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ISOLATION AND IDENTIFICATION OF STAPHYLOCOCCUS AUREUS IN DIABETIC ULCERS AS A RISK FACTOR FOR INFECTION

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Abstract

Diabetic foot ulcers are chronic complications of diabetes mellitus prone to bacterial infection, increasing the risk of amputation and mortality. This study aims to analyze the relationship between patient characteristics and the presence of Staphylococcus aureus in diabetic ulcers as a risk factor for infection. This study used an analytic cross-sectional design and was conducted at Bunda Thamrin Clinic Laboratory, Medan, in April 2025. A total of 30 diabetic foot ulcer samples were collected using total sampling. Bacterial cultures were grown on Blood Agar, followed by Gram staining, catalase, coagulase tests, and identification using the Vitek 2 Compact system. Statistical analysis was performed using Chi-square and Spearman correlation tests with a significance level of $p<0.05$. Of the 30 samples examined, Staphylococcus aureus was found in 14 (46.7%) cases, Staphylococcus epidermidis in 6 (20%), Citrobacter freundii in 5 (16.7%), and Proteus mirabilis in 5 (16.7%). The Chi-square test showed a significant association between gender and S. aureus infection ($p=0.042$) and between age group and infection ($p=0.031$). The Spearman test showed a positive correlation between fasting blood glucose level and the presence of S. aureus ($r=0.428$; $p=0.018$). There is a significant relationship between patient characteristics (gender, age, and blood glucose level) and Staphylococcus aureus infection in diabetic ulcers. Early bacterial identification is crucial for targeted antibiotic therapy and prevention of complications such as osteomyelitis and amputation.

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Introduction

Diabetic foot ulcers are non-traumatic lesions on the skin of people with diabetes mellitus that arise due to repeated pressure accompanied by complications of peripheral neuropathy or peripheral artery disease. The healing process for these ulcers is difficult because they are prone to developing infections (Rahmawati, 2022). The World Health Organization (WHO) reports an increase in cases of diabetic foot ulcers in the adult population, with the number of sufferers reaching 122 million people worldwide. In middle- and low-income countries, an estimated 2.2 million deaths under the age of 70 are caused by diabetic foot ulcers, and this figure has the potential to increase to 600 million by 2035 (Anggraini et al., 2023). In Indonesia, the prevalence of diabetic foot ulcers in people with diabetes mellitus reaches 15%, with an amputation rate of 30% and a mortality rate of 32%. Approximately 15–25% of people with diabetes mellitus experience diabetic foot ulcers (Lenny Putri Yunita Gayatri et al., 2024). Data from the 2018 Basic Health Research (Riskesdas) shows that there are 202,872 people with diabetes mellitus in North Sumatra Province, and diabetic foot ulcer complications are estimated to occur in 10–60% of patients (RISKESDAS, 2018).

One of the serious complications of diabetes mellitus is diabetic foot problems that cause open wounds on the skin surface. Poor blood circulation and nerve damage due to high blood sugar levels facilitate the growth of bacteria, both aerobic and anaerobic, causing wounds to develop more quickly into diabetic foot ulcers (Ismail et al., 2023). The infection can be caused by various pathogenic bacteria, including *Staphylococcus aureus*, which plays a major role in worsening the condition of the wound (Zubir et al., 2024). Identifying the bacteria causing the infection in diabetic ulcers is crucial for determining the appropriate antimicrobial therapy, preventing further complications, and reducing the rate of amputation and mortality in people with diabetes mellitus (Nisak, 2021).

Pathogenic bacteria that infect diabetic ulcers can originate from both gram-negative and gram-positive aerobic groups. According to Erlia (Narulita et al., 2019), gram-negative aerobic bacteria that often cause contamination include *Citrobacter sp*., *Klebsiella sp*., *Escherichia coli*, and Proteus mirabilis. Meanwhile, gram-positive bacteria include *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Streptococcus sp*. Among these bacteria, Staphylococcus aureus is the most commonly found pathogen in diabetic foot wounds because it is part of the normal flora on the skin surface (Mita Zuliana et al., 2023). When its growth is excessive, this bacterium triggers an infection characterized by inflammation, the formation of pus-filled abscesses, and tissue damage that can lead to cell death. Skin, mucosal, and open wound infections such as diabetic ulcers can develop into systemic infections, thereby worsening the patient's condition (Narulita et al., 2019).

