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Identification of Gram-Negative Bacteria in Diabetic Ulcers of Diabetes Mellitus Patients at Daha Husada Kediri General Hospital

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ABSTRACT

Diabetes mellitus is a chronic disease often complicated by diabetic ulcers, which may be exacerbated by nosocomial infections caused by pyogenic bacteria. This study aimed to identify Gram-negative bacteria in diabetic ulcers among patients with diabetes mellitus. This study employed a descriptive cross-sectional design to identify Gram-negative bacteria isolated from diabetic ulcer wounds. The research was conducted in the Bacteriology Laboratory of Institut Ilmu Kesehatan Bhakti Wiyata, Kediri, East Java, Indonesia. This descriptive study used a total sampling technique with 22 samples. Wound swabs were cultured on EMB agar, followed by biochemical tests (Indole, MR-VP, Citrate, TSIA) and Gram staining. The results showed the presence of Gram-negative bacteria, namely *Escherichia coli* (59.1%), *Klebsiella* sp. (31.8%), and *Proteus* sp. (9.1%). The predominance of *Escherichia coli* and *Klebsiella* sp. suggests that Gram-negative pathogens play a major role in moderate to severe diabetic ulcer infections (grade 3–4). These findings highlight that *Escherichia coli* was the most dominant Gram-negative bacterium, indicating its significant role in diabetic ulcer infections in this hospital setting.

Keywords: diabetes mellitus, diabetic ulcers, gram negative

INTRODUCTION

Diabetic ulcers are one of the most common chronic complications in patients with diabetes mellitus and are a major cause of morbidity and reduced quality of life (Habibu et al., 2022; Shofi, 2021). These wounds tend to heal slowly due to vascular impairment, peripheral neuropathy, and high blood glucose levels, which create an ideal environment for the growth of pathogenic microorganisms (Nikola et al., 2025). Such conditions make the wounds highly susceptible to infection by various bacteria, both Gram-positive and Gram-negative, which can aggravate the wound condition and delay the healing process (Novelni, 2019; Nur & Marissa, 2016).

Gram-negative bacteria are known to be the most dominant pathogens in diabetic ulcer infections. Several studies have shown that *Escherichia coli*, *Klebsiella* sp., and *Proteus* sp. are frequently isolated from the wounds of diabetic patients (Rinaldo & Farhanah, 2017; Zuliana et al., 2023). Baral et al. (2024) reported that 82.97% of isolates from diabetic ulcer patients in

Dhaka were Gram-negative bacteria, with *Klebsiella pneumoniae* being the most predominant isolate. Similarly, Coşkun et al. (2024) found that 57% of microorganisms responsible for diabetic foot infections were Gram-negative, primarily *Escherichia coli* and *Pseudomonas aeruginosa*. In the study conducted by Shi et al. (2022), the microbiological analysis of diabetic wound specimens demonstrated that the predominant bacterial isolates were *Staphylococcus aureus* (38.2%), *Staphylococcus epidermidis* (29.2%), and *Escherichia coli* (28.1%), indicating a polymicrobial nature of infection commonly associated with chronic diabetic ulcers.

The identification and characterization of microorganisms from diabetic ulcer wounds are essential for determining the bacterial species responsible for infection, which assists in selecting effective antimicrobial therapy (Mulyati et al., 2024; Zambelli et al., 2025). Common examinations include observing colony morphology, performing Gram staining, and conducting biochemical tests to accurately identify bacterial species (Khan et al., 2023; Wu et al., 2024). This information is crucial for hospital infection control efforts, encouraging the rational use of antibiotics, and helping to prevent the escalation of antimicrobial resistance (Wang et al., 2025; Zambelli et al., 2025).

