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Association Between Hygiene Level Among Diabetic Ulcer Patients and the Incidence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infection

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Abstract: This study aimed to analyze the association between hygiene level and the incidence of *Methicillin-Resistant Staphylococcus aureus* (MRSA) infection in diabetic ulcers. An analytical observational study with a *cross-sectional* approach was conducted. The study included 20 patients with diabetic ulcers at RSUD Prof. Dr. Margono Soekarjo Purwokerto, selected using an accidental sampling technique. Bacterial identification was performed through culture on *Mannitol Salt Agar* (MSA), Gram staining, biochemical tests, and antibiotic susceptibility testing using the disk diffusion method. MRSA identification was determined based on cefoxitin susceptibility testing. Hygiene levels were assessed using an observation checklist developed based on wound care principles and WHO guidelines. Data were analyzed using Fisher's Exact test with a significance level of $p < 0.05$. Among the 20 patients with diabetic ulcers, MRSA infection was identified in 17 patients (85%), while 3 patients (15%) were not infected with MRSA. In the group with good hygiene practices, 2 patients (10%) were infected with MRSA and 3 patients (15%) were not infected, whereas among patients with poor hygiene practices, 15 patients (75%) were infected with MRSA. Statistical analysis revealed a significant association between hygiene level and MRSA infection incidence ($p = 0.009$). These findings indicate that hygiene practices in diabetic ulcer care are associated with the occurrence of MRSA infection among patients with diabetic ulcers.
Keywords: Hygiene; MRSA; antibiotic resistance; diabetic ulcer

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by impaired glucose metabolism and insulin dysfunction, resulting in elevated blood glucose levels or hyperglycemia (Sholikah et al., 2021). DM is often considered a silent killer because many patients are unaware of their condition until chronic complications develop (Fortuna et al., 2023). One of the most common complications of DM is diabetic ulcer, a chronic wound that develops due to vascular impairment and peripheral neuropathy, making it highly susceptible to bacterial colonization and infection (Enikmawati et al., 2025). Diabetic ulcers represent a major clinical concern because they are associated with prolonged wound healing, increased risk of amputation, and reduced quality of life. In Indonesia, the prevalence of diabetic ulcers has been reported to reach approximately 12%, with an amputation risk of 14–24% and a recurrence rate of up to 50% within three years (Nurwahidah et al., 2018; Cahyaningtyas and Werdiningsih, 2022). Bacterial infection in diabetic ulcers can further delay wound healing and increase the risk of systemic complications. Among the bacterial pathogens commonly isolated from diabetic ulcers, *Staphylococcus aureus* is one of the most frequently identified species (Macdonald et al., 2021; Wahyuni et al., 2024).

A major concern in the management of diabetic ulcer infections is the emergence of *Methicillin-Resistant Staphylococcus aureus* (MRSA). MRSA is an opportunistic pathogen characterized by resistance to methicillin and other β-lactam antibiotics, including penicillin, ampicillin, amoxicillin, oxacillin, cephalosporins, carbapenems, and monobactams (Lestari et al., 2020). Initially recognized as an important cause of healthcare-associated infections, MRSA has subsequently spread widely in community settings and become increasingly difficult to control due to its antimicrobial resistance characteristics (Kurniawan et al., 2021). The presence of MRSA in diabetic ulcers may complicate clinical management, prolong treatment duration, increase healthcare costs, and contribute to higher morbidity among affected patients (Octifani et al., 2025).

Previous studies have reported a considerable prevalence of MRSA among patients with diabetic ulcers. A global meta-analysis conducted by Zhou et al. (2024), involving 12,924 patients from 20 countries, reported that the prevalence of MRSA among diabetic foot ulcer patients was 17%. Similarly, Khariunnisa et al. (2020) reported that 14 out of 21 diabetic ulcer patients (66.7%) were positive for *S. aureus*, with 42.9% of isolates showing resistance to cefoxitin, indicating MRSA characteristics. Furthermore, Sugireng and Rosdarni (2020) demonstrated a high prevalence of MRSA in diabetic ulcers, with all examined samples (100%) carrying the *mecA* gene, a molecular marker associated with MRSA resistance.

