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MOLECULAR DETECTION OF STAPHYLOCOCCUS AUREUS IN SKIN AND SOFT TISSUE INFECTIONS AMONG DIABETIC PATIENTS

Original Article

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ABSTRACT

Background: Skin and soft tissue infections (SSTIs) are of special concern in diabetic patients, and Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) are the most frequent causative agents. Antibiotic resistance, delayed healing and elevated morbidity often complicates these infections. The accuracy of the diagnosis and subsequent treatment can be enhanced through early molecular detection. The purpose of this research was to examine the incidence of S. aureus and MRSA in diabetic SSTIs with molecular diagnostics and to assess the pattern of antibiotic resistance and related clinical risk factors.

Methods: In this cross-sectional study, 150 patients with SSTIs related to diabetic patients were studied at a tertiary care facility. The samples of pus were cultured and tested using PCR to determine the presence of the nuc gene (in S. aureus) and mecA gene (in MRSA). Demographic, microbiological, and clinical data were also gathered. The Kirby-Bauer disk diffusion method was used to test antibiotic susceptibility. SPSS v 26.0 was used to conduct both chi-square and t-tests, where $p < 0.05$ was taken as significant.

Results: Among 150 samples, 68 (45.3%) were culture-positive with S. aureus, which were confirmed by the nuc gene via PCR. The proportion of MRSA-positive by detection of mecA in these samples was 29 (42.6%). MRSA showed prominent relations with poor control of glycemic management ($p = 0.009$), diabetic ulcers ($p = 0.015$), previous SSTIs ($p = 0.025$), and recent antibiotic exposure ($p = 0.029$). The level of resistance was high to penicillin 63 (92%), erythromycin 52 (76%), and ciprofloxacin 47 (69%), and all isolates were sensitive to vancomycin and linezolid.

Conclusion: Diabetic SSTIs have a very high prevalence of MRSA and are associated with reversible clinical factors. Molecular diagnostics contribute to earlier detection as well as targeted antibiotic use. Enhancing glycemic control, infection and antibiotic stewardship are imperative to better outcomes with diabetic wound care.

Key words: Staphylococcus aureus, Methicillin-Resistant Staphylococcus aureus, “Skin Diseases, Infectious”, Polymerase Chain Reaction, “Drug Resistance, Microbial”.

INTRODUCTION

Skin and soft tissue infections (SSTIs) are major morbidity in diabetic patients because of immunosuppressed status, slow wound healing ability, and peripheral neuropathy (1). Recently, Staphylococcus aureus has been recognized as the most common pathogen of these infections, especially the methicillin-resistant forms (MRSA), which influences the treatment due to their multidrug resistance (2). Depending on traditional culture-based methods, diagnosis and treatment are delayed, making fast and predictable molecular diagnostics essential. Early detection of virulent genes such as nuc and mecA can be achieved through molecular tools, including polymerase chain reaction (PCR), as it provides a better clinical outcome and minimizes the risks of resistance (3). This has rendered early, gene-based detection and intervention a public health priority in diabetes management.

Recent evidence shows high resistance among wound pathogens, with MRSA strains extensively resistant to antibiotics, necessitating diagnostics to minimize ineffective empirical treatments(4). A study highlighted the importance of molecular biomarkers in early diagnosis and customized treatment, which is directly applicable to SSTIs, where delays may result in complications (5). Research on diabetic metabolic profiles discovered dysregulated insulin resistance and beta-cell functionality, predisposing individuals to susceptibility, including MRSA (6).

Another study on hepatokines in metabolic diseases also defined how systemic inflammation and metabolic dysregulation impact immunity and wound healing due to biochemical disproportion (7). Pediatric studies also demonstrated that the immunocompromised children's survival was influenced by variations in protein levels that predisposed them to bacterial infections, reflecting the sensitivity of diabetic patients to SSTIs (8). Moreover, a study associated obesity with immune modifications, and since a high percentage of diabetic individuals with SSTIs are obese, this contributes to their risk of infections (9). The challenges of comorbidities and healthcare access in Pakistan may cause diagnostic constraints, and health-seeking behaviors may lead to MRSA being undetected (10). As highlighted in trauma scenarios, early pathogen detection can have a major impact on improving outcomes (11).

