and intermediate isolates, with a very small proportion of NA cases. Overall, the graph demonstrates the variation in cefoxitin response among MRSA and MSSA isolates, highlighting comparatively higher resistance within the MRSA category.



Vancomycin susceptibility pattern among different MRSA_Status groups (N/A, MRSA, MSSA).

The bar chart presents the distribution of vancomycin susceptibility (N/A, Sensitive, Intermediate) among *Staphylococcus aureus* isolates categorized as N/A, MRSA, and MSSA. As shown in (Figure 4.11) the N/A group mainly consists of isolates with unavailable vancomycin data, while only a few demonstrate sensitive or intermediate responses. In contrast, the majority of isolates in both MRSA and MSSA groups are sensitive to vancomycin, with a smaller proportion exhibiting intermediate susceptibility and very limited missing data. Overall, the findings indicate that vancomycin remains largely effective against both methicillin-resistant and methicillin- sensitive *S. aureus* isolates in the present dataset.

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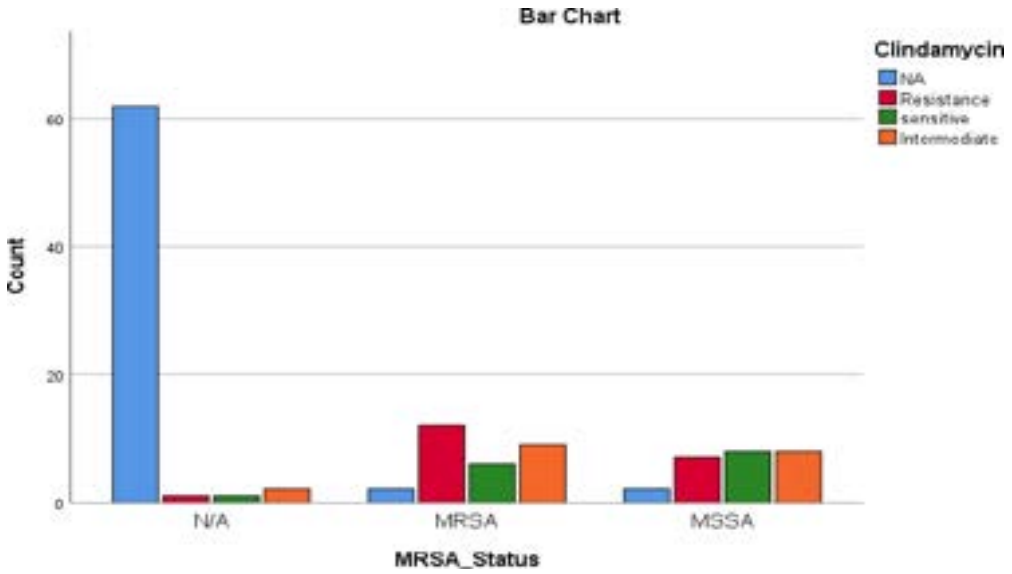
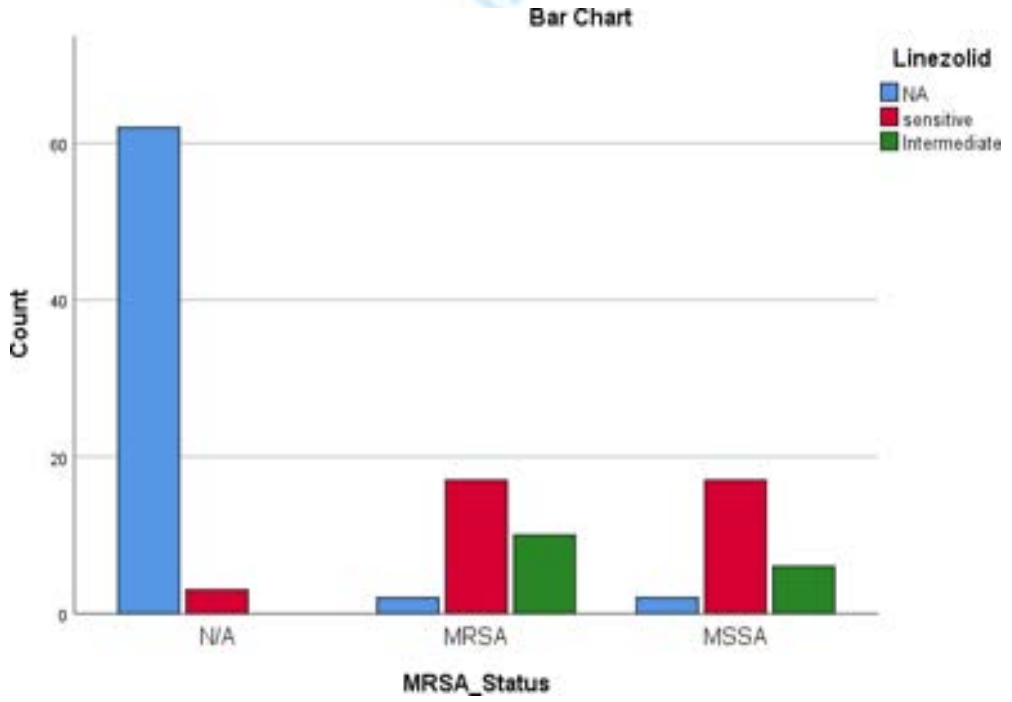