Although previous studies have extensively investigated MRSA prevalence, antimicrobial susceptibility patterns, and bacterial characteristics in diabetic ulcers, limited information is available regarding the relationship between wound care hygiene practices and MRSA occurrence at the individual patient level. Previous studies by Fau (2024) and Lestari et al. (2020) primarily focused on the occurrence of MRSA and risk factors associated with bacterial infection in diabetic ulcers. However, hygiene practices during wound management represent potentially modifiable factors that may influence bacterial contamination and colonization. Practices such as hand hygiene before wound care, routine wound cleansing with NaCl solution, regular dressing replacement, the use of sterile equipment, and maintaining cleanliness around the wound area may reduce microbial contamination and support the wound healing process (Hamidah and Maksum, 2025).

Appropriate wound care and wound cleansing are essential strategies to prevent the progression of infection and minimize bacterial dissemination (Cahyaningtyas and Werdiningsih, 2022). The occurrence of MRSA in diabetic ulcers may be influenced by multiple factors, including poor glycemic control, inadequate wound hygiene, prolonged duration of diabetes, and inappropriate antibiotic use (Sugireng and Rosdarni, 2020). In addition to patient-related factors, MRSA transmission may occur during wound care procedures through contact with healthcare workers, contaminated wound care equipment, dressings, or healthcare environments. Therefore, maintaining proper wound hygiene and implementing optimal wound care procedures are important strategies to reduce bacterial transmission and control MRSA occurrence in diabetic ulcer patients.

Based on this background, this study aimed to analyze the association between wound hygiene levels among diabetic ulcer patients and the occurrence of *Methicillin-Resistant Staphylococcus aureus* (MRSA). Unlike previous studies that have mainly focused on MRSA prevalence and antimicrobial resistance patterns, this study integrates microbiological identification with evaluation of wound care hygiene practices at the individual patient level. This approach is expected to provide additional evidence regarding modifiable factors associated with MRSA occurrence in diabetic

ulcers and contribute to the development of preventive strategies in clinical wound management. Furthermore, the findings may serve as a reference for future studies investigating risk factors and preventive approaches for MRSA in diabetic ulcer patients.

METHOD

Research Design

This study employed an analytical observational design with a cross-sectional approach. The study was conducted from October 2025 to May 2026. Sample collection was performed at RSUD Prof. Dr. Margono Soekarjo Purwokerto, while microbiological analyses were conducted at the Microbiology Laboratory of the Diploma IV Medical Laboratory Technology Program, Universitas Muhammadiyah Purwokerto.

Study Subjects

The study subjects were patients with diabetes mellitus (DM) complicated by diabetic ulcers who met the predetermined inclusion and exclusion criteria. The inclusion criteria were patients with DM-associated diabetic ulcers at RSUD Prof. Dr. Margono Soekarjo Purwokerto who were eligible for wound swab collection, agreed to participate by signing an informed consent form, and had not received antibiotic therapy within the previous 72 hours. The exclusion criteria included patients undergoing other medical treatments, patients with extensive debridement wounds, and patients with unstable clinical conditions.

Samples were collected using an accidental sampling technique. During the study period, 20 respondents met the inclusion and exclusion criteria and were included in the analysis. The independent variable in this study was the level of hygiene during wound care procedures among diabetic ulcer patients, while the dependent variable was the occurrence of *Methicillin-Resistant Staphylococcus aureus* (MRSA) infection. The samples analyzed were clinical specimens from diabetic ulcer patients that were identified as *S. aureus* and fulfilled the inclusion and exclusion criteria.