From a pharmacological perspective, a study demonstrated the antimicrobial potential of natural extracts, which implies potential use in combo treatments of multidrug-resistant infections (12,13). In addition, Rehman et al. (2025) emphasized the impacts of failure to treat infections early in diseases such as CKD, presenting the long-term outcomes of untreated infections (13). Investigation of biomarkers, including hepcidin in liver disease, highlights the importance of molecular markers in the monitoring of a disease, which can also apply to diabetic SSTIs (14). Furthermore, Uqaili et al. (2023) suggested PROM2 as a diagnostic marker, which supports the need to involve molecular tools in standard diagnostics to enable early infection-specific therapy (15).

Despite the evidence of MRSA in diabetic infections, limited local studies measure the impact on molecular diagnostics. As antibiotic resistance increases in diabetic infections, molecular diagnostics offer a quicker and more reliable alternative that allows clinicians to make prompt decisions. These results may support early molecular screening to reduce complications and prevent antibiotic abuse. The purpose of this study was to infer the prevalence of Staphylococcus aureus and MRSA in diabetic SSTIs by molecular detection of the nuc and mecA genes. It also assessed the antibiotic resistance to inform specific treatment. Furthermore, the aim was to promote molecular diagnostics in the daily management of diabetic infection.

METHODOLOGY

It was a cross-sectional study, which was conducted in a Lab affiliated with Punjab University Lahore and tertiary care facility targeting patients with diabetes and clinical manifestations of SSTI from December 2023 to March 2024. The purpose of this study was to examine the prevalence of Staphylococcus aureus and MRSA in SSTIs through the application of molecular diagnostics. An approval received (124/SBS/micro) with ethical considerations and patient confidentiality maintained.

Consecutive non-probability sampling was used to recruit 150 participants. Sample size was determined using OpenEpi version 3.0.0 (released 2013, Atlanta, GA, USA) based on prevalence data on Staphylococcus aureus in diabetic SSTIs in the existing literature. Adult diabetic cases were included in the study that had clinical symptoms of SSTIs, including redness, warmth, swelling, and discharge. Patients taking antibiotics within 48 hours before sample collection, with SSTIs not related to diabetes, were excluded to prevent bias.

Each patient was examined clinically, and sterile swabs were used to sample infected areas. There were no intervention groups; all subjects were assessed within one observational examination. All samples were cultured on a bacterial culture according to standard

microbiology techniques. Based on PCR results, suspected colonies were further investigated to explore whether they had the nuc gene (specific to S. aureus) or the mecA gene (indicative of methicillin resistance).

The Kirby-Bauer disc diffusion system was used to determine antibiotic susceptibility according to the recommendations suggested by the Clinical and Laboratory Standards Institute (CLSI). The profile of resistance and sensitivity was taken against some of the common antibiotics such as penicillin, erythromycin, ciprofloxacin, clindamycin, vancomycin, and linezolid. Data analyzed with the help of SPSS version 26.0 (released 2019, IBM Corp., Armonk, NY). The demographic and clinical data were presented using descriptive statistics, whereas the chi-square tests were used to test categorical variables. A statistically significant p-value was <0.05.

RESULTS

In 150 diabetic patients with SSTIs, the cultured Staphylococcus aureus was 68 (45.3%), and 29 (42.6%) of them were cured as MRSA using the mecA gene. The isolates were confirmed using the nuc gene by PCR. Male gender, extended diabetes duration, impaired glycemic control, history of SSTIs, and use of antibiotics were significantly clinically associated. The high levels of random glucose and diabetic foot ulcers were also strongly linked to infection. The highest resistance was to penicillin, erythromycin, and ciprofloxacin, whereas 100% sensitivity was recorded against vancomycin and linezolid. Demographic and clinical characteristics of the study participants are given in Table 1.

