Bioscientist: Jurnal Ilmiah Biologi
https://e-journal.undikma.ac.id/index.php/bioscientist

September 2026 Vol. 14, No. 3
e-ISSN: 2654-4571
pp. 1356-1374

Analysis of the Sensitivity Pattern of *Staphylococcus aureus* in Diabetic Ulcers with Different Severity Levels

[1]Diana Yuli Lestari, [2*]Kurniawan, [3]Kurnia Ritma Dhanti, [4]Margono
[1,2,3]Department of Medical Laboratory Technology, Faculty of Health Sciences, Universitas Muhammadiyah Purwokerto, Banyumas, Indonesia.
[4]Microbiology Laboratory, RSUP Dr. Soeradji Tirtonegoro, Klaten, Indonesia.
*Corresponding Author e-mail: kurniawan@ump.ac.id
Received: July 2026; Revised: August 2026; Accepted: September 2026; Published: September 2026

Abstract: This study aimed to describe the antibiotic susceptibility patterns of *Staphylococcus aureus* isolated from diabetic ulcer patients with varying degrees of ulcer severity at Prof. Dr. Margono Soekarjo Hospital, Purwokerto. This study employed a descriptive observational design with a *cross-sectional* approach. A total of 20 diabetic ulcer patients were enrolled using an *accidental sampling* technique from October to November 2025. Bacterial identification was performed through microbiological examination, while antibiotic susceptibility testing was conducted using the *Kirby–Bauer* disk diffusion method. Ulcer severity was classified according to the Wagner classification system. The results showed that 70 bacterial isolates were obtained from the 20 diabetic ulcer patients, of which 46 isolates were identified as *S. aureus*. The susceptibility testing revealed that the susceptibility rates of *S. aureus* to linezolid, cefoxitin, and ciprofloxacin were 43.5%, 15.2%, and 26.1%, respectively, with variations observed across different Wagner grades. In contrast, most *S. aureus* isolates exhibited resistance to the tested antibiotics, including linezolid (54.3%), cefoxitin (80.4%), and ciprofloxacin (63.0%). The antibiotic susceptibility patterns of *Staphylococcus aureus* isolated from diabetic ulcers varied according to ulcer severity. Therefore, continuous monitoring of antibiotic susceptibility patterns and the availability of local bacterial resistance data are essential to support rational and effective antibiotic selection.
Keywords: *Staphylococcus aureus*; diabetic ulcer; antibiotic susceptibility; Wagner grade

How to Cite: Lestari, D. Y., Kurniawan, Dhanti, K. R., & Margono. (2026). Analysis of the Sensitivity Pattern of Staphylococcus aureus in Diabetic Ulcers with Different Severity Levels. *Bioscientist: Jurnal Ilmiah Biologi*, *14*(3), 1356–1374. https://doi.org/10.33394/bioscientist.v14i3.22210

https://doi.org/10.33394/bioscientist.v14i3.22210

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from impaired insulin secretion and/or insulin action (Khariunnisa & Tri, 2020). DM may lead to various chronic complications, including diabetic ulcers, which are open wounds commonly occurring on the lower extremities. Diabetic ulcers represent a major clinical challenge due to their prolonged healing process and potential progression to infection, necrosis, and gangrene, which may increase the risk of amputation and mortality among patients with DM (Septiana et al., 2024).

Epidemiologically, the prevalence of diabetic ulcers among patients with DM worldwide and in Asia has been reported to reach 6.3% and 5.5%, respectively (McDermott et al., 2023). In Indonesia, the prevalence of diabetic ulcers is estimated at 12–15% of the total DM population (Kemenkes RI, 2025). Diabetic ulcers remain a significant clinical concern because delayed or inadequate management may result in disease progression and severe complications. The development and severity of diabetic ulcers are influenced by multiple factors, including glycemic control, peripheral neuropathy, vascular impairment, reduced immune function, and microbial infection of the wound (Safira et al., 2023).

Microbial infections in diabetic ulcers may involve various microorganisms, including bacteria, fungi, viruses, and parasites. Among these microorganisms, bacteria are the predominant pathogens identified in diabetic ulcers, as demonstrated by the diversity of bacterial species isolated from infected wounds. Previous studies by BA et al. (2024) and Zuliana et al. (2023) reported that bacterial infections in diabetic ulcers are frequently dominated by Gram-positive bacteria, with *Staphylococcus aureus* (*S. aureus*) being one of the most commonly identified species. Lee et al. (2023) reported that *S. aureus* represents one of the major bacterial pathogens isolated from diabetic ulcers, with a prevalence ranging from 15–38%. The presence of *S. aureus* in diabetic ulcers is clinically important because this bacterium can act as an opportunistic pathogen and contribute to soft tissue infection.

S. aureus is a commensal bacterium commonly found on human skin, nasal cavities, and upper respiratory tracts. However, under specific conditions, it can become an opportunistic pathogen capable of causing infection in damaged tissues, including diabetic ulcers (Sinaga and Mahyudi, 2022). This bacterium possesses several virulence mechanisms, including strong adhesion capacity, biofilm formation, and production of extracellular enzymes and toxins, which contribute to bacterial persistence, impaired immune clearance, and delayed wound healing. These characteristics, combined with extensive antibiotic exposure during infection management, may contribute to the emergence of antibiotic-resistant *S. aureus* isolates.

One of the major antimicrobial resistance concerns associated with *S. aureus* is methicillin-resistant *Staphylococcus aureus* (MRSA), which refers to *S. aureus* isolates resistant to specific β-lactam antibiotics, resulting in limited therapeutic options. Cefoxitin is widely used in antimicrobial susceptibility testing as a phenotypic indicator for detecting methicillin resistance in *S. aureus* (CDC, 2025). In addition, linezolid exhibits activity against Gram-positive bacteria, including *S. aureus*, whereas ciprofloxacin, a fluoroquinolone antibiotic, requires careful consideration due to the increasing occurrence of resistance among bacterial pathogens. Therefore, susceptibility testing against linezolid, cefoxitin, and ciprofloxacin was selected in this study to characterize different aspects of *S. aureus* antimicrobial susceptibility patterns in diabetic ulcers (Senneville et al., 2023).

Previous investigations of bacterial infections in diabetic ulcers from different regions have demonstrated variations in bacterial composition according to ulcer severity. Tehrani (2022) reported that more severe diabetic ulcers were more frequently associated with Gram-negative bacteria, whereas less severe ulcers showed a higher proportion of Gram-positive bacteria. Nevertheless, *S. aureus* was detected across different levels of ulcer severity. These findings indicate that characterization of bacterial profiles and antibiotic susceptibility patterns according to ulcer severity is essential for improving understanding of local microbial epidemiology.

Previous studies have also demonstrated variations in the antimicrobial susceptibility patterns of *S. aureus* isolated from diabetic ulcers. Khariunnisa et al. (2020) reported that *S. aureus* isolates from diabetic ulcers remained susceptible to cefoxitin and vancomycin but exhibited resistance to amoxicillin and erythromycin. Another study showed that *S. aureus* isolates were susceptible to meropenem and amikacin but resistant to ciprofloxacin and ampicillin (Triani et al., 2022). These differences highlight the importance of continuous surveillance of *S. aureus* antimicrobial susceptibility patterns within specific healthcare settings.

Antibiotic susceptibility patterns may differ among geographical regions and healthcare facilities due to variations in patient characteristics, ulcer severity, antibiotic

prescribing practices, and antimicrobial stewardship programs implemented by hospitals (Barlam et al., 2016; Wayne, 2023). Therefore, local surveillance data are required to provide an accurate representation of *S. aureus* susceptibility patterns and support evidence-based antimicrobial management in specific healthcare facilities.