Microbiological Examination

Identification of *S. aureus* was performed using diabetic ulcer swab samples cultured on selective Mannitol Salt Agar (MSA) medium and incubated at 37°C for 24 hours. The growing colonies were subsequently examined based on colony morphology, Gram staining characteristics, and biochemical tests, including catalase, oxidase, and coagulase assays.

MRSA identification was based on the initial antibiotic susceptibility screening results obtained from a collaborative research project conducted by Lestari et al. (2026). The study by Lestari et al. (2026) focused on the microbiological identification of *S. aureus* and its antibiotic susceptibility profile. MRSA detection was performed using the disk diffusion method with 30 µg cefoxitin disks on Mueller-Hinton Agar (MHA) medium.

The *S. aureus* isolates were initially cultured in Tryptic Soy Broth (TSB) and incubated for 24 hours. After bacterial growth was observed, indicated by turbidity in the broth medium, bacterial cell density was determined using a turbidimetric method with a spectrophotometer at a wavelength of 600 nm. The spectrophotometer was adjusted to obtain an absorbance value of 0.1, corresponding to the McFarland standard turbidity (1.5×10^8 bacterial cells/mL). The obtained absorbance values were converted into bacterial cell concentrations (cells/mL) using the web-based software OD600 Calculator (https://www.agilent.com) (Kamalah et al., 2023).

Subsequently, the bacterial suspension was inoculated onto MHA plates, followed by placement of 30 µg cefoxitin antibiotic disks and dimethyl sulfoxide (DMSO) as a negative control. After incubation at 37°C for 16–18 hours, the inhibition zones surrounding the cefoxitin disks were measured using a caliper and recorded in millimeters (mm). The interpretation of antibiotic susceptibility results followed the Clinical and Laboratory Standards Institute (CLSI) M100, 32nd edition (2022) guidelines. *S. aureus* isolates with inhibition zone diameters ≤21 mm were interpreted as MRSA, whereas isolates with inhibition zone diameters ≥22 mm were classified as Methicillin-Susceptible *Staphylococcus aureus* (MSSA).

Based on the identification results from 20 diabetic ulcer swab samples, a total of 70 bacterial isolates were obtained. Conventional culture-based identification revealed 46 isolates of *S. aureus*. In some cases, a single diabetic ulcer swab yielded more than one bacterial colony with different morphological characteristics. Colonies showing distinct morphological features were selected and purified for subsequent *S. aureus* identification and MRSA screening using cefoxitin disks. Therefore, a single patient could yield more than one *S. aureus* isolate. Further screening using cefoxitin disks identified 37 isolates as MRSA, and these isolates were used as test isolates to analyze the relationship between hygiene levels among diabetic ulcer patients and MRSA infection occurrence.

Measurement of Hygiene Level

In this study, the hygiene level of diabetic ulcer patients was defined as the degree of adherence to hygiene principles during wound care procedures received by patients in the hospital setting. Hygiene levels were assessed using a direct observation checklist during wound care procedures, before completion of specimen (*swab*) processing and before the culture and MRSA identification results were available. Therefore, the observer was blinded to the MRSA infection status of the respondents during the observation process.

Direct observations were conducted while patients underwent wound care procedures at RSUD Prof. Dr. Margono Soekarjo Purwokerto. The assessed indicators included handwashing practices before wound care procedures, the use of sterile gloves during wound management, wound cleansing using 0.9% NaCl solution, and the use of sterile gauze during wound dressing. These indicators were developed based on the World Health Organization (WHO) hand hygiene guidelines (WHO, 2025) and the principles of Diabetic Foot Ulcer management (IWGDF, 2023) to prevent infection in diabetic ulcers.

Each indicator that complied with appropriate wound care procedures was assigned a score of 1, while non-compliant indicators received a score of 0. The total hygiene score ranged from 0 to 4. Hygiene levels were subsequently categorized as good (score 3–4) and poor (score 0–2) using the modified Bloom's cut-off approach (Wahidiyat *et al.*, 2021), where fulfillment of ≥75% of the indicators was classified as good hygiene. The observation instrument was developed based on WHO and IWGDF guidelines; however, it was not subjected to a validity test prior to implementation. The observation results were used to describe the hygiene practices of diabetic ulcer patients during wound care procedures.