The increasing prevalence of diabetes mellitus (DM) in the community is directly proportional to the risk of *Staphylococcus aureus* infection. High blood sugar levels cause damage to blood vessels and nerves, thereby reducing local immunity and facilitating bacterial colonization (Susanto et al., 2020). *Staphylococcus aureus* can rapidly infect diabetic foot ulcers, hindering the healing process and increasing the risk of serious complications such as osteomyelitis and amputation. Early identification of the bacteria causing the infection is crucial for selecting the appropriate antibiotic therapy and controlling further complications (Mita Zuliana et al., 2023).

Previous research by (Zubir et al., 2024) on 40 samples of ulcer wounds in patients with diabetes mellitus found various gram-positive and gram-negative bacteria. The results showed gram-positive bacteria such as *Staphylococcus aureus* (22.5%) and *Staphylococcus epidermidis* (22.5%), while gram-negative bacteria included Proteus mirabilis (7.5%), *Pseudomonas aeruginosa* (7.5%), *Klebsiella pneumoniae* (15%), *Enterobacter agglomerans* (2.5%), *Enterobacter aerogenes* (5%), *Escherichia coli* (12%), and *Citrobacter freundii* (5%). Based on these data and the high incidence of DM in North Sumatra, this study was conducted to isolate and identify Staphylococcus aureus in diabetic ulcers to assess its role as a risk factor for infection.

Method

This study employed an analytic cross-sectional design aimed at analyzing the relationship between patient characteristics (age, gender, and fasting blood glucose levels) and the presence of Staphylococcus aureus in diabetic ulcers as a risk factor for infection (Hardani et al., 2020), The research was conducted at Bunda Thamrin General Hospital, Medan, and laboratory examinations were performed at the Bunda Thamrin Clinical Laboratory in April 2025. The population consisted of all diabetic foot ulcer patients who visited the hospital during the study period. Sampling was conducted using a total sampling technique, resulting in 30 samples that met the inclusion criteria: patients diagnosed with type 2 diabetes mellitus with lower extremity ulcer lesions, who had not received antibiotic therapy within the previous seven days, and who agreed to participate by signing an informed consent form.

Samples were collected aseptically from diabetic foot ulcers using sterile cotton swabs after cleaning the wound area with sterile physiological saline. The specimens were cultured on Blood Agar (BA) medium and incubated for 24 hours at 37°C. The morphology of the bacterial colonies was observed macroscopically, followed by Gram staining, catalase test, and coagulase test. The isolates were then identified automatically using the Vitek 2 Compact system. A diabetic ulcer was defined as a non-traumatic skin lesion with or without pus, confirmed by clinical examination. The presence of Staphylococcus aureus was determined based on the appearance of golden-yellow colonies with hemolysis on Blood Agar, Gram-positive clustered cocci morphology, positive catalase and coagulase reactions, and confirmation using the Vitek 2 Compact device.

Data were analyzed both descriptively and analytically. Descriptive statistics were used to determine the distribution of bacterial species, while analytical statistics were applied to test the relationships between variables (Prema Hapsari Hidayati et al., 2024).The Chi-square test was used to evaluate associations between categorical variables (gender, age group) and the presence of S. aureus infection, while the Spearman correlation test analyzed the relationship between fasting blood glucose levels and S. aureus infection. A p-value < 0.05 was considered statistically significant (Septiana et al., 2024, Lasmini & Margaretta, 2022).

This research has received ethical clearance and exemption from the Health Research Ethics Committee of Universitas Sari Mutiara Indonesia, with registration number No. 3371/F/KEP/USM/IV/2025, valid from April 14, 2025, to April 14, 2026. All participants provided written informed consent prior to data collection, and confidentiality of patient data was fully maintained throughout the study.

Results and Discussion

The results of bacterial culture on Blood Agar showed the growth of colonies with different morphological characteristics. The colonies were then identified using Gram staining and biochemical tests. From a total of 30 diabetic ulcer samples examined, Staphylococcus aureus was found as the dominant bacterium, detected in 14 isolates (46.7%). Other bacteria identified included Staphylococcus epidermidis (6 isolates, 20%), Citrobacter freundii (5 isolates, 16.7%), and Proteus mirabilis (5 isolates, 16.7%).