Daha Husada General Hospital in Kediri serves as one of the main referral centers for managing patients with diabetes mellitus and its chronic complications, including diabetic ulcers. Diabetic ulcers represent a significant clinical challenge due to impaired wound healing and frequent polymicrobial infections that complicate treatment outcomes. Despite the high prevalence of diabetic ulcer cases, data regarding the distribution and antimicrobial susceptibility profiles of Gram-negative bacteria isolated from these wounds at Daha Husada General Hospital remain scarce. Therefore, this study aims to identify and characterize Gram-negative bacterial isolates obtained from pus samples of diabetic ulcer patients with diabetes mellitus treated at Daha Husada General Hospital, Kediri. The outcomes of this study are expected to provide essential microbiological insights that will support healthcare professionals in selecting appropriate antimicrobial therapy, implementing effective infection control strategies, and mitigating the growing threat of antibiotic resistance among diabetic ulcer patients.

METHOD

Study Design and Location

This study employed a descriptive cross-sectional design to identify Gram-negative bacteria isolated from diabetic ulcer wounds. The research was conducted in the Bacteriology Laboratory of Institut Ilmu Kesehatan Bhakti Wiyata, Kediri, East Java, Indonesia.

Population and Sampling

The study population consisted of patients diagnosed with diabetes mellitus presenting with diabetic ulcer wounds who were treated at Daha Husada General Hospital, Kediri. Sampling was conducted using the total sampling technique, involving all patients who met the inclusion criteria during the study period. A total of 22 pus samples were collected between May and June 2022.

Sample Collection

Pus samples were collected from patients diagnosed with diabetes mellitus presenting with diabetic ulcer wounds after obtaining informed consent. Samples were taken using sterile cotton swabs from the wound base after cleaning with sterile saline solution to avoid surface contaminants. Each sample was immediately transported to the laboratory in sterile transport media for further analysis (Zami & Shofi, 2024).

Culture and Isolation Procedures

Samples were inoculated onto MacConkey Agar (MCA), Blood Agar (BA), and Eosin Methylene Blue (EMB) Agar plates, then incubated at 37°C for 24–48 hours under aerobic conditions. Colony growth was observed based on shape, size, margin, surface, color, and consistency (Zuliana et al., 2023).

Gram Staining and Microscopic Examination

Isolates were subjected to Gram staining to differentiate Gram-negative bacteria. Microscopic examination was performed using an oil immersion lens (1000× magnification) to confirm cell shape and arrangement (Zami & Shofi, 2024).

Biochemical Identification

Presumptive Gram-negative isolates were further characterized through a series of biochemical tests, including oxidase, catalase, indole, citrate utilization, triple sugar iron (TSI), and motility tests. The results were compared with standard biochemical profiles according to Bergey's Manual of Determinative Bacteriology and Clinical and Laboratory Standards Institute (CLSI) guidelines (2024) (Zami & Shofi, 2024).

Data Analysis

All bacterial isolates were identified and grouped according to species and antibiotic susceptibility patterns. Data were analyzed descriptively and presented as percentages and frequency distributions using Microsoft Excel 2021 (Zami & Shofi, 2024).

RESULT AND DISCUSSION

In this study, bacterial identification was conducted on the pus of diabetic ulcer patients at Daha Husada Kediri General Hospital, involving 22 individuals from May to June 2022, consisting of 13 male patients and 9 female patients. The age of the research respondents was dominated by the age range of 50 – 59 years. This research was conducted using qualitative bacterial culture tests with growth media. The characteristics of the respondents based on the informed consent results can be seen in Table 1 below.

Based on the results of the questionnaire and informed consent, the majority of respondents are in the age range of 50-59 years old, which falls into the elderly category. The data can be seen in Table 1. Diabetes Mellitus complications with neuropathy can affect elderly patients due to the declining physiological functions of the human body, especially the ability of pancreatic β cells to produce insulin (Mildawati et al., 2019). Based on the data in Table 1, the respondents in the study were predominantly male patients. This situation is caused by the fact that men have a higher average level of outdoor and indoor activity compared to women in hot and humid weather. In addition, the use of insulin in men increases the risk of developing diabetic ulcers fivefold. In a study with DM patients as respondents, it was found that men had a low assessment level regarding drying their feet, periodic foot and nail hygiene, footwear use, and foot hygiene (Rizqiyah et al., 2020). As individuals age, there is a decrease in insulin receptor sensitivity and an increase in insulin resistance, which contribute to elevated blood glucose levels (Maltese et al., 2022). Chronic hyperglycaemia in elderly patients exacerbates vascular and peripheral nerve damage, ultimately increasing the risk of developing diabetic ulcers (Mancin et al., 2025). In addition, the wound healing process in elderly patients tends to be slower due to reduced tissue regeneration, impaired microcirculation, and a weakened immune response (Erwanto & Lanni, 2023; Nunan et al., 2014). This explains why elderly patients with diabetic ulcers are more susceptible to infections and complications. Therefore, age is considered one of the critical factors influencing the severity and healing duration of diabetic ulcers.