A previous study by Kusumah (2024) investigated *S. aureus* and its antibiotic resistance patterns among diabetic ulcer patients at RSUD Banyumas. However, that study did not describe the distribution of *S. aureus* susceptibility patterns according to different ulcer severity levels based on the Wagner classification. Consequently, the descriptive pattern of *S. aureus* susceptibility to antibiotics across different Wagner grades, particularly against linezolid, cefoxitin, and ciprofloxacin, remains insufficiently characterized. Therefore, the novelty of this study lies in describing the antimicrobial susceptibility patterns of *S. aureus* isolated from diabetic ulcers across different severity levels according to the Wagner classification at RSUD Prof. Dr. Margono Soekarjo Purwokerto.

This study was conducted at RSUD Prof. Dr. Margono Soekarjo Purwokerto, a referral hospital serving patients from southern Central Java and surrounding regions, including parts of West Java Province. This healthcare facility was selected because of the availability of diabetic ulcer patients and the need for local data regarding *S. aureus* susceptibility patterns across different ulcer severity levels. The findings of this study are expected to provide additional information regarding local antimicrobial susceptibility profiles of *S. aureus* in diabetic ulcers. Therefore, this study aimed to describe the antimicrobial susceptibility patterns of *Staphylococcus aureus* isolated from diabetic ulcers with different severity levels at RSUD Prof. Dr. Margono Soekarjo Purwokerto.

METHOD

Study Design and Study Period

This study employed a descriptive observational design with a cross-sectional approach. Clinical specimens were collected from patients with diabetes mellitus (DM) complicated by diabetic ulcers at Prof. Dr. Margono Soekarjo Regional General Hospital, Purwokerto. Laboratory examinations and data analyses were conducted at the Microbiology Laboratory, Medical Laboratory Technology Study Program, Universitas Muhammadiyah Purwokerto, from October 2025 to May 2026. The entire study protocol received ethical approval from the Ethics Committee of Prof. Dr. Margono Soekarjo Regional General Hospital (RSMS) Purwokerto prior to study initiation (Ethical Approval No. 420/08874).

Study Subjects

The study subjects consisted of patients with DM-associated diabetic ulcers classified as Wagner grade 0–5. The inclusion criteria were: patients with DM who were hospitalized at Prof. Dr. Margono Soekarjo Regional General Hospital, patients with diabetic ulcer complications accompanied by infection allowing wound swab collection, patients who agreed to participate as research respondents, and patients who had not received systemic antibiotics within the previous 72 hours. The exclusion criteria included patients whose wounds had undergone extensive debridement, patients receiving other treatments that could interfere with the evaluation of diabetic ulcer infection, and patients with contamination or other unstable clinical conditions.

Samples were collected using an accidental sampling technique during the sampling period of October–November 2025. All patients who met the inclusion criteria and agreed to participate during the study period were included as respondents. A total of 20 respondents were enrolled, and wound swab samples were collected for bacterial

diversity analysis and antibiotic susceptibility testing. From these samples, bacterial isolation and purification were performed, resulting in 70 bacterial isolates. Subsequent bacterial identification identified 46 isolates as *Staphylococcus aureus*.

Study Object and Variables

The study object consisted of *S. aureus* bacterial isolates obtained from patients with DM-associated diabetic ulcers. The study variables included the severity level of diabetic ulcers and the antibiotic susceptibility pattern of *S. aureus*. The severity of diabetic ulcers was determined based on the Wagner classification system, while antibiotic susceptibility patterns were assessed using the Kirby–Bauer disk diffusion method on Mueller–Hinton Agar (MHA) and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) standards applied in this study. Because this study used a descriptive design, ulcer severity and antibiotic susceptibility patterns were analyzed based on isolate distribution without performing statistical tests to determine associations or differences between variables.

Equipment and Materials

The equipment used in this study included laboratory coats, test tubes, sterile Petri dishes, incubators, Laminar Air Flow (LAF) cabinets, inoculating loops, forceps, Bunsen burners, autoclaves, glass slides, cool boxes, Erlenmeyer flasks, beakers, gloves, masks, and stirring rods. The materials used included Amies transport medium, diabetic ulcer swab samples, Mannitol Salt Agar (MSA), Tryptic Soy Broth (TSB), Tryptic Soy Agar (TSA), Mueller–Hinton Agar (MHA), Gram staining reagents, 0.9% NaCl solution, 3% hydrogen peroxide (H_2O_2), oxidase reagent, plasma, and antibiotic disks consisting of linezolid 30 µg (LZD30), cefoxitin 30 µg (FOX30), and ciprofloxacin 5 µg (CIP5) (Oxoid Limited), obtained through PT. Dipa Puspa Labsains (Dipalabs), Jakarta. Dimethyl sulfoxide (DMSO) solution was used as a negative control.

Research Procedures

1. **Collection of diabetic ulcer swab samples**

   Prior to sample collection, wounds were cleaned using sterile physiological saline (0.9% NaCl) to remove surface debris. The surrounding skin area was disinfected using 70% alcohol while avoiding contact with the wound bed. Samples were collected from the wound base using sterile cotton swabs, which were subsequently placed into Amies transport medium and immediately transported to the laboratory in a cool box. Previous antibiotic exposure was recorded based on the history of antibiotic use, whereas the inclusion criterion of no systemic antibiotic use within the previous 72 hours was applied to minimize the potential influence of recent antibiotic administration on microbiological examination results.

2. **Bacterial isolation from diabetic ulcer samples**

   Diabetic ulcer swab samples were processed using serial dilution methods and inoculated onto Mannitol Salt Agar (MSA). The plates were incubated at 37°C for 18–24 hours. Following incubation, bacterial colony morphology was examined. *S. aureus* colonies on MSA were characterized by yellow pigmentation, round morphology, and convex surfaces.

3. **Purification of bacterial isolates**

   Obtained bacterial isolates were subcultured onto fresh Mannitol Salt Agar (MSA). A single yellow bacterial colony was collected using a sterile inoculating loop and streaked onto MSA using the quadrant streak method. The plates were then incubated at 37°C for 48 hours, followed by morphological observation of bacterial

colonies. Individual yellow colonies were subsequently inoculated onto slanted Tryptic Soy Agar (TSA) media to establish pure bacterial culture stocks.

4. Gram staining

Gram staining was performed by transferring a bacterial colony onto a glass slide containing 1–2 drops of distilled water. The smear was air-dried and heat-fixed over a Bunsen flame. The staining procedure consisted of crystal violet application for 1 minute, followed by rinsing, iodine solution application for 1 minute, rinsing, decolorization using 90% ethanol for 30 seconds, and counterstaining with safranin for 1 minute. The slides were then rinsed, dried, and examined microscopically. *Staphylococcus aureus* was identified microscopically as Gram-positive cocci appearing purple and arranged in grape-like clusters. Further biochemical tests were subsequently performed (Kurniawan et al., 2021).

5. Catalase test

Catalase is an enzyme responsible for catalyzing the decomposition of hydrogen peroxide (H_2O_2). Hydrogen peroxide is toxic to bacterial cells because it can inactivate intracellular enzymes (Hidayati et al., 2024). The catalase test was performed by placing 3% hydrogen peroxide solution onto a clean glass slide. A bacterial isolate was then mixed with the hydrogen peroxide using an inoculating loop. A positive reaction was indicated by the formation of visible gas bubbles due to oxygen release. In *S. aureus*, a positive catalase reaction was characterized by rapid and prominent bubble formation following exposure to 3% hydrogen peroxide, indicating catalase enzyme production.

6. Oxidase test

The oxidase test was conducted to determine the ability of bacteria to produce cytochrome c oxidase, an enzyme involved in the electron transport system during aerobic respiration (Hidayati et al., 2024). The test was performed by applying bacterial isolates onto filter paper, followed by the addition of 1–2 drops of oxidase reagent (*tetramethyl-p-phenylenediamine*). A positive reaction was indicated by a color change to purple or dark blue within 10–30 seconds, whereas the absence of color change indicated a negative reaction. *S. aureus* produces a negative oxidase reaction because it does not produce cytochrome c oxidase; therefore, no color change occurs after oxidase reagent application.