Data Analysis

The collected data were analyzed using descriptive and analytical approaches. The unit of analysis in this study was diabetic ulcer patients (n = 20). Descriptive analysis was performed to determine the frequency distribution and percentage of MRSA infection occurrence. Bivariate analysis was initially conducted using the Chi-

square test to evaluate the association between hygiene level and MRSA infection occurrence, and the Odds Ratio (OR) with a 95% Confidence Interval (95% CI) was calculated.

The Fisher's Exact test was applied because some cells had an expected count of <5, indicating that the assumptions for the Chi-square test were not fulfilled. Statistical significance was determined at $p < 0.05$. This study was conducted after fulfilling ethical requirements and obtaining ethical approval from the Health Research Ethics Committee of RSUD Prof. Dr. Margono Soekarjo Purwokerto (Approval Number: 420/08874).

RESULTS AND DISCUSSION

Macroscopic and Microscopic Characteristics of *S. aureus*

Identification of *S. aureus* isolates was performed based on the macroscopic characteristics of bacterial colonies grown on culture media and microscopic characteristics observed through Gram staining. These characteristics were used as preliminary criteria to support bacterial identification prior to further analysis. The macroscopic and microscopic observations of *S. aureus* isolates are presented in Figure 1.

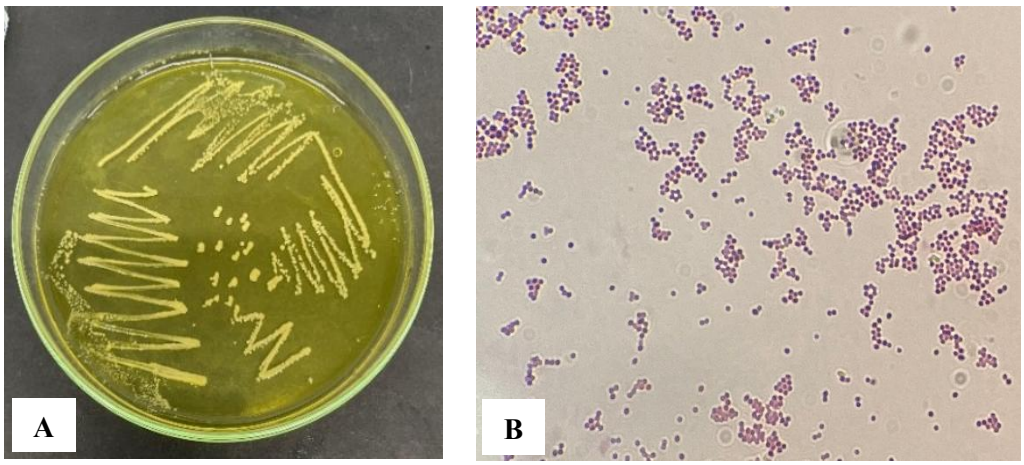

Figure 1. (A) *S. aureus* bacterial colonies on *Mannitol Salt Agar* (MSA) medium after 24 h incubation at 37°C; (B) Gram staining results of *S. aureus* observed using a binocular microscope (100× objective lens with *oil immersion*)

Based on the identification of 70 bacterial isolates obtained from 20 diabetic ulcer swab samples, 46 isolates were previously identified as *S. aureus* in a study conducted by Lestari *et al.* (2026). Following cultivation on *Mannitol Salt Agar* (MSA) medium and incubation at 37°C for 24 h, all isolates demonstrated good growth characteristics. The colonies exhibited round, *glistening, opaque,* and *convex* morphology, with smooth edges and yellow pigmentation. Gram staining revealed Gram-positive cocci arranged in grape-like clusters, which is a characteristic feature of *S. aureus*.