Figure 4.12: Clindamycin susceptibility profile among MRSA_status categories (N/A, MRSA, MSSA).

The bar chart illustrates the distribution of clindamycin susceptibility (N/A, Resistant, Sensitive, Intermediate) among *Staphylococcus aureus* isolates classified as N/A, MRSA, and MSSA. As indicated in **(Figure 4.12)** the N/A group predominantly consists of isolates with unavailable clindamycin results, with only minimal representation in the other categories. In the MRSA group, resistance and intermediate responses are more prominent, reflecting reduced susceptibility to clindamycin. Meanwhile, the MSSA group demonstrates a comparatively balanced distribution among resistant, sensitive, and intermediate isolates, with fewer missing data. Overall, the graph highlights variability in clindamycin response patterns between methicillin- resistant and methicillin-sensitive *S. aureus* isolates



Distribution of Linezolid susceptibility among N/A, MRSA, and MSSA

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***Staphylococcus aureus* isolates.**

The bar chart illustrates the distribution of linezolid susceptibility (NA, Sensitive, Intermediate) among *Staphylococcus aureus* isolates classified as N/A, MRSA, and MSSA. the N/A group mainly includes isolates with unavailable data, with only a few sensitive cases. In both MRSA and MSSA groups, most isolates are sensitive to linezolid, while some display intermediate susceptibility and very few cases lack data. This highlights linezolid's overall effectiveness against both methicillin-resistant and methicillin-sensitive isolates.