7. Coagulase test

The coagulase test was performed to detect the presence of coagulase enzyme in *Staphylococcus* spp., which converts fibrinogen into fibrin and induces plasma clot formation (Purnamasari & Suwarno, 2023). Approximately 10 µL of sterile physiological saline was placed on a glass slide, followed by the addition of bacterial colonies using a sterile inoculating loop. The suspension was homogenized, and 10 µL of plasma was added adjacent to the bacterial suspension and mixed gently. The reaction was observed within 10–30 seconds. A positive result was indicated by visible clot or granular agglutination, whereas a negative result was indicated by the absence of visible changes and maintenance of a homogeneous suspension. This test was used to differentiate coagulase-positive *S. aureus* from other coagulase-negative *Staphylococcus* species, such as *S. epidermidis* and *S. saprophyticus*.

8. Antibiotic susceptibility testing

Bacterial isolates were suspended in Tryptic Soy Broth (TSB) and incubated at 37°C for 24 hours. The resulting bacterial suspension was subsequently diluted and

standardized to a turbidity equivalent to 0.5 McFarland (approximately 1.5 × 10^8 CFU/mL). Inoculum standardization was performed using turbidity measurement at a wavelength of 600 nm. The absorbance values were used to estimate bacterial concentration and converted into cell numbers using the web-based software OD600 Calculator (https://www.agilent.com).

The standardized bacterial suspension (0.5 McFarland; approximately 1.5 × 10^8 CFU/mL) was evenly inoculated onto Mueller–Hinton Agar (MHA) plates and allowed to absorb into the medium. Antibiotic disks containing linezolid 30 µg, cefoxitin 30 µg, and ciprofloxacin 5 µg (Oxoid Limited), along with DMSO-containing disks as negative controls, were placed on the agar surface at appropriate distances.

The plates were incubated at 37°C for 18–24 hours. Following incubation, inhibition zones surrounding the antibiotic disks were observed and measured using a vernier caliper in millimeters (mm). The inhibition zone diameters were interpreted according to the CLSI M100 (2020) breakpoint criteria and categorized as susceptible, intermediate, or resistant.

Table 1. Interpretation of inhibition zone diameters based on CLSI 2020

No	Antibiotic	Disk Content	Susceptible	Intermediate	Resistant
1	Linezolid	30 µg	≥21 mm	–	≤20 mm
2	Cefoxitin	30 µg	≥22 mm	–	≤21 mm
3	Ciprofloxacin	5 µg	≥21 mm	16–20 mm	≤15 mm

Source: https://clsi.org/shop/standards/m100/

Data Analysis

The collected data were analyzed descriptively and presented as frequencies and percentages. The analysis was conducted to describe the distribution of diabetic ulcer severity according to the Wagner classification, the presence of *S. aureus* isolates, and the antibiotic susceptibility patterns of *S. aureus* against linezolid, cefoxitin, and ciprofloxacin across different ulcer severity levels. The results were presented in tables and descriptive narratives.

RESULTS AND DISCUSSION

Patient Characteristics and Clinical Characteristics of Diabetic Foot Ulcers

Based on the isolation and identification results of samples obtained from diabetic foot ulcer swabs at Prof. Dr. Margono Soekarjo Hospital, Purwokerto, a total of 20 patients with diabetes mellitus (DM) and diabetic foot ulcers who fulfilled the inclusion criteria were enrolled during the initial study period (October–November 2025). Data were collected from the Medical Record Unit of Prof. Dr. Margono Soekarjo Hospital and respondent questionnaires. These data provided information regarding sex, age, duration of diabetes mellitus, duration of diabetic foot ulcers, wound location, ulcer severity according to the Wagner classification, and other clinical characteristics required for the study.

Patient Characteristics and Clinical Characteristics of Diabetic Foot Ulcers

The characteristics of the 20 patients with DM-associated diabetic foot ulcers enrolled in this study are presented in Table 2.

Table 2. Characteristics of patients with diabetic foot ulcers

Characteristics	Frequency (n)	Percentage (%)
Sex		
Male	13	65
Female	7	35
Age		
15–24 years (Adolescents)	1	5
25–59 years (Adults)	12	60
>60 years (Elderly)	7	35
Duration of Diabetes Mellitus		
0–5 years	16	80
6–10 years	2	10
>10 years	2	10
Duration of Diabetic Foot Ulcer		
0–5 years	19	95
6–10 years	1	5

As shown in Table 2, diabetic foot ulcer patients in this study were predominantly male (65%). This finding is consistent with previous studies by Rachmawati et al. (2021) and Hu et al. (2024), which reported a higher proportion of diabetic foot ulcers among male patients. This predominance may be associated with higher levels of physical activity and mobility among males, potentially increasing the risk of foot trauma. However, Fadlilah (2018) reported that diabetic foot ulcer complications were more frequently observed among females. These differences may be influenced by variations in participant characteristics across studies, including age, diabetes duration, glycemic control, and hormonal factors. Nevertheless, diabetic foot ulcer development is multifactorial and may be influenced by several factors, including age, duration of diabetes mellitus, vascular status, peripheral neuropathy, and lifestyle-related factors.

Regarding age distribution, the majority of participants (60%) were adults aged 25–59 years, indicating that diabetic foot ulcers also commonly occur among individuals of productive age. This finding is consistent with Khariunnisa & Tri (2020), who reported that most diabetic foot ulcer patients were within the 45–55-year age range (57.1%), categorized as adults. The similarity between these findings suggests that adults to pre-elderly individuals represent a vulnerable population for diabetic foot ulcer development. Nevertheless, increasing age remains an important risk factor because aging processes may contribute to physiological decline, impaired microcirculation, and delayed tissue regeneration (American Diabetes Association, 2024).

Based on the duration of diabetes mellitus, most participants had been diagnosed with diabetes for 0–5 years (80%). This finding indicates that, in this study population, diabetic foot ulcers were more frequently observed among patients with relatively shorter diabetes duration. This condition may be influenced by several factors, including suboptimal glycemic control, delayed diagnosis, and treatment adherence. However, longer diabetes duration may increase the risk of diabetic foot ulcers through chronic hyperglycemia, which contributes to peripheral neuropathy, vascular impairment, and delayed wound healing (Zhao et al., 2024; Yan et al., 2025).

Regarding ulcer duration, 95% of participants had experienced diabetic foot ulcers for 0–5 years. A prolonged ulcer duration may increase the risk of infection and complications and may interfere with the wound healing process. Successful diabetic foot ulcer healing is influenced by various factors, including glycemic control, peripheral vascular condition, neuropathy, nutritional status, and the presence of infection (Jeffcoate & Harding, 2023). Therefore, continuous wound monitoring and comprehensive management are required to accelerate healing and prevent further complications.

The clinical characteristics of diabetic foot ulcers provide important information regarding disease severity, clinical conditions, and factors influencing wound healing. Evaluation of wound characteristics is also essential to support appropriate management and prevent complications. In this study, wound characteristics assessed included ulcer location, ulcer severity according to the Wagner classification, signs of infection, antibiotic use, and the presence of wound odor. The characteristics of diabetic foot ulcers are presented in Table 3.

Table 3. Characteristics of diabetic foot ulcers

Characteristics	Frequency (n)	Percentage (%)
Ulcer Location		
Right foot	12	60
Left foot	8	40
Ulcer Grade (Wagner Classification)		
Grade 0 (Pre-ulcerative)	0	0
Grade 1 (Superficial ulcer)	4	20
Grade 2 (Deep ulcer)	5	25
Grade 3 (Ulcer with complication/abscess)	1	5
Grade 4 (Localized gangrene)	4	20
Grade 5 (Entire foot gangrene)	6	30
Signs of Infection		
Redness	1	5
Pus	13	65
Swelling	3	15
Redness, pus, and swelling	3	15
Use of Antibiotics/Wound Healing Agents		
Yes	4	20
No	12	60
Honey	4	20
Wound Odor		
Present	12	60
Absent	8	40

Bacterial Isolate Characteristics from Diabetic Foot Ulcers

Based on ulcer location, most participants experienced diabetic foot ulcers on the right foot (60%), while 40% had ulcers on the left foot. The predominance of right-foot involvement may be associated with the dominant foot receiving greater mechanical

pressure, shearing forces, and repeated trauma during daily activities. In patients with diabetes mellitus, minor unnoticed trauma due to peripheral neuropathy may progress into diabetic foot ulcers if not properly managed (Jeffcoate & Harding, 2023). This finding is consistent with Holcomb & Millward (2025), who reported that approximately 61% of initial neuropathic ulcers occurred on the right foot. Therefore, routine foot examinations, particularly of the dominant foot, and the use of appropriate footwear are important preventive measures for diabetic foot ulcers.