Biochemical analysis showed that all isolates were positive for catalase and coagulase production but negative for oxidase activity. The observed colony characteristics were consistent with previous findings reported by Kurniawan et al. (2021), who described *S. aureus* colonies as round, *glistening, opaque,* and *convex* with yellow pigmentation. Furthermore, Wahyuni et al. (2024) reported that *S. aureus* is a Gram-positive coccus frequently isolated from diabetic ulcers.

Characteristics of Research Subjects

Based on observations and questionnaire assessments conducted among 20 diabetic ulcer patients treated at RSUD Prof. Dr. Margono Soekarjo Purwokerto, several characteristics of the study subjects were identified, including sex, age, frequency of wound care, and wound hygiene level. The detailed characteristics of the respondents are presented in Table 1.

Table 1. Characteristics of study subjects

Variable	Frequency (n)	Percentage (%)
Sex		
Male	13	65.0
Female	7	35.0
Age		
<60 years	13	65.0
≥60 years	7	35.0
Frequency of diabetic ulcer care		
Daily routine care	17	85.0
Occasionally	3	15.0
Signs of diabetic ulcer infection		
Present	20	100.0
Absent	0	0.0

Based on Table 1, male patients with diabetic ulcers were more prevalent than female patients, with 13 individuals (65%) and 7 individuals (35%), respectively. This finding is consistent with Ayu et al. (2022), who reported that male patients with diabetes mellitus have a higher risk of developing diabetic ulcers compared with female patients. This difference may be influenced by several factors, including variations in clinical characteristics, metabolic factors, and duration of diabetes. In addition, differences in hormonal regulation between males and females may also contribute to the higher incidence of diabetic ulcers among males. Rosa et al. (2019) suggested that female hormones may play a role in regulating blood glucose levels, thereby reducing the risk of chronic complications such as diabetic ulcers.

Regarding age distribution, the majority of respondents were younger than 60 years. This finding indicates that diabetic ulcer complications were more frequently observed among individuals of productive age in this study population. This result differs from the findings of Detty et al. (2020), who reported that patients aged ≥60 years had a higher risk of developing diabetes mellitus accompanied by diabetic ulcer complications. According to Cahyaningtyas and Werdiningsih (2022), diabetic ulcers in elderly patients may be associated with physiological decline, including reduced insulin secretion and increased insulin resistance.

Regarding the frequency of diabetic ulcer care, 17 patients (85%) performed wound care routinely every day, whereas 3 patients (15%) did not perform wound care regularly. This finding indicates that adherence to wound care practices among patients was relatively high. This result supports the findings of Cahyaningtyas and Werdiningsih (2022), who stated that diabetic foot ulcer care provides important benefits, including maintaining foot cleanliness to prevent pathogenic bacterial infections and enabling early detection of abnormalities in the foot.

Based on clinical signs of diabetic ulcer infection, all patients (100%) exhibited signs of infection. This finding indicates that all patients had clinically infected ulcers. The infections were mainly characterized by local inflammatory signs, including

purulent discharge accompanied by unpleasant odor, while some patients also presented with redness and swelling. This observation is consistent with Herdani et al. (2023), who reported that diabetic ulcer infections are characterized by local inflammatory manifestations, including erythema, swelling, increased local temperature, pain, changes in granulation tissue, and foul odor. These clinical signs represent responses to bacterial colonization, particularly by *S. aureus*, which may contribute to pus formation and progression into abscesses.

Characteristics of Diabetic Ulcer Care Hygiene Level

The distribution of hygiene level observations during diabetic ulcer care is presented in Table 2.