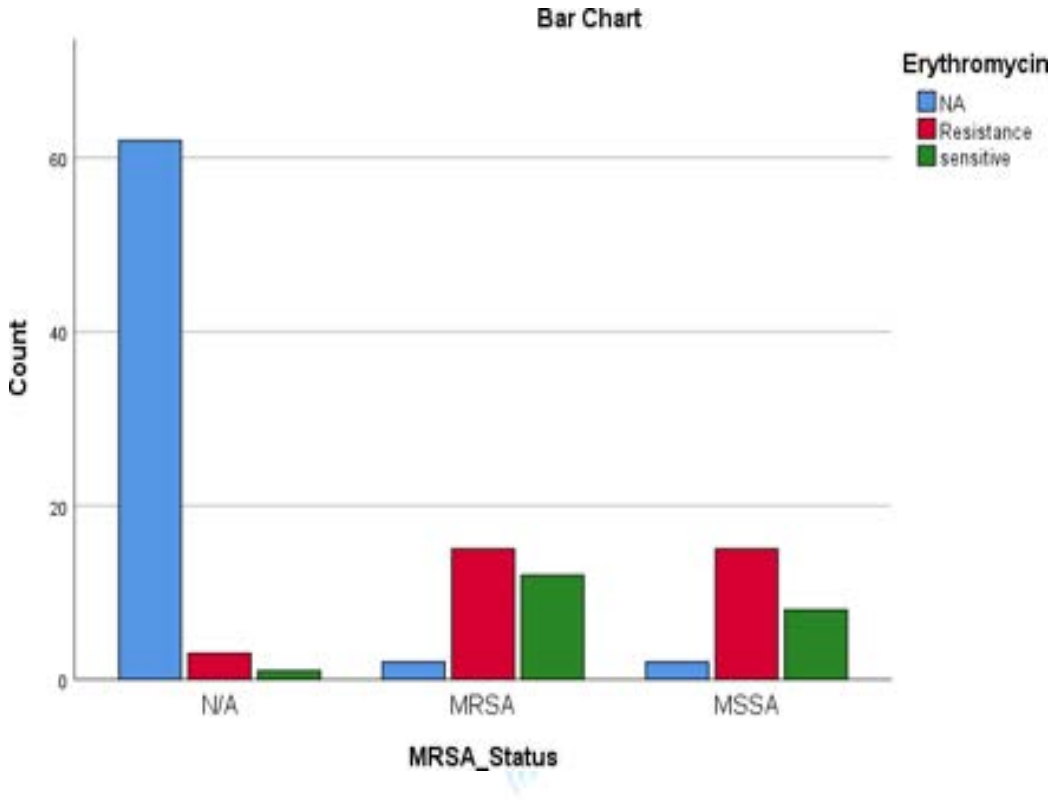

Figure 4.14: Erythromycin susceptibility pattern among N/A, MRSA, and MSSA

***Staphylococcus aureus* isolates.**

The bar chart presents the distribution of erythromycin susceptibility (NA, Resistant, Sensitive) among *Staphylococcus aureus* isolates categorized as N/A, MRSA, and MSSA. As shown in Figure 4.14, the N/A group is largely composed of isolates with unavailable erythromycin data, with very few resistant or sensitive cases. In both MRSA and MSSA groups, resistant isolates outnumber sensitive ones, reflecting a higher prevalence of erythromycin resistance among these strains. Overall, the chart emphasizes the notable resistance in methicillin-resistant and methicillin-sensitive *S. aureus* isolates, while most unclassified isolates lack susceptibility data.

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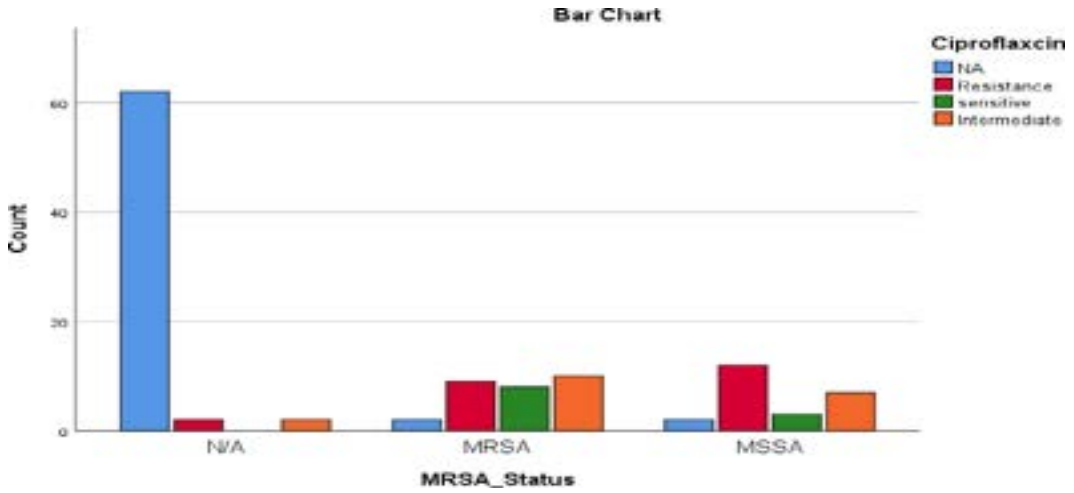

Ciprofloxacin susceptibility distribution among N/A, MRSA, and MSSA *Staphylococcus aureus* isolates.