According to the Wagner classification, the distribution of ulcer severity was dominated by Wagner grade 5 ulcers (entire foot gangrene), accounting for 30% of cases, followed by grade 2 ulcers (25%), and grade 1 and grade 4 ulcers (20% each). Wagner grade 3 ulcers represented the lowest proportion (5%). The high proportion of grade 4 and grade 5 ulcers indicates that most patients referred to Prof. Dr. Margono Soekarjo Hospital had severe and chronic wound conditions. This condition may be associated with delayed detection, delayed treatment, referral processes, and limitations in primary healthcare management. This finding is consistent with Kurniawan et al. (2024), who reported that referral hospitals tend to receive diabetic foot ulcer patients with more severe conditions.

In this study, diabetic foot ulcer severity was assessed using the Wagner classification, which evaluates wound depth, tissue involvement, and the presence of gangrene (Frykberg, 2020). The relatively high proportion of grade 1 and grade 2 ulcers indicates that some patients presented with superficial and deep ulcers before progressing to abscess formation or osteomyelitis. These findings highlight the importance of early detection and appropriate management to prevent ulcer progression to gangrene and reduce the risk of amputation among patients with diabetic foot ulcers.

Regarding clinical signs of infection, most participants presented with pus formation in the ulcer wound (65%). The presence of pus is considered one of the clinical indicators of infection in diabetic foot ulcers and reflects inflammatory responses caused by bacterial colonization or tissue invasion (IWGDF/IDSA, 2023). Infection in diabetic foot ulcers may delay wound healing and increase the risk of further complications.

Based on the history of antibiotic use or wound healing agent application, most participants did not receive antibiotics or wound healing agents (60%), whereas 20% received antibiotics and 20% used honey as wound therapy. These differences indicate variations in wound management among participants. Antibiotic administration in diabetic foot ulcers is generally determined based on clinical conditions and the presence of infection; therefore, not all patients with diabetic foot ulcers require antibiotic therapy.

Regarding wound odor, 60% of participants had malodorous wounds, while 40% did not. Wound odor may be associated with infection, tissue necrosis, or increased microbial colonization. This finding suggests that many participants had wound characteristics that may support microbial persistence and require appropriate monitoring and wound management.

Bacterial Isolate Characteristics from Diabetic Foot Ulcers

Bacterial identification was performed on isolates obtained from diabetic foot ulcer swab samples through a series of microbiological procedures, including bacterial isolation, purification, colony morphology analysis (color, size, and shape), cellular morphology examination, biochemical tests (catalase, oxidase, and coagulase), and antibiotic susceptibility testing (Putri et al., 2024). The overall identification results are presented in Figures 1 and 2.

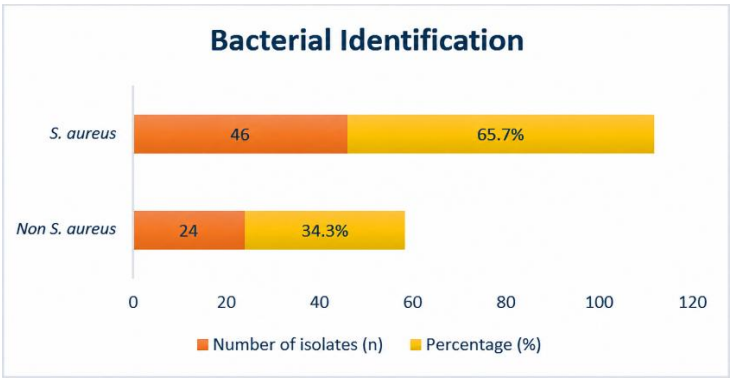

Figure 1. Identification results of *Staphylococcus aureus*

Isolation of bacteria from 20 diabetic foot ulcer swab samples cultured on Mannitol Salt Agar (MSA) yielded a total of 70 bacterial isolates. Following bacterial identification, 46 isolates (65.7%) were identified as *S. aureus*, whereas the remaining 24 isolates (34.3%) were identified as non-*S. aureus* bacteria. This finding is consistent with Sinaga et al. (2021), who reported that *S. aureus* was among the most frequently isolated bacteria from diabetic foot ulcers (24%), followed by other bacterial species such as *Klebsiella pneumoniae* (13%) and *Escherichia coli* (13%).

The predominance of *S. aureus* isolates in this study indicates that this bacterium was the most frequently isolated species from diabetic foot ulcers among patients with diabetes mellitus at Prof. Dr. Margono Soekarjo Hospital. This finding suggests that *S. aureus* represents an important bacterial species involved in diabetic foot ulcer infections in this hospital setting. However, the presence of 24 non-*S. aureus* isolates indicates that diabetic foot ulcer infections may involve diverse bacterial populations. These isolates may include bacteria other than *Staphylococcus* that are capable of growing on MSA due to their tolerance to high salt concentrations.

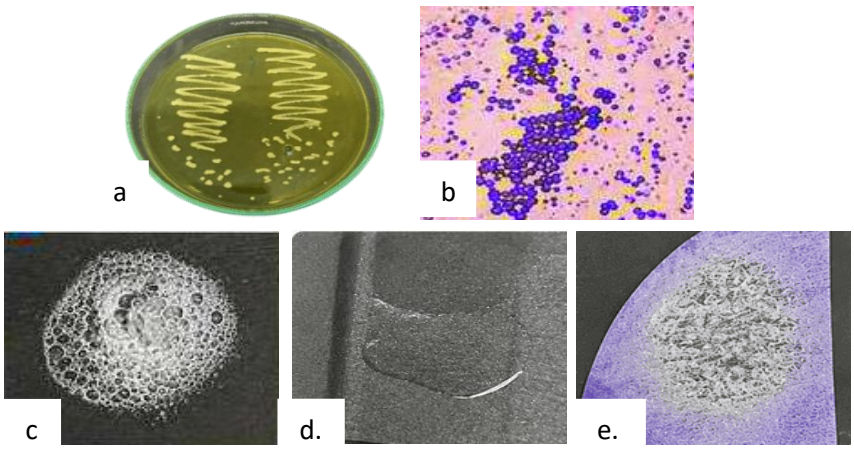

Figure 2. Characteristics of *Staphylococcus aureus* (a. *S. aureus* colony showing mannitol fermentation (morphology); b. Gram staining showing clustered cocci morphology of *S. aureus*; c. Catalase-positive reaction indicated by bubble formation; d. Coagulase-positive reaction indicated by agglutination; e. Oxidase-negative reaction indicated by the absence of color change after bacterial streaking)

The morphological characteristics of *S. aureus* colonies on MSA included yellow-colored, opaque, circular colonies with convex elevation, smooth edges, and a shiny surface. Microscopically, *S. aureus* appeared as Gram-positive cocci with purple staining arranged in grape-like clusters. These characteristics are consistent with

Kurniawan et al. (2021), who described *S. aureus* colonies as large, circular, shiny, opaque colonies with convex elevation, yellow pigmentation, and a diameter of approximately 2–3 mm. Similarly, Chaves et al. (2025) reported that *S. aureus* is a Gram-positive coccus arranged in grape-like clusters and produces yellow/golden colonies on MSA under aerobic or anaerobic conditions.