Table 2. Distribution of hygiene practice indicators during diabetic ulcer care

Hygiene indicator	Performed n (%)	Not performed n (%)	Total n (%)
Hand washing before wound care procedures	1 (5%)	19 (95%)	20 (100%)
Wound cleansing using 0.9% NaCl solution	16 (80%)	4 (20%)	20 (100%)
Use of sterile gloves during wound care procedures	12 (60%)	8 (40%)	20 (100%)
Use of sterile gauze/dressing materials for wound closure	15 (75%)	5 (25%)	20 (100%)

Note: Each indicator was assigned a score of 1 when performed and 0 when not performed

The hygiene level assessment instrument consisted of an observation checklist containing wound care practice indicators before the procedure. The checklist included four hygiene indicators in healthcare services based on diabetic foot ulcer (DFU) management principles and World Health Organization (WHO) guidelines: hand washing before wound care procedures, wound cleansing using 0.9% NaCl solution, the use of sterile gloves during wound care procedures, and the use of sterile gauze/dressing materials for wound closure. Each indicator was assessed using a dichotomous scoring system (performed = 1; not performed = 0).

The total score obtained by each patient ranged from 0 to 4, which was subsequently used to categorize hygiene levels. Based on the total score, hygiene level was categorized as poor when the score ranged from 0–2 and as good when the score ranged from 3–4.

Table 3. Distribution of hygiene levels among diabetic ulcer patients

Hygiene level	Number (n)	Frequency (%)
Good	5	25.0
Poor	15	75.0
Total	20	100.0

Based on Table 3, most diabetic ulcer patients had poor hygiene levels, accounting for 15 patients (75%), whereas only 5 patients (25%) demonstrated good hygiene practices. These findings indicate that hygiene practices during diabetic ulcer wound care procedures were generally inadequate, despite the important role of proper hygiene in preventing infection among diabetic ulcer patients.

According to Piko et al. (2020), the primary objectives of wound care are to maintain tissue viability, inhibit necrotic processes, and stimulate new tissue regeneration through appropriate wound cleansing and dressing procedures to prevent infection. Therefore, implementing proper hygiene practices during wound management is an essential component in reducing the risk of bacterial contamination and infection in diabetic ulcers.

Diabetic Ulcer Patients Infected with MRSA

The distribution of diabetic ulcer patients infected with MRSA and the distribution of MRSA isolates are presented in Tables 4 and 5.

Table 4. Distribution of diabetic ulcer patients infected with MRSA

Category	Frequency (n)	Percentage (%)
MRSA	17	85.0
Non-MRSA	3	15.0
Total	20	100.0

Based on Table 4, among the 20 diabetic ulcer patients included in this study, 17 patients (85%) were identified as infected with MRSA, whereas the remaining 3 patients (15%) were not infected with MRSA. This finding is consistent with Sugireng and Rosdarni (2020), who reported a high prevalence of MRSA infection among diabetic ulcer patients.

The high proportion of MRSA observed in both studies indicates that MRSA is one of the predominant pathogens found in diabetic ulcers, particularly among patients with chronic wounds that are susceptible to colonization by antibiotic-resistant bacteria. However, the proportion of MRSA infection observed in the present study was higher than that reported in the meta-analysis by Zhou et al. (2024), which estimated a global MRSA prevalence of 17% among diabetic foot ulcer patients. This difference may be attributed to variations in population characteristics, healthcare facilities, and antibiotic resistance patterns across different countries included in the meta-analysis. In addition, the relatively small sample size in the present study (20 patients) may have contributed to the higher observed MRSA proportion and limited the representativeness of the findings for a broader population.

Table 5. Distribution of MRSA isolates among diabetic ulcer patients

Category	Frequency (n)	Percentage (%)
MRSA	37	80.4
MSSA	9	19.6
Total	46	100.0

Based on Table 5, among the 46 *S. aureus* isolates obtained from 20 diabetic ulcer patients, 37 isolates (80.4%) were identified as MRSA based on cefoxitin screening, whereas 9 isolates (19.6%) were classified as methicillin-susceptible *Staphylococcus aureus* (MSSA). Among the 17 patients identified with MRSA infection, several patients yielded more than one MRSA isolate.