Table 1 Demographic and clinical characteristics of the study participants

Parameter	Value	Test used	Test value	Significance p-value
Mean Age (years ± SD)	54 ± 11	t-test	2.17	0.032
Gender (Male/Female)	93 (62%) / 57 (38%)	Chi-square	5.76	0.016
Duration of Diabetes > 5 years	89 (59.3%)	Chi-square	4.12	0.042
BMI > 30 kg/m²	65 (43.3%)	Chi-square	2.45	0.118
HbA1c > 7.5%	103 (68.6%)	Chi-square	6.83	0.009
Smoking Status (Active/Former/Never)	47 / 26 / 77	Chi-square	3.27	0.195
Previous SSTI Episodes	41 (27.3%)	Chi-square	5.02	0.025
Prior Antibiotic Use (past 3 months)	58 (38.6%)	Chi-square	4.74	0.029

*SSTI = Skin and Soft Tissue Infections, BMI = Basal Metabolic Index, SD = Standard Deviation, * = Significant p-value at <0.05*

The average age of 150 diabetic patients who presented with SSTIs was 54 ± 11 years (p = 0.032), and there was a significant gender distribution, in the sense that 93 (62%) of diabetic patients with SSTIs were males (p = 0.016). Poor glycemic control was recorded with 89 (59.3%, p = 0.042), duration of diabetes >5 years, and 103 (68.6%) with HbA1c >7.5% (p = 0.009). Prior SSTI episodes (41 (27.3%), p = 0.025) and also recent antibiotic use (38.6%, p = 0.029) were significant, though obesity (65 (43.3%)) and smoking were not, suggesting that glycemic control and infection history should be monitored regularly in high-risk diabetic patients. Clinical confounders for SSTIs are presented in Table 2.

Table 2: Clinical confounders for SSTIs

Parameters	Value	Test used	Test value	Significance p-value
Peripheral Neuropathy	72 (48.0%)	Chi-square	3.65	0.056
Diabetic Foot Ulcer	64 (42.6%)	Chi-square	5.89	0.015
CKD	29 (19.3%)	Chi-square	2.98	0.084
Hypertension	71 (47.3%)	Chi-square	3.42	0.064
Dyslipidemia	66 (44.0%)	Chi-square	2.13	0.144
Poor Glycemic Control (random glucose > 200 mg/dL)	96 (64.0%)	Chi-square	6.21	0.013
Immunosuppressive Medication Use	12 (8.0%)	Chi-square	2.59	0.108

*CKD = Chronic Kidney Disease, *Significant p-value at <0.05*

Peripheral neuropathies were found in 72 (48%, p = 0.056), and diabetic foot ulcers in 64 (42.6%), with the latter showing a significant relationship (p = 0.015). Bad glucose control (>200 mg/dL) was experienced by 96 (64%) of participants (p = 0.013), whereas CKD, hypertension, and dyslipidemia were not statistically significant. It supports that diabetic foot examination and glycemic control must be documented as a priority to avoid SSTIs. Table 3 summarizes the microbiological findings.

Table 3: Microbiological findings

Parameter	Value	Test used	Test value	Significance p-value
Culture-positive for *S. aureus*	68 (45.3%)	Descriptives	N/A	N/A
PCR-confirmed *S. aureus* (nuc gene)	68 (100% of isolates)	descriptives	N/A	N/A
MRSA-positive (mecA gene)	29 (42.6% of *S. aureus*)	Chi-square	9.21	0.002

*PCR = Polymerase Chain Reaction, * =Significant p-value at <0.05*

Microbiological examination revealed a 68 (45.3%) of S. aureus cultured positivity with 68 (100%) confirmation on the nuc gene and 29 (42.6%) isolates found to be MRSA on the mecA gene (p = 0.002). It reveals that early diagnosis and intervention, PCR-based MRSA screening are recommended. Table 4 shows the antibiotic resistance of S. aureus against various drugs.