Mannitol Salt Agar (MSA) is a selective medium containing 7.5% NaCl, which inhibits the growth of many bacterial species while supporting the growth of *Staphylococcus* species and other salt-tolerant bacteria (Abdilah & Kurniawan, 2021; Sugireng & Rosdarni, 2021). *Staphylococcus aureus* is a Gram-positive bacterium with cocci-shaped cells (Figure 2b) that retain the primary stain, crystal violet (Suliati et al., 2022). Hayati et al. (2019) explained that differences in Gram staining characteristics are associated with variations in bacterial cell wall structure and thickness. Gram-positive bacteria possess a thicker peptidoglycan layer than Gram-negative bacteria, allowing the crystal violet–iodine complex to remain trapped during the decolorization process, resulting in purple-stained cells. According to Rashed & Husein (2021), *S. aureus* is a Gram-positive bacterium with spherical cells measuring approximately 0.5–1.5 µm in diameter and appearing blue or purple after Gram staining.

Biochemical tests demonstrated positive results for catalase and coagulase, while the oxidase test showed negative results. A positive catalase reaction is indicated by gas bubble formation after bacterial colonies are exposed to hydrogen peroxide (H_2O_2), demonstrating the ability of the isolate to break down toxic H_2O_2 into water (H_2O) and oxygen (O_2) through catalase enzyme activity (Nugroho et al., 2024). The positive coagulase reaction, indicated by plasma coagulation, confirmed the ability of the isolates to produce coagulase, an extracellular enzyme characteristic of *S. aureus* that converts fibrinogen into fibrin. This virulence factor contributes to bacterial survival by facilitating fibrin formation, thereby helping bacteria evade phagocytosis and maintain infection (Hayati et al., 2019).

The oxidase-negative reaction was demonstrated by the absence of color change following oxidase reagent application, indicating that the isolates did not produce cytochrome c oxidase. This characteristic is consistent with the typical biochemical profile of *S. aureus*. Therefore, the combination of catalase-positive, coagulase-positive, and oxidase-negative results further supports the identification of the isolates as *Staphylococcus aureus*, distinguishing them from other Gram-positive and Gram-negative bacteria (Shettigar & Murali, 2020).

The distribution of *S. aureus* isolates according to diabetic foot ulcer severity based on the Wagner classification is presented in Table 4.

Table 4. Distribution of *Staphylococcus aureus* isolates according to diabetic foot ulcer grade based on the wagner classification

Wagner Ulcer Grade	Number of Isolates	*S. aureus* Isolates, n (%)
Grade 1	11	11 (100%)
Grade 2	14	10 (71.4%)
Grade 3	7	0 (0.0%)
Grade 4	14	10 (71.4%)
Grade 5	24	15 (62.5%)
Total	**70**	**46 (65.7%)**

Antibiotic Susceptibility Patterns of *Staphylococcus aureus*

Antibiotic susceptibility testing was performed on bacterial isolates obtained from diabetic foot ulcer patients that had been identified as *S. aureus*. Three antibiotics

commonly used against *S. aureus*, namely linezolid, cefoxitin, and ciprofloxacin, were evaluated. Dimethyl sulfoxide (DMSO) was used as a negative control. The antibiotic susceptibility test was conducted using the Kirby–Bauer disk diffusion method on Mueller–Hinton Agar (MHA).

Based on the measurement of inhibition zone diameters, variations in the susceptibility patterns of *S. aureus* isolates to linezolid, cefoxitin, and ciprofloxacin were observed. Differences in inhibition zone diameters reflected variations in the ability of each antibiotic to inhibit the growth of *S. aureus*. The results of the susceptibility test are presented in Figure 3, which demonstrates the formation of inhibition zones around antibiotic disks in *S. aureus* isolates, while the DMSO disk served as the negative control. The distribution of susceptibility patterns for the three antibiotics is presented in Table 5.

Figure 3. Antibiotic susceptibility test results of *S. aureus* (a. DMSO as negative control; b. Linezolid; c. Cefoxitin; d. Ciprofloxacin)

Table 5. Distribution of *Staphylococcus aureus* susceptibility patterns to antibiotics

Antibiotic	Susceptible, n (%)	Intermediate, n (%)	Resistant, n (%)	Total, n (%)
Linezolid	20 (43.5%)	1 (2.2%)	25 (54.3%)	46 (100%)
Cefoxitin	7 (15.2%)	2 (4.3%)	37 (80.4%)	46 (100%)
Ciprofloxacin	12 (26.1%)	5 (10.9%)	29 (63.0%)	46 (100%)

Note: S = susceptible; I = intermediate; R = resistant.

Based on Table 5, the 46 *S. aureus* isolates tested against the three antibiotics exhibited varying susceptibility profiles. Linezolid demonstrated the highest proportion of susceptible isolates (43.5%), followed by ciprofloxacin (26.1%) and cefoxitin (15.2%). In contrast, more than half of the *S. aureus* isolates demonstrated resistance to all three tested antibiotics. The highest resistance rate was observed for cefoxitin (80.4%), followed by ciprofloxacin (63.0%) and linezolid (54.3%). Therefore, resistance represented the predominant category for all tested antibiotics, with the highest resistance proportion observed for cefoxitin. These variations in susceptibility patterns may reflect differences in antibiotic activity against *S. aureus*, which can be influenced by antibiotic mechanisms of action, previous antibiotic exposure, and characteristics of diabetic foot ulcer infections.

Wainwright et al. (2019) reported that linezolid is an antibiotic used for the treatment of skin and soft tissue infections because it inhibits bacterial protein synthesis by preventing the formation of the 70S initiation complex during translation. This mechanism provides activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). In diabetic foot infections, linezolid may be considered, particularly in patients with MRSA risk factors or moderate-to-severe infections requiring activity against resistant Gram-positive bacteria (IWGDF/IDSA,

2023). Because of its selective use, linezolid is generally not recommended as an empirical first-line therapy for all diabetic foot ulcer cases but may be considered in infections with MRSA risk or in patients with previous antibiotic treatment failure (Lipsky et al., 2020).

Nevertheless, in the present study, resistance to linezolid remained relatively high (54.3%), whereas susceptible isolates accounted for 43.5%. This finding may be influenced by several factors, including possible previous antibiotic exposure, which may create selective pressure and contribute to the emergence of isolates with more complex resistance patterns (Ramirez-Acuña et al., 2019). However, the underlying mechanism of linezolid resistance could not be directly determined in this study because genetic factors and previous antibiotic exposure were not analyzed.

The *S. aureus* isolates exhibited the lowest susceptibility and the highest resistance toward cefoxitin. The high proportion of cefoxitin resistance (80.4%) suggests the possibility of isolates exhibiting phenotypic characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA), which are frequently associated with severe diabetic foot ulcers. Cefoxitin is used as a phenotypic indicator for detecting methicillin resistance in *S. aureus* because its susceptibility results generally provide better interpretative performance compared with oxacillin. Phenotypic resistance to cefoxitin is associated with β-lactam resistance mechanisms, one of which may involve the *mecA* gene encoding penicillin-binding protein 2a (PBP2a), resulting in reduced bacterial affinity toward β-lactam antibiotics (Osok et al., 2025).

However, the presence of *mecA* or *mecC* genes was not evaluated through molecular analysis in this study; therefore, these findings cannot directly confirm the presence of specific resistance genes. Purwati et al. (2021) reported that patients with severe diabetic foot ulcers often experience prolonged hospitalization, which may increase the risk of MRSA colonization commonly associated with healthcare environments. Similarly, Sari et al. (2021) reported that the high prevalence of MRSA infections in chronic severe diabetic foot ulcers may be associated with repeated and inappropriate antibiotic exposure. Increasing ulcer severity may therefore be accompanied by a higher risk of MRSA infection and colonization.

Ciprofloxacin demonstrated greater inhibitory activity against *S. aureus* compared with cefoxitin but lower activity compared with linezolid. In diabetic foot ulcers with higher severity, a greater proportion of *S. aureus* isolates resistant to ciprofloxacin was observed compared with some lower Wagner grades. Previous antibiotic exposure may contribute to resistance development; however, this factor was not specifically analyzed in the present study and therefore cannot be confirmed as the cause of resistance among the obtained isolates.