Table 1. The correlation between gender, age, and blood glucose levels with Staphylococcus aureus infection in diabetic ulcers

Variable	Category	n	S. aureus (+)	S.aureus (–)	p-value
Gender	Male	12	8 (66.7%)	4 (33.3%)	0.042*
	Female	18	6 (33.3%)	12 (66.7%)	
Age Group	<50 years	10	3 (30%)	7 (70%)	0.031*
	≥50 years	20	11 (55%)	9 (45%)	
Fasting Glucose	(Mean ± SD)	_	204 ± 52 mg/dL	168 ± 47 mg/dL	0.018*

Table 1 presents the distribution of S. aureus according to gender, age group, and fasting blood glucose levels. The Chi-square test showed a significant association between gender and S. aureus infection ($p = 0.042$) and between age group and infection ($p = 0.031$). The Spearman correlation test revealed a positive relationship between fasting blood glucose level and the presence of S. aureus ($r = 0.428$; $p = 0.018$).

Figure 1. Growth of Staphylococcus aureus colonies on Blood Agar medium showing golden-yellow pigmentation and clear β-hemolysis zones at 37°C after 24 hours incubation.

Culture results on Blood Agar media showed that out of 30 diabetic foot ulcer samples, Staphylococcus aureus was identified as the dominant bacterium, found in 14 isolates (46.7%). The colonies appeared round, smooth, convex, with golden-yellow pigmentation and complete β-hemolysis, which are typical characteristics of S. aureus. Other isolates included Staphylococcus epidermidis (6 isolates, 20%) with creamy-white colonies, Citrobacter freundii (5 isolates, 16.7%) with grayish-white colonies, and Proteus mirabilis (5 isolates, 16.7%) with irregular grayish colonies without hemolysis.

These findings support the report by (Lasmini & Margaretta, 2022) "Identification of S*taphylococcus aureus* Bacteria in Nasal Swabs of Food Handlers in Pekanbaru City," which described that S. aureus colonies on Blood Agar produce a golden-yellow pigment and clear β-hemolysis zones after 24–48 hours of incubation. The Gram-positive cocci morphology observed in this study was also in accordance with their findings.

Microscopic examination using Gram staining of 30 diabetic foot ulcer samples showed that 20 samples (66.7%) exhibited Gram-positive cocci arranged in irregular clusters, indicating Staphylococcus aureus, while 10 samples (33.3%) showed Gram-negative bacilli morphology. The clustered Gram-positive cocci appearance is characteristic of S. aureus, whereas the Gram-negative rods may represent species such as Citrobacter freundii or Proteus mirabilis, which were also confirmed through the Vitek 2 Compact identification test.

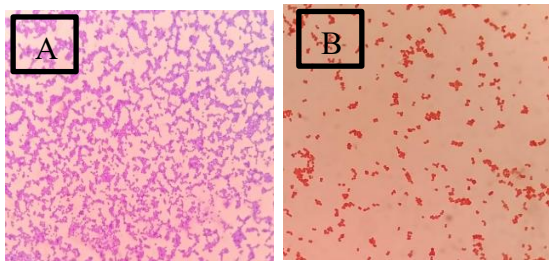

Figure 2. Gram staining results: (A) Staphylococcus aureus showing Gram-positive cocci in grape-like clusters (1000× magnification); (B) Gram-negative bacilli as comparison (1000× magnification).

The dominance of Gram-positive cocci observed in this study is consistent with the characteristics of Staphylococcus aureus as the principal pathogen associated with diabetic ulcers. Among the 30 samples examined, S. aureus was identified in 14 isolates (46.7%), followed by Staphylococcus epidermidis (6 isolates; 20%), Citrobacter freundii (5 isolates; 16.7%), and Proteus mirabilis (5 isolates; 16.7%).These results support the findings of (Nur Mita Zuliana et al., 2023) study, "Identification of Bacteria in Diabetic Ulcer Wounds," which examined 40 ulcer samples and found S. aureus (22.5%) and Staphylococcus epidermidis (22.5%) to be the most common Gram-positive bacteria. Meanwhile, Gram-negative bacteria such as Proteus mirabilis and Citrobacter freundii were also reported in Zuliana's study, with prevalences of 7.5% and 5%, respectively.