The high number of MRSA isolates recovered from individual diabetic ulcer patients indicates that diabetic ulcer wounds at RSUD Prof. Dr. Margono Soekarjo Purwokerto were colonized or infected by bacterial strains exhibiting resistance to specific β-lactam antibiotics. These findings highlight the importance of appropriate antimicrobial management and the consideration of alternative therapeutic options for infections caused by resistant bacterial strains.

Association between Hygiene Level among Diabetic Ulcer Patients and MRSA Infection

The association between hygiene level among diabetic ulcer patients and MRSA infection is presented in Table 6. Based on the results, among patients with good hygiene levels, 2 patients (10.0%) were infected with MRSA, whereas 3 patients

(15.0%) were classified as non-MRSA infections. In contrast, among patients with poor hygiene levels, 15 patients (75.0%) were infected with MRSA, and no patients were identified with non-MRSA infections.

Table 6. Association between hygiene level among diabetic ulcer patients and MRSA infection

Hygiene level	MRSA n (%)	Non-MRSA n (%)	Total n (%)	OR (95% CI)	*p*-value
Good	2 (10.0)	3 (15.0)	5 (25.0)	43.40 (1.64–1,148.90)	0.009
Poor	15 (75.0)	0 (0.0)	15 (75.0)		
Total	17 (85.0)	3 (15.0)	20 (100.0)		

Note: The p-value was obtained using Fisher's Exact Test. The Odds Ratio (OR) was calculated using the Haldane–Anscombe correction because of the presence of a zero-frequency cell in the 2×2 contingency table.

The association between hygiene level and MRSA infection was analyzed using Fisher's Exact Test because several cells had an expected count of less than 5; therefore, the assumptions required for the Chi-square test were not fulfilled. The Fisher's Exact Test showed a statistically significant association between hygiene level and MRSA infection ($p = 0.009$; $p < 0.05$). Because one cell had a frequency of zero, the Odds Ratio was calculated using the Haldane–Anscombe correction, resulting in an OR of 43.40 (95% CI: 1.64–1,148.90). This finding indicates that patients with poor hygiene levels had higher odds of MRSA infection compared with patients with good hygiene levels. However, the wide confidence interval indicates that the OR estimate has limited precision and should therefore be interpreted cautiously.

Although most patients performed wound care routinely, the prevalence of MRSA infection remained high. This finding suggests that the frequency of wound care alone may not be sufficient to prevent MRSA infection if it is not accompanied by appropriate hygiene practices. Proper wound management, including adequate cleansing, aseptic techniques, and appropriate dressing procedures, is an important component in preventing infection among diabetic foot ulcer patients (IWGDF, 2023). The high prevalence of MRSA observed in this study may indicate the predominance of antibiotic-resistant bacterial strains in diabetic ulcers or may reflect the influence of antibiotic exposure during diabetic ulcer management (Macdonald et al., 2021; Zhou et al., 2024).

The findings of this study are consistent with Fau (2024), who reported that personal hygiene was significantly associated with infection symptoms among diabetic ulcer patients. Patients with poor personal hygiene had a higher risk of experiencing infection symptoms compared with those who maintained good hygiene practices. Similarly, Macdonald et al. (2021) reported that diabetic patients with ulcer complications have an increased risk of MRSA colonization and infection due to chronic wound conditions, vascular impairment, reduced immune function, and prolonged antibiotic exposure during diabetic ulcer management.

Furthermore, Lestari et al. (2020) demonstrated that the high prevalence of *S. aureus* infection in diabetic ulcers is associated with the presence of MRSA. As *S. aureus* is commonly found as part of normal skin flora, disruption of the skin barrier caused by diabetic ulcers may facilitate bacterial entry into damaged tissues and promote infection. In addition, inadequate infection control practices and exposure to contaminated wound care equipment may increase the risk of bacterial transmission and MRSA colonization in chronic wounds (Lestari et al., 2020; WHO, 2025).