According to Uçkay et al. (2019), resistance of *S. aureus* to ciprofloxacin, a fluoroquinolone antibiotic, may occur through mutations in the *gyrA* and *grlA* genes, which encode subunits of DNA gyrase and topoisomerase IV, respectively. These enzymes represent the primary targets of ciprofloxacin. Mutations in these genes may alter antibiotic target structures, reducing ciprofloxacin binding efficiency and resulting in bacterial resistance. Khan et al. (2019) further explained that fluoroquinolone resistance in *S. aureus* may occur through mutations affecting DNA gyrase and topoisomerase IV.

In addition to these mechanisms, chronic diabetic foot ulcers characterized by extensive necrotic tissue and impaired circulation or ischemia may influence ciprofloxacin penetration and distribution into infected tissues. Severe diabetic foot ulcers accompanied by necrotic tissue and vascular impairment may therefore represent important factors affecting antibiotic delivery to infection sites. Furthermore,

the chronic wound environment may facilitate bacterial persistence and increase the risk of resistance development. However, these resistance mechanisms were not directly investigated in this study and therefore cannot be confirmed as the underlying mechanisms responsible for resistance in the obtained isolates.

Distribution of Antibiotic Susceptibility Patterns of *Staphylococcus aureus* According to Diabetic Foot Ulcer Severity Based on the Wagner Classification

The distribution of antibiotic susceptibility patterns of *Staphylococcus aureus* according to diabetic foot ulcer severity based on the Wagner classification is presented in Table 6.

Table 6. Antibiotic Susceptibility of *Staphylococcus aureus* by Wagner Grade

Antibiotic	Grade 1 (n = 11)	Grade 2 (n = 10)	Grade 3 (n = 0)	Grade 4 (n = 10)	Grade 5 (n = 15)
Linezolid	S: 3 (27.3%) R: 8 (72.7%)	S: 6 (60.0%) R: 4 (40.0%)	–	S: 5 (50.0%) R: 5 (50.0%)	S: 6 (40.0%) I: 1 (6.7%) R: 8 (53.3%)
Cefoxitin	S: 4 (36.4%) R: 7 (63.6%)	S: 2 (20.0%) R: 8 (80.0%)	–	I: 1 (10.0%) R: 9 (90.0%)	S: 1 (6.7%) I: 1 (6.7%) R: 13 (86.7%)
Ciprofloxacin	S: 3 (27.3%) I: 2 (18.2%) R: 6 (54.5%)	S: 4 (40.0%) I: 1 (10.0%) R: 5 (50.0%)	–	S: 3 (30.0%) R: 7 (70.0%)	S: 2 (13.3%) I: 2 (13.3%) R: 11 (73.3%)

Note: S = susceptible; I = intermediate; R = resistant; n = number of *S. aureus* isolates in each Wagner grade; – = no *S. aureus* isolates obtained from Wagner grade 3 ulcers.

Based on Table 6, the susceptibility patterns of *S. aureus* against the tested antibiotics varied across different diabetic foot ulcer severity levels according to the Wagner classification. Descriptively, cefoxitin resistance increased from 63.6% in Wagner grade 1 ulcers to 80.0% in grade 2, 90.0% in grade 4, and 86.7% in grade 5 ulcers. A similar pattern was observed for ciprofloxacin, with resistance proportions of 54.5%, 50.0%, 70.0%, and 73.3% in Wagner grades 1, 2, 4, and 5, respectively. In contrast, linezolid resistance did not show a consistent increase according to Wagner grade, with resistance proportions of 72.7%, 40.0%, 50.0%, and 53.3% in Wagner grades 1, 2, 4, and 5, respectively.

Therefore, descriptively, resistance to cefoxitin and ciprofloxacin tended to be higher among isolates obtained from more severe Wagner grades, whereas linezolid susceptibility patterns varied among ulcer severity categories. These findings should be interpreted cautiously because the study was descriptive and did not include statistical analysis to determine associations between ulcer severity and antibiotic resistance.

The absence of susceptibility data for Wagner grade 3 ulcers was not due to missing data but rather because *S. aureus* was not identified among the seven bacterial isolates obtained from this group. Therefore, comparisons of susceptibility patterns among Wagner grades, particularly grade 3, should be interpreted carefully. Among *S. aureus* isolates from Wagner grade 5 ulcers, the highest resistance proportions were observed against cefoxitin (86.7%) and ciprofloxacin (73.3%), whereas linezolid demonstrated a relatively higher proportion of susceptible isolates (40.0%).

The high proportion of resistant *S. aureus* isolates obtained from severe Wagner-grade ulcers may be discussed in the context of wound characteristics, antibiotic exposure, and environmental conditions that support bacterial persistence. Previous

antibiotic exposure and chronic wound environments that promote biofilm formation are among the factors that may contribute to increased bacterial tolerance toward antibiotics (Senneville et al., 2023). Biofilm formation by *S. aureus* plays an important role in enhancing bacterial tolerance to antibiotics and contributes to the difficulty of eradicating infections in chronic wounds (Asaad et al., 2021; Wu et al., 2024). However, these factors were not specifically analyzed in this study and therefore cannot be confirmed as direct causes of resistance in the obtained isolates.

The findings of this study are consistent with Tehrani (2022), who reported that increasing diabetic foot ulcer severity was associated with changes in bacterial composition and more complex antibiotic resistance patterns. In addition, Khariunnisa et al. (2020) demonstrated that *S. aureus* isolates from diabetic foot ulcers exhibited variable antibiotic susceptibility patterns, including resistance to several commonly used antibiotics. These findings highlight the importance of considering ulcer severity when selecting antimicrobial therapy, as more severe ulcers may be accompanied by repeated antibiotic exposure and an increased risk of resistant bacterial development (Dwedar, 2022).

More severe clinical conditions and repeated antibiotic exposure may represent factors contributing to increased bacterial resistance. However, these factors were not specifically investigated in the present study and therefore cannot be confirmed as determinants of resistance among the isolated bacteria. Purwati et al. (2021) reported that the prevalence of resistant bacteria in type A or type B referral hospitals in Central Java tends to increase due to high exposure to β-lactam antibiotics. Therefore, bacterial culture and antibiotic susceptibility testing according to diabetic foot ulcer severity should be considered as important components in selecting more appropriate and rational antimicrobial therapy.

CONCLUSION

The antibiotic susceptibility patterns of *Staphylococcus aureus* isolated from diabetic foot ulcers varied across different severity levels according to the Wagner classification. Descriptively, the proportion of resistance to cefoxitin and ciprofloxacin tended to be higher among isolates obtained from more severe Wagner grades, whereas the susceptibility pattern of linezolid varied across ulcer severity categories. These findings highlight the importance of continuous monitoring of antibiotic susceptibility patterns and the availability of local bacterial resistance data as a basis for selecting rational and effective antibiotic therapy. Potential factors influencing antibiotic resistance, including previous antibiotic exposure, biofilm formation, and wound characteristics, were not specifically analyzed in this study. Therefore, these factors cannot be used to determine the underlying causes of resistance among the obtained isolates.

RECOMMENDATION

Future studies are recommended to include larger sample sizes and broader research locations to improve the representativeness and generalizability of the findings. In addition, molecular analyses of antibiotic resistance-associated genes are needed to provide a more comprehensive understanding of the resistance mechanisms of *S. aureus* in diabetic foot ulcers with varying severity levels. Further studies are also recommended to evaluate the susceptibility of *S. aureus* to other antibiotics and to conduct periodic surveillance of antibiotic susceptibility patterns as a basis for developing rational antimicrobial use policies.