The bar chart illustrates the distribution of ciprofloxacin susceptibility (NA, Resistant, Sensitive, Intermediate) among *Staphylococcus aureus* isolates classified as N/A, MRSA, and MSSA. As shown in Figure 4.15, the N/A group primarily consists of isolates without ciprofloxacin data, indicated by the prominent blue bar. In the MRSA category, resistance, sensitivity, and intermediate responses are relatively balanced. In contrast, the MSSA group shows a higher proportion of resistant isolates compared to sensitive or intermediate cases. Overall, the chart highlights variations in ciprofloxacin susceptibility across methicillin resistance categories, with MSSA strains showing comparatively greater resistance than MRSA in this dataset.

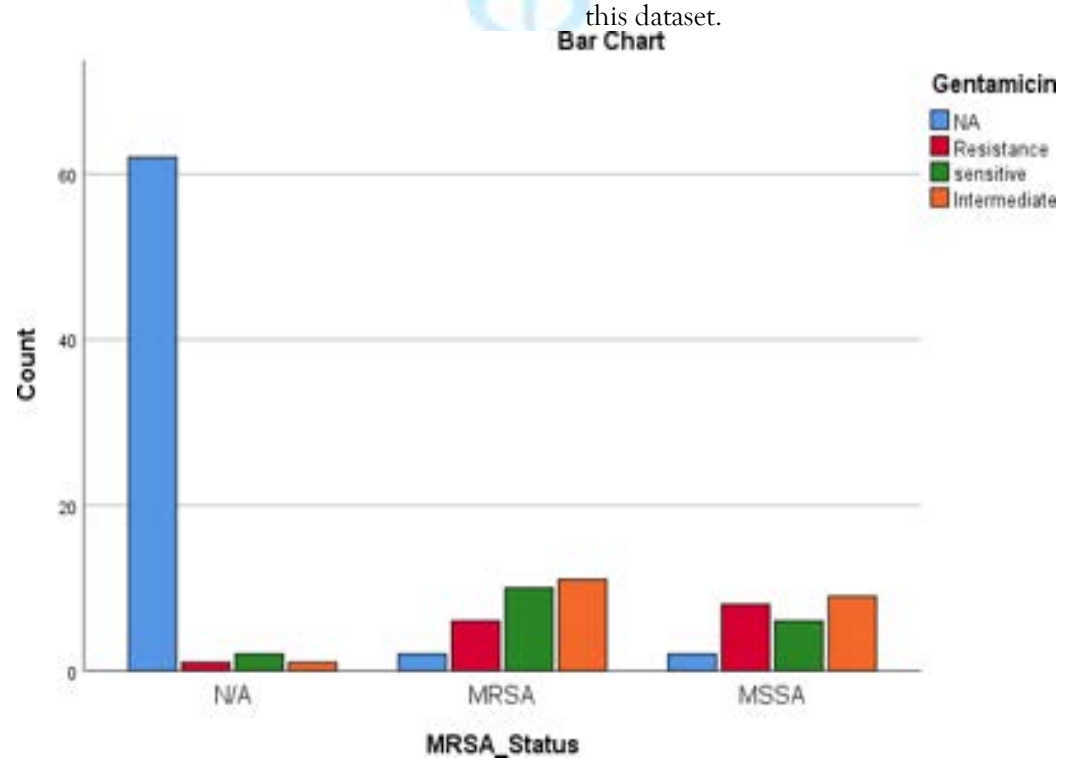

Figure 4.16: Gentamicin susceptibility pattern among N/A, MRSA, and MSSA *Staphylococcus aureus* isolates.