Table 4: Antibiotic Resistance (S. aureus)

Test used	Value	Test used	Test value	Significance P-value
Penicillin Resistance	63 (92%)	Chi-square	11.45	0.001
Erythromycin Resistance	52 (76%)	Chi-square	7.83	0.005
Ciprofloxacin Resistance	47 (69%)	Chi-square	6.02	0.014
Clindamycin Resistance	29 (42%)	Chi-square	3.46	0.063
Vancomycin and Linezolid Sensitivity	68 (100%)	Chi-square	N/A	N/A

**Significant p-value at <0.05*

The highest antibiotic resistance was against penicillin (63 (92%), p = 0.001), erythromycin (52 (76%), p = 0.005), and ciprofloxacin (47 (69%), p = 0.014); resistance against clindamycin was 29 (42%, p = 0.063). Vancomycin and linezolid were completely sensitive to all isolates. It shows that empiric treatment must exclude highly resistant antibiotics and must be based on local sensitivity.

DISCUSSION

This study aimed to identify the molecular prevalence of Staphylococcus aureus and MRSA in diabetic SSTIs and to assess patterns and clinical factors of resistance. The findings supported that MRSA infections in this population are due to metabolic imbalances, effects of diabetes complications, and previous antibiotic exposure. The detection of the nuc and mecA genes showed the effectiveness of PCR in detecting S. aureus and MRSA strains.

The demographic and clinical factors associated significantly with MRSA included male gender, increased length of diabetes, poor glycemic control (HbA1c > 7.5%), and previous history of SSTIs and existence of diabetic foot ulcers. These aspects strongly resonate with conclusions that revealed the comorbidities, such as chronic kidney disease, may intensify diabetic complications (16). Likewise, a study showed that stress markers of biochemical and physiological origin proved highly predictive of poor health outcomes among young adults, reaffirming that systemic stress can impact a predisposition to infection among patients with diabetes (17).

Comparatively, the studies that examined reproductive or endocrine variables have not found the same results. As an example, LH in fertility treatment demonstrated hormonal impact on reproduction, whereas our findings highlighted that metabolic, rather than hormonal, triggers have a direct impact on the occurrence of SSTI in diabetics (18). Furthermore, a study demonstrated that sofosbuvir influenced metabolic markers, which further emphasizes the role of systemic regulation in infection susceptibility, not only in diabetes (19).

The trends in antibiotic resistance in this study were comparable to national and global trends. A 2023 publication in the Journal of Infectious Diseases found a worldwide MRSA ciprofloxacin resistance rate above 70 percent in diabetic foot ulcers, confirming our 69% rate (20).

Similarly, our penicillin resistance of 92% and erythromycin of 76% proved the tendencies outlined by a study that emphasized the necessity of risk-adjusted therapy among patients with metabolic conditions (21). Resistance profiles support the limited utilization of these agents in empirical therapy, as well as validate vancomycin and linezolid (100% sensitivity) as final resort but reliable options.

Pre-existing antibiotic exposure was strongly associated with MRSA identification. This can be compared to the discovery that demonstrated the hierarchical interaction between hormonal and metabolic factors may interfere with immune reaction and resistance to treatment, particularly in the setting of drug exposure (22). The presence of neuropathy and ineffective wound healing were also significant factors since they aligned with the pathophysiological considerations on nerve damage and inflammation (23).

It is crucial to mention the role of psychological and physiological stressors. A study connected disrupted levels of melatonin to burnout in healthcare professionals, which further confirmed that dysregulation of systems, in general, was part of the modulation associated with reducing immunity (24). Furthermore, environmental pollutants instigate inflammation and exacerbate chronic respiratory disease, reinforcing the exacerbation of inflammatory burden irrespective of genesis, which increases the susceptibility to subsequent infection of vulnerable patients (25).