ACKNOWLEDGMENT

The authors would like to express their gratitude to the Higher Education, Research, and Development Council (*Majelis Pendidikan Tinggi, Penelitian, dan Pengembangan*; Diktilitbang) of the Central Executive Board of Muhammadiyah for providing research funding through the Muhammadiyah National Research Grant Batch IX 2025 under the Fundamental Lecturer Research Program II (Grant Number: 0259.615/I.3/D/2025). The authors also thank the research institution for granting permission and providing support throughout the study, as well as all individuals and organizations who contributed to the completion of this research.

The authors used AI-assisted technologies solely to improve the readability and language quality of the manuscript. The authors reviewed and edited the final content and take full responsibility for the manuscript.

REFERENCES

Abdilah, F., & Kurniawan, K. (2021). Morphological characteristics of air bacteria. *Borneo Journal of Medical Laboratory Technology, 3*(2), 353–359.

American Diabetes Association Professional Practice Committee. (2024). Retinopathy, neuropathy, and foot care: Standards of care in diabetes—2024. *Diabetes Care, 47*, S231–S243. https://doi.org/10.2337/dc24-S012

Asaad, A., Ansari, & Ajlan. (2021). Epidemiology of biofilm-producing *Acinetobacter baumannii* nosocomial isolates from a tertiary care hospital in Egypt: A cross-sectional study. *Infection and Drug Resistance*, 6973. https://doi.org/10.2147/IDR.S261939

BA, O'Connor, M. J., Bartler, A. V., Ho, K. C., Zhang, K., Casas Fuentes, R. J., Melnick, B. A., Huffman, K. N., Hong, S. J., & Galiano, R. D. (2024). Understanding *Staphylococcus aureus* in hyperglycaemia: A review of virulence factor and metabolic adaptations. *Wound Repair and Regeneration*, 5, 661–670. https://doi.org/10.1111/wrr.13192

Barlam, T. F., Cosgrove, S. E., Abbo, L. M., MacDougall, C., Schuetz, A. N., Septimus, E. J., Srinivasan, A., Falck-Ytter, Y. T., Fishman, N. O., Hamilton, C. W., Jenkins, T. C., Lipsett, P. A., Malani, P. N., May, L. S., Moran, G. J., Neuhauser, M. M., Newland, J. G., Ohl, C. A., Seo, S. K., & Trivedi, K. K. (2016). Implementing an antibiotic stewardship program: Guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clinical Infectious Diseases, 62*(10), 51–77. https://doi.org/10.1093/cid/ciw118

Centers for Disease Control and Prevention. (2025). *Laboratory testing for methicillin (oxacillin)-resistant Staphylococcus aureus (MRSA).* https://www.cdc.gov/mrsa/php/laboratories/index.html

Chaves, C. R. S., Salamandane, C., Maurício, B. D. S., Machamba, A. A. L., Salamandane, A., & Brito, L. (2025). Multidrug-resistant *Staphylococcus aureus* in diabetic foot infections (DFI) from Beira, Mozambique: Prevalence and virulence profile. *Infection and Drug Resistance, 18*, 779–2796. https://doi.org/10.2147/IDR.S521876

Dwedar. (2022). Prevalence of multidrug-resistant bacteria in diabetic foot infections in a tertiary care hospital. *Journal of Clinical Medicine, 11*(4), 1063. https://doi.org/10.3390/jcm11041063

Fadlilah. (2018). Faktor-faktor yang berhubungan dengan derajat ulkus kaki diabetik di RSUD Dr. Moewardi Surakarta. *Jurnal Ilmiah Rekam Medis dan Informatika Kesehatan, 8*(1), 37–43.

Fitria, E., Nur, A., Marissa, N., & Ramadhan, N. (2017). Karakteristik ulkus diabetikum pada penderita diabetes mellitus di RSUD Dr. Zainal Abidin dan RSUD Meuraxa Banda Aceh. *Buletin Penelitian Kesehatan, 45*(3), 153–160. https://doi.org/10.22435/bpk.v45i3.6818.153-160

Frykberg. (2020). The Wagner and University of Texas diabetic foot ulcer classification systems: A reliability study. *The Journal of Foot & Ankle Surgery, 59*(3), 589–593. https://doi.org/10.1053/j.jfas.2019.10.004

Hayati, M., Tyasningsih, W., Praja, R. N., Chusniati, S., & Yunita, M. N. (2019). Isolasi dan identifikasi *Staphylococcus aureus* pada susu kambing Peranakan Etawah penderita mastitis subklinis di Kelurahan Kalipuro, Banyuwangi. *Jurnal Medik Veteriner, 2*(2), 76–82. https://doi.org/10.20473/jmv.vol2.iss2.2019.76-82

Hidayati, M., Asrar, M. F., Chaniago, H., & Iskandar, D. (2024). Hubungan ankle brachial index dengan keparahan ulkus kaki diabetik derajat Wagner pada penderita diabetes melitus. *Fakumi Medical Journal, 4*(3), 172–180. https://doi.org/10.33096/fmj.v4i3.385

Holcomb, A., & Millward, D. (2025). Investigation into a predominant laterality for the development of lower-extremity neuropathic ulceration and amputation. *Journal of the American Podiatric Medical Association, 115*(2). https://doi.org/10.7547/22-223

Hu, X., Kang, R., Chen, L., & Hu, X. (2024). Gender difference as the risk of diabetic foot ulcers in T2DM patients: A meta-analysis. *Journal of Men's Health, 20*(1), 1–6. https://doi.org/10.22514/jomh.2024.002

International Working Group on the Diabetic Foot. (2023). *Practical guidelines on the prevention and management of diabetes-related foot disease*. International Working Group on the Diabetic Foot.

Jeffcoate, & Harding. (2023). Diabetic foot ulcers. *The Lancet, 361*(9368), 1545–1551. https://doi.org/10.1016/S0140-6736(03)13169-8

Kementerian Kesehatan Republik Indonesia. (2025). *Laporan tahunan pengendalian penyakit tidak menular: Fokus komplikasi diabetes melitus di Indonesia*. Kementerian Kesehatan Republik Indonesia.

Khairunnisa, R., Soleha, T. U., & Ramadhian, M. R. (2020). Identifikasi dan uji resistensi *Staphylococcus aureus* pada ulkus diabetik di Instalasi Penyakit Dalam RSUD Dr. H. Abdul Moeloek. *Jurnal Agromedicine Unila, 7*(1), 1–6.

Khan, Z. A., Ahmad, J., Nasim, O., & Rustam, Z. (2019). Antimicrobial susceptibility pattern of isolates from diabetic foot ulcers. *Journal of Infection and Public Health, 14*, 105–110.

Kurniawan, A. R., Hidayat, Rimawati, V. E., & Putri, D. F. (2024). Analisis karakteristik klinis dan profil bakteri pada pasien ulkus diabetikum di rumah sakit rujukan: Studi observasional. *Jurnal Bedah Indonesia, 52*(1), 88–95.

Kurniawan, Tyas, E. A., & Supriyadi. (2021). Prevalensi bakteri *methicillin-resistant Staphylococcus aureus* (MRSA) pada peralatan laboratorium. *The Journal of Muhammadiyah Medical Laboratory Technologist, 4*(2), 188–200. https://doi.org/10.30651/jmlt.v4i2.7554

Kusumah, F. P. (2024). *Identifikasi dan pola resistensi Staphylococcus pada pasien penderita ulkus diabetikum di RSUD Banyumas*. Universitas Bakti Tunas Husada Tasikmalaya.