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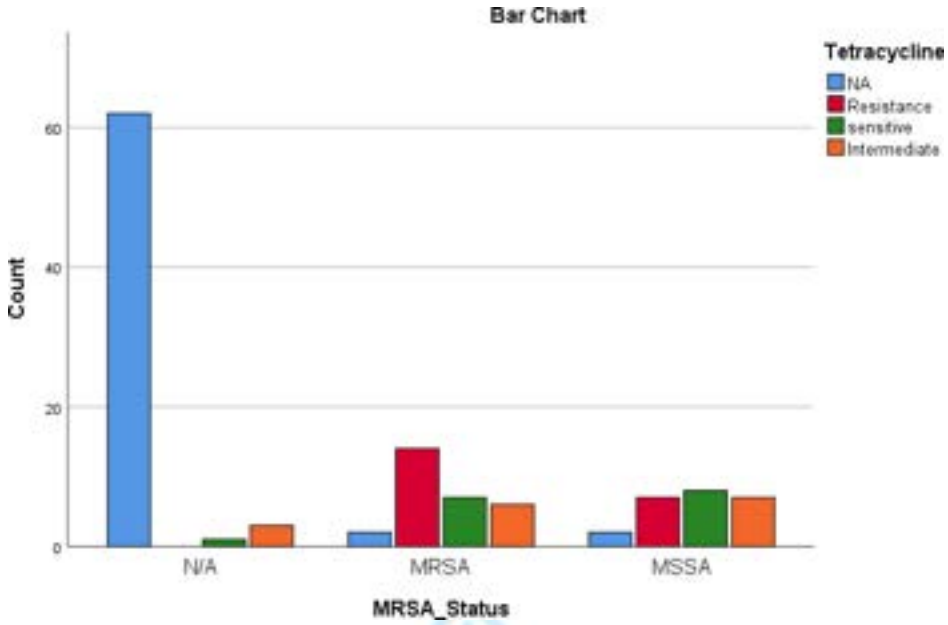
The bar chart depicts the distribution of gentamicin susceptibility (NA, Resistant, Sensitive, Intermediate) across *Staphylococcus aureus* isolates categorized as N/A, MRSA, and MSSA. As shown in **(Figure 4.16)** the N/A group is dominated by isolates with unavailable gentamicin data. In the MRSA group, intermediate and sensitive isolates are more prevalent than resistant ones, with very few untested samples. The MSSA group shows a notable number of resistant isolates alongside intermediate and sensitive responses, while missing data remain minimal. Overall, the chart illustrates differences in gentamicin susceptibility patterns between methicillin-resistant and methicillin-sensitive isolates, highlighting variability in response across categories.



Tetracycline susceptibility distribution among N/A, MRSA, and MSSA

***Staphylococcus aureus* isolates.**

The bar chart reveals distinct tetracycline susceptibility patterns among *Staphylococcus aureus* isolates, with the MRSA group showing predominantly high resistance, the MSSA group displaying more balanced proportions of resistant, sensitive, and intermediate isolates, and the N/A group largely composed of untested samples. Across all tested antibiotics, a consistently high proportion of isolates (approximately 55%) were categorized as NA, indicating testing was not performed for a substantial subset. Among the antibiotics evaluated, Vancomycin and Linezolid demonstrated the highest sensitivity rates (31.9% and 31.1%, respectively), confirming their continued effectiveness as last-line therapeutic options. In contrast, Erythromycin showed the highest resistance rate (27.5%), followed by Penicillin (21%) and Ciprofloxacin (19.3%), reflecting increasing resistance toward commonly used antibiotics due to selection pressure. Moderate resistance levels (15–18%) were observed for Cefoxitin, Tetracycline, Clindamycin, and Oxacillin, while notable intermediate susceptibility percentages—particularly for Oxacillin (19.2%), Gentamicin (17.5%), and Ciprofloxacin (16%)—suggest the possible emergence of reduced susceptibility and gradual resistance development. Overall, these findings highlight a concerning resistance trend toward frequently used antibiotics while underscoring the preserved effectiveness of reserve agents, emphasizing the critical need for continuous antimicrobial resistance surveillance and rational antibiotic stewardship

Discussion

Diabetic foot ulcers (DFUs) are highly susceptible to bacterial colonization due to compromised vascular supply and impaired immune response in diabetic patients. In the