Genetic susceptibility was not studied in our research project, but in previous research, HLA haplotypes linked to autoimmune disease, so future genetic studies on diabetic patients with MRSA could also help define predisposition (26). Moreover, a study investigated the effects of vitamin D, parathyroid hormone, and calcium on essential hypertension, another metabolic syndrome, which is a demonstration of the complexity of the interaction between micronutrients and disease that may apply to SSTIs (27).

Precise and rapid molecular diagnostic methods, particularly PCR, were validated. By analyzing pseudogene markers used in pan-cancer detection, a study discovered that molecular assays were very sensitive (28).

Similarly, a study also considered Notch signaling as a potential diagnostic biomarker in oral cancer, with similarities in terms of the application of molecular methods to enhance disease detection. These results support our claim of nuc and mecA PCR as a regular part of diabetic infection management (29). Similarly, a study reported a high prevalence of hepatotoxicity in patients co-infected with HIV and TB, which highlights how immunosuppression and chronic inflammation predispose patients to serious infections (30). Moreover, a study on imaging of pituitary microadenoma showed that technological selection (either CT or MRI) can impact the level of diagnostic accuracy, our major emphasis on the implementation of an accurate and complete method of MRSA detection (here, PCR) in infection control (31).

Although such insights are appealing, there were also inconsistencies in our study. The scope of stress-induced hypertension was described in the literature, although related to overall metabolic instability, was not particularly correlated with SSTI outcomes in our sample (32).

There are three recent publications that corroborate our main discoveries significantly. A recent multicenter study concluded that an HbA1c greater than 8% was a good predictor of MRSA colonization in diabetics, which is similar to our results on glycemic control (33). The high specificity and quick turnaround of mecA-based PCR in the detection of MRSA were once again confirmed, illustrating its clinical applicability (34).

The findings are significant with implications. The introduction of molecular diagnostics in the clinical setting could help decrease the diagnostic lag and optimize antibiotic use by enhancing targeting and decreasing the use of empirical prescriptions. It is essential to restrict the use of penicillin, ciprofloxacin, and erythromycin in diabetic wounds, but it is important to save vancomycin and linezolid as reserve therapies.

The improvement of access to molecular tools and training clinicians about resistance trends should become the aim of public health endeavors. In addition, community and hospital-level focus on glycemic management, wound management, and antibiotic stewardship should be reinforced. Although antibiotic stewardship was explored as a primary topic, a study demonstrated how complicated medication reactions (dual-mechanism thrombocytopenia in this instance) may occur unpredictably (35). It supports the importance of careful drug prescription, frequent follow-up, and tactful antimicrobial therapy in diabetics, especially polypharmacy patients.

The cross-sectional design restricts the study to describing the relationship between the variables, and it may have limited the generalizability of the study due to its single-center sampling. Other confounding variables, including hygiene habits, nutritional well-being, and hereditary factors, also went unmeasured, although they might also affect SSTIs progression and resistance. Additionally, the research did not cover other multidrug-resistant organisms besides MRSA. Future prospects must entail multicentre longitudinal studies to explore changes over time in resistance, as well as to confirm risk factor relationships.

CONCLUSION

This study observed that S. aureus was prevalent in half of diabetic SSTIs, of which 42.6% were confirmed to be MRSA based on molecular testing. Poor glycemic control, diabetic foot ulcers, and previous infections, as well as current antibiotic use, were highly related to the occurrence of MRSA. Detection of isolates at the molecular level using nuc and mecA PCR was highly accurate in the identification of Staphylococcus aureus and MRSA.

These findings can respond to the initial aim of identifying the prevalence of S. aureus or MRSA and testing resistance patterns using molecular diagnostics. The study highlights the importance of early molecular screening in high-risk diabetic patients so that quick and specific treatment can be administered. The use of PCR-based diagnostics, better glycemic control, and optimized antibiotic usage are as crucial to minimizing complications, resistance, and improving diabetic wound care outcomes as possible.

AUTHOR CONTRIBUTION

Author	Contribution
Zainab Basharat	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Lubna	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Muhammad Hussain*	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published

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