Lipsky, B. A., Senneville, E., Abbas, Z. G., et al. (2020). IWGDF guideline on the diagnosis and treatment of foot infection in persons with diabetes. *Diabetes/Metabolism Research and Reviews, 36*, e3280. https://doi.org/10.1002/dmrr.3280

McDermott, K., Fang, M., Boulton, A. J., Selvin, E., & Hicks, C. W. (2023). Etiology, epidemiology, and disparities in the burden of diabetic foot ulcers. *Diabetes Care, 46*, 209–221. https://doi.org/10.2337/dci22-0043

Nugroho, R. B., Wibawa, D. A. A., & Prasetyanto, M. E. (2024). Profil sensitivitas antibiotik terhadap isolat bakteri swab luka penderita diabetes melitus. *Gema Kesehatan, 16*(1), 23–33. https://doi.org/10.47539/gk.v16i1.448

Osok. (2025). Karakteristik fenotipik dan molekuler *Staphylococcus aureus* resisten metisilin (MRSA) pada infeksi ulkus diabetikum. *Jurnal Mikrobiologi Klinik Indonesia, 12*, 15–28. https://doi.org/10.xxxx/jmki.2025.v12i1.46

Purnamasari, & Suwarno. (2023). Identifikasi *Staphylococcus* sp. dan resistensi antibiotik di Kecamatan Tutur, Pasuruan. *Jurnal Medik Veteriner, 6*(1), 93–104. https://doi.org/10.20473/jmv.vol6.iss1.2023.93-104

Purwati, Darwis, I., Hidayat, H., & Wisnu, G. N. P. P. (2021). Antibiotic resistance pattern of *Staphylococcus aureus* isolated from diabetic foot ulcer in a tertiary care hospital, Indonesia. *Journal of Clinical Microbiology and Infectious Diseases, 5*(2), 45–51.

Putri, A. F., Kamilla, L., & Tumpuk, S. (2024). Isolasi, identifikasi dan profil resistensi antibiotik bakteri *Staphylococcus aureus* dari air tahu yang dijual di berbagai kecamatan di Kota Pontianak. *Journal of Social Science Research, 4*(4), 1598–1614.

Rachmawati, Y., Mukti, A. W., Efendi, M., Syavadillah, R., & Fitria, P. (2021). Studi penggunaan antibiotik terhadap pasien ulkus diabetikum di Rumat Gedangan Sidoarjo—Spesialis Luka Diabetes. *FARMASIS: Jurnal Sains Farmasi, 2*(2), 28–35. https://doi.org/10.36456/farmasis.v2i2.5187

Ramirez-Acuña, J. M., Cardenas-Cadena, S. A., Marquez-Salas, P. A., Garza-Veloz, I., Perez-Favila, A., Cid-Baez, M. A., Flores-Morales, V., & Martinez-Fierro, M. L. (2019). Diabetic foot ulcers: Current advances in antimicrobial therapies and emerging treatments. *Antibiotics, 8*. https://doi.org/10.3390/antibiotics8040193

Rashed, & Husein. (2021). *Staphylococcus aureus*: An overview of pathogenesis, identification, and antimicrobial resistance. *Journal of Clinical Medicine and Biochemistry, 7*(1), 1–10. https://doi.org/10.xxxx/jcmb.2021.7.1

Safira, S. J., Decroli, E., & Alioes, Y. (2023). Pola bakteri dan sensitivitas antibiotik pada pasien ulkus kaki diabetik di RSUP Dr. M. Djamil Padang. *Jurnal Ilmu Kesehatan Indonesia, 4*(3), 181–189. https://doi.org/10.25077/jikesi.v4i3.1061

Sari, M., Dendy, Nasrul, E., & Alia, E. (2021). Pola bakteri dan resistensi antibiotik pada ulkus diabetikum. *Jurnal Kesehatan Andalas, 10*(2), 85–92.

Senneville, É., Albalawi, Z., van Asten, S. A., Abbas, Z. G., Allison, G., Aragón-Sánchez, J., Embil, J. M., Lavery, L. A., Alhasan, M., Oz, O., Uçkay, I., Urbančič-Rovan, V., Xu, Z.-R., & Peters, E. J. G. (2024). IWGDF/IDSA guidelines on the diagnosis and treatment of diabetes-related foot infections (IWGDF/IDSA 2023). *Clinical Infectious Diseases*, 1–23. https://doi.org/10.1093/cid/ciad527

Septiana, & Ajeng. (2024). Identifikasi dan uji sensitivitas *Staphylococcus* sp. terhadap beberapa antibiotik pada ulkus diabetikum. *Media Kesehatan Politeknik Kesehatan Makassar, 19*(1), 91–95. https://doi.org/10.32382/medkes.v19i1.571

Shettigar, & Murali. (2020). Virulence factors and clonal diversity of *Staphylococcus aureus* in colonization and wound infection with emphasis on diabetic foot infection. *European Journal of Clinical Microbiology & Infectious Diseases, 39*.

Sinaga, & Mahyudi. (2022). Identification of *Staphylococcus aureus* in patients with diabetic ulcers in Bunda Thamrin Hospital Medan. *Jurnal Analis Laboratorium Medik, 7*, 86–91.

Sinaga, J. T., Liani, F. N., & Pratiwi, D. I. N. (2021). Pola kepekaan bakteri dari kultur pus pasien ulkus kaki diabetikum berdasarkan tingkat keparahan di RSUD Ulin Banjarmasin. *Hemostasis: Jurnal Mahasiswa Kedokteran, 4*(2), 297–304. https://doi.org/10.20527/ht.v4i2.3998

Sugireng, & Rosdarni. (2021). Deteksi gen *nuc* isolat bakteri *Staphylococcus aureus* dari pasien ulkus diabetikum dengan metode PCR. *Prosiding Seminar Nasional Biologi, 7*(1), 133–136. https://doi.org/10.24252/psb.v7i1.23435

Suliati, Sasongkowati, R., Endarini, L. H., & Anggraini, A. D. (2022). Gen exfoliative A (EtA) *Staphylococcus aureus* pada isolat luka pasien diabetes mellitus. *Tunas-Tunas Riset Kesehatan, 12*(5), 126–130. https://doi.org/10.33846/2trik12204

Tehrani. (2022). Microbial profile and antibiotic susceptibility pattern in diabetic patients with mild, moderate, and severe foot infections in Tehran. *77*(5), 1925–1933. https://doi.org/10.22092/ARI.2022.359759.2476

Triani, Amanah, & Wibisono. (2022). Uji sensitivitas antibiotik terhadap bakteri patogen pada pasien ulkus diabetikum di RSUD Waled Cirebon. *Journal of Biomedicine and Health Science*.

Uçkay, I., Gariani, K., Pataky, Z., Lipsky, B. A., Jornayvaz, F. R., & Gastaldi, G. (2019). Antibiotic therapy of diabetic foot infections: A comprehensive review. *Expert Opinion on Pharmacotherapy, 20*, 1361–1377. https://doi.org/10.1080/14656566.2019.1621290

Wainwright, Wiskirchen, D. E., Shepard, A., Kuti, J. L., & Nicolau, D. P. (2019). Linezolid for the treatment of methicillin-resistant *Staphylococcus aureus* infections in diabetic foot ulcers. *Diabetes Care & Metabolism Journal, 10*(2).

Wayne. (2023). *Performance standards for antimicrobial susceptibility testing*. Clinical and Laboratory Standards Institute.

Wijaya, L., Budiyanto, A., Astuti, I., & Mustofa. (2019). Pathogenesis, evaluation, and recent management of diabetic foot ulcer. *Journal of the Medical Sciences, 51*(1), 82–97. https://doi.org/10.19106/JMedSci005101201910

Yan, T., Dou, Z., Claire, M., Ellen, K., & Caroline, M. (2025). Risk factors for first-ever diabetes-related foot ulcer: A systematic review and meta-analysis. *International Wound Journal, 22*(8). https://doi.org/10.1111/iwj.70728

Zhao, Y. N., Tang, W. H., Cheng, Z. X., Xu, J. X., Zhang, Y., & Liu, X. M. (2024). Risk factors for diabetic foot ulcer. *Vascular, 3*(3), 661–669. https://doi.org/10.1177/17085381231154805

Zuliana, N. M., Suliati, & Endarini, L. H. (2023). Identifikasi bakteri pada luka ulkus pasien diabetes mellitus. *Jurnal Kesehatan Poltekkes Palembang, 18*(2), 205–211. https://doi.org/10.36086/jpp.v18i2.1835