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* that exhibits resistance to methicillin and most β-lactam antibiotics. This resistance mechanism is generally associated with the presence of the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a), resulting in reduced affinity for β-lactam antibiotics (Lutpiatina and Erina, 2018). Although this mechanism has been extensively described in previous studies, molecular detection of the *mecA* gene was not performed in the present study. Therefore, MRSA identification was based on phenotypic detection using the cefoxitin disk diffusion method according to the CLSI interpretation criteria.

Inadequate hygiene practices during wound care may increase the risk of contamination by pathogenic microorganisms. Failure to perform hand hygiene before wound procedures, inappropriate use of protective equipment, and the use of non-sterile dressing materials may serve as potential routes for bacterial transmission. This finding is consistent with WHO recommendations, which emphasize that hand hygiene and aseptic techniques are fundamental principles in infection prevention (WHO, 2025).

In addition, successful wound healing is strongly influenced by the quality of wound care practices. The use of physiological solutions such as NaCl, appropriate sterile dressing materials, and sterile equipment may support tissue regeneration and reduce bacterial contamination. Proper diabetic ulcer care not only accelerates wound healing but also plays an important role in preventing bacterial infections, including MRSA infection (Nurlina et al., 2025). The International Working Group on the Diabetic Foot (IWGDF) (2023) also emphasizes that prevention and management of diabetic foot infections should not rely solely on antibiotic therapy but should involve optimal wound care practices, including wound cleansing, aseptic techniques, and appropriate specimen collection procedures.

Although this study demonstrated a significant association between hygiene level and MRSA infection, the findings should be interpreted cautiously. One possible explanation is reverse causality, in which ulcers with greater severity may be more susceptible to colonization or infection by pathogenic bacteria, including MRSA, while simultaneously requiring more intensive wound care procedures (IWGDF, 2023). Furthermore, several potential confounding factors were not controlled in this study and may have influenced the association between hygiene level and MRSA infection. These factors include diabetic ulcer severity, duration of diabetes, glycemic control, history of antibiotic use, and comorbidities (Aung et al., 2025).

This study also has several limitations. The relatively small sample size (20 patients) and the use of accidental sampling limit the generalizability of the findings to a broader population. In addition, hygiene level was assessed only through direct observation during the sampling period and may not fully represent the consistency of wound care practices throughout the healing process. Furthermore, MRSA identification was performed using phenotypic methods based on cefoxitin susceptibility testing without molecular confirmation, such as detection of the *mecA* gene; therefore, possible misclassification of MRSA status cannot be completely excluded.

CONCLUSION

This study demonstrates that hygiene practices during diabetic ulcer care are significantly associated with the occurrence of *Methicillin-Resistant Staphylococcus aureus* (MRSA) infection among patients with diabetic ulcers. Patients with poor hygiene practices showed a higher proportion of MRSA-positive cultures compared

with those with good hygiene practices, indicating that appropriate wound care hygiene may play an important role in reducing the risk of MRSA infection in diabetic ulcer management. However, due to the cross-sectional design and relatively small sample size, this study cannot establish a causal relationship between hygiene practices and MRSA infection. Therefore, the findings should be interpreted within the context of the study population and healthcare setting. Further studies with larger sample sizes, longitudinal designs, and comprehensive assessment of potential confounding factors are needed to confirm these findings.

RECOMMENDATION

The findings of this study should be interpreted cautiously considering the relatively small sample size, the use of an *accidental sampling* technique, and the identification of MRSA based solely on culture-based methods without molecular confirmation. Therefore, further studies involving larger sample sizes are required to obtain stronger evidence and improve the representativeness of the findings. Future research is recommended to employ multicenter study designs involving various healthcare facilities and longitudinal assessments of hygiene practices. In addition, future investigations should consider potential confounding factors, including ulcer severity, duration of diabetes mellitus, glycemic control, history of antibiotic use, and comorbidities that may influence the presence of MRSA. Molecular confirmation of MRSA identification, such as detection of the *mecA* gene, is also recommended to improve identification accuracy and strengthen the validity of the findings.

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