Similar findings were reported by (Rezania Febiola, 2022) in "Identification of Pathogenic Bacteria by Culture Testing in Cases of Diabetic Ulcers at Yogyakarta City Hospital," where S. aureus was the most frequently isolated Gram-positive bacterium (27.2%), followed by various Gram-negative bacilli such as *Klebsiella* and *Proteus spp*.

Identification of bacteria using the Vitek 2 Compact system confirmed these results, with S. aureus detected in 14 samples (46.7%), S. epidermidis in 6 samples (20%), C. freundii in 5 samples (16.7%), and P. mirabilis in 5 samples (16.7%). These findings reinforce that S. aureus remains the dominant etiological agent of diabetic ulcer infections due to its virulence factors—such as coagulase, hemolysin, and biofilm formation—that enhance adhesion, colonization, and tissue invasion.

The predominance of S. aureus observed in this study supports the clinical pattern of diabetic ulcers described by (Fadhilah Zalfa Cahyaningrum et al., 2025) in "Analysis of Bacterial Sensitivity Test Results in Ulcers of Type 2 Diabetes Mellitus Patients at a Hospital in Yogyakarta", which reported S. aureus as the most common Gram-positive bacterium isolated (22.5%) together with S. epidermidis.

This finding is also consistent with (Rezania Febiola, 2022) "Identification of Pathogenic Bacteria with Culture Tests in Cases of Diabetic Ulcers at Yogyakarta City Hospital," which noted S. aureus as the most dominant Gram-positive bacterium (27.2%), followed by several Gram-negative bacteria such as *Klebsiella* and *Proteus spp*.

The bacteria C. freundii and P. mirabilis in this study indicate the presence of polymicrobial infection, which is common in chronic wounds in diabetic patients. (Wu et al., 2024) "Analysis of Distribution and Drug Susceptibility Test Results of Pathogenic Bacteria in Diabetic Foot Ulcers" reported that mixed infections involving both Gram-positive and Gram-negative organisms delay wound healing and increase the risk of osteomyelitis and amputation.

Pathophysiologically, chronic hyperglycemia impairs neutrophil function and blood circulation, facilitating colonization by S. aureus and other bacteria (Lipinwati et al., 2023), "Staphylococcus Aureus Antibiotics Resistance's pattern in Osteomyelitis Cases at Hospital in Jambi City". which explained that prolonged hyperglycemia weakens host defense, enabling S. aureus to act as a primary pathogen and major risk factor for severe infections in diabetic ulcer patients.

Based on gender, this study found that female patients experienced diabetic ulcers more frequently than males. This observation supports the results of (Rezania Febiola, 2022) who reported that 60.7% of diabetic ulcer patients were female and 39.3% were male. Hormonal changes during menopause, particularly reduced estrogen levels, may decrease vascular elasticity and promote atherosclerosis and hypertension, increasing ulcer susceptibility. Furthermore, postmenopausal metabolic changes may elevate body mass index (BMI), a factor linked to insulin resistance and delayed wound healing (Fasero & Coronado, 2025).

Age also plays a significant role. According to Indonesia's Basic Health Research (Riskesdas, 2018), the highest prevalence of diabetes occurs in the 55–64-year age group (6.3%). Individuals in this age range tend to develop severe macroangiopathy and microangiopathy, which lead to peripheral neuropathy and impaired circulation. These conditions, together with chronic hyperglycemia and declining insulin function, make this age group particularly vulnerable to diabetic foot ulcers (Purwandari et al., 2022).

Conclusion

This study confirms that diabetic foot ulcers are chronic wounds highly susceptible to infection by pathogenic bacteria, particularly Staphylococcus aureus. Among the 30 samples examined at the Bunda Thamrin Clinic Laboratory in Medan, S. aureus was identified as the dominant pathogen (46.7%), characterized by golden-yellow

colonies producing β-hemolysis on Blood Agar and Gram-positive cocci morphology under microscopic examination.

The isolation and identification of S. aureus are essential for guiding rational antibiotic therapy and preventing serious complications such as osteomyelitis, sepsis, and limb amputation. These findings emphasize the importance of rapid and accurate microbiological diagnosis in every case of diabetic foot ulcer to ensure targeted treatment, minimize antibiotic resistance, and ultimately reduce morbidity and mortality among diabetic patients.

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