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present study, 120 tissue samples were collected from patients with DFUs, and 90 samples (75%) were culture-positive for bacterial growth. Among these, *Staphylococcus aureus* was isolated from 56 samples, indicating a prevalence of 46.7%. This finding aligns with prior research reporting *S. aureus* as a predominant pathogen in DFUs, reflecting its capacity to adapt to wound environments and resist host defenses (Lipsky *et al.*, 2012) reported that *S. aureus* accounted for approximately 40–50% of isolates from DFU infections, emphasizing its clinical significance as a major causative agent.In the present study, all isolates presumptively identified as *Staphylococcus aureus* demonstrated Gram-positive cocci arranged in clusters, with yellow colonies on Mannitol Salt Agar indicating mannitol fermentation and predominant β-hemolysis on blood agar. Additionally, all isolates were catalase-positive and coagulase-positive, while oxidase-negative. These findings are consistent with previous microbiological investigations of diabetic foot infections. (Selvarajan *et al.*, 2021) reported that *Staphylococcus aureus* isolates recovered from diabetic foot ulcer specimens were identified as Gram-positive cocci in clusters and demonstrated positive catalase and coagulase reactions, confirming their phenotypic identity through conventional laboratory methods. Similarly, their study observed characteristic growth on selective media supporting mannitol fermentation, which parallels the morphological and biochemical profile observed in the present study.

In the present study, *Staphylococcus aureus* isolates demonstrated variable antimicrobial susceptibility patterns. High sensitivity was observed to Vancomycin (31.9%) and Linezolid (31.1%), whereas increased resistance was noted against Erythromycin (27.5%), Penicillin (21%), and Ciprofloxacin (19.3%). Furthermore, MRSA accounted for 24.2% of the total study samples, indicating a considerable prevalence of methicillin resistance among diabetic foot ulcer isolates. Similarly, (Shanmugam & Jeya, 2013) reported that *S. aureus* isolates from diabetic foot infections exhibited high susceptibility to vancomycin and linezolid, while showing marked resistance to erythromycin and penicillin. Their findings support the preserved efficacy of glycopeptides and oxazolidinones against resistant Gram-positive pathogens.

In the present study, a total of 120 diabetic foot ulcer samples were processed, with 90 (75%) showing culture positivity, indicating a high burden of bacterial colonization. Within the positive cultures, Staphylococcus aureus was isolated from 56 samples (46.7%), highlighting it as a predominant pathogen in this patient population. Comparing the culture-positive isolates among themselves, MRSA accounted for 29 out of 56 S. aureus isolates (51.8%), while MSSA represented 25 isolates (44.6%). This shows that more than half of the S. aureus-positive cases were methicillin-resistant, emphasizing the clinical importance of monitoring MRSA prevalence in diabetic foot infections.

Examining the antibiotic susceptibility patterns within the S. aureus isolates, Vancomycin and Linezolid demonstrated the highest sensitivity rates, with 31.9% and 31.1% of isolates being susceptible, respectively. In contrast, Erythromycin, Penicillin, and Ciprofloxacin showed the highest resistance levels at 27.5%, 21%, and 19.3%, respectively. When comparing MRSA and MSSA isolates, MRSA strains exhibited consistently higher resistance across almost all tested antibiotics, while MSSA isolates showed relatively greater sensitivity, particularly to beta-lactams and glycopeptides.

The internal comparison also highlighted intermediate susceptibility levels for Cefoxitin, Oxacillin, Tetracycline, and Clindamycin, with percentages ranging between 15% and 18% across the isolates. Notably, a substantial proportion of isolates (approximately 55%) were categorized as NA for certain antibiotics, reflecting either non-tested samples or data not applicable for specific antimicrobial agents. Despite these limitations, the trends indicate that MRSA isolates remain the major contributors to multidrug resistance, whereas MSSA isolates maintain partial sensitivity to commonly used therapeutic agents.

Conclusion

Diabetic foot ulcers are heavily colonized by *Staphylococcus aureus*, with 46.7% of samples testing positive. Among these, MRSA accounted for 24.2%, highlighting a significant prevalence of methicillin resistance. Antibiotic susceptibility patterns showed highest sensitivity to

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Vancomycin (31.9%) and Linezolid (31.1%), while resistance was highest for Erythromycin (27.5%), Penicillin (21%), and Ciprofloxacin (19.3%). MRSA isolates consistently displayed higher resistance across almost all tested antibiotics compared to MSSA. These findings emphasize the importance of culture and susceptibility testing for selecting appropriate therapy, monitoring MRSA prevalence, and implementing effective antibiotic stewardship in the management of diabetic foot infections

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