In elderly patients with high blood glucose levels, the ulcer wound environment becomes richer in nutrients such as glucose and proteins, which can support the growth of pathogenic microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Macdonald et al., 2021). Hyperglycemia also inhibits leukocyte phagocytic function and decreases the body's natural antibacterial activity, allowing bacteria to colonize more easily and form biofilms on the wound surface (Mamdoh et al., 2023). As a result, infections become more difficult to control and prolong the wound healing time in elderly diabetic patients.

Table 1. Characteristics of the Respondents

Characteristic	Category	Frequency	%
Age	≤ 39 year	1	4.55%
	40 – 49 year	6	27.27%
	50 –59 year	11	50.00%
	60 – 69 year	4	18.18%
	≥ 70 year	-	-
Gender	Men	13	59.1%
	Women	9	40.9%
Degree of Ulcer	Grade 0	-	-
	Grade 1	-	-
	Grade 2	3	13.64%
	Grade 3	9	40.91%
	Grade 4	8	36.36%
	Grade 5	2	9.09%

Based on the characteristics in Table 1, the respondents in this study have varying degrees of wounds. The most common degree of injury is degree 3 (40.91%), followed by degree 4 (36.36%), degree 2 (13.64%), and then degree 5 (9.09%), with no patients found with degrees 0 and 1. The wound severity levels were classified based on the degree of ulcer severity according to the Wagner Ulcer Classification System (Monteiro-Soares et al., 2024). The differences in wound degrees among patients can be caused by several factors. Several factors that may influence the severity of wounds include treatment factors, a person's quality of life, awareness of blood sugar control, the use of safe and appropriate foot insoles or protectors, sensory neuropathy, calluses that cause recurrent diabetic ulcers, and other factors (Manungkalit, 2018). Zambelli et al. (2025) reported that more than 55% of isolates in severe diabetic ulcers were Gram-negative bacteria, while Gonçalves et al. (2024) found that 31.4% of Gram-negative isolates were dominant in moderate to severe ulcers. This finding reinforces that the increase in ulcer grade correlates with the higher proportion of Gram-negative bacteria found in the wounds.

Table 2. Morphological Characteristics of Gram-Negative Bacterial Colonies Isolated from Diabetic Ulcer Samples

Sample Code	Shape	Size	Margin	Surface	Color	Consistency
A, B, E, F, G, H, I, M, O, P, Q, R, T	Circular	Small	Entire	Convex	Metallic green	Semi mucoid
C, D, J, K, L, N, S	Circular	Large	Entire	Convex	Pink	Mucoid
U, V	Circular	Large	Entire	Convex	Transparent	Mucoid

The results shown in Table 2 indicate that three types of bacterial colonies were formed. In 13 samples, colonies with distinctive characteristics formed, exhibiting a metallic green color, semi-mucoid consistency, small size, flat edges, and convex surfaces. Then, in 7 samples, large bacterial colonies formed, round in shape, pink in color, mucoid consistency, with flat edges and convex surfaces. In 2 other samples, colonies formed that were colorless, small in size, transparent, with smooth edges and a mucoid consistency.

The next step is Gram staining, which will indicate the type of bacterial colonies isolated from the EMB Agar medium. Table 3 shows the microscopic results of Gram staining. The characteristics of the bacteria obtained are red, rod-shaped, and dispersed in arrangement, so it can be concluded that the bacteria growing on EMB Agar are Gram-negative.

Table 3. Gram Staining

Sample Code	Shape	Arrangement	Color	Characteristic
A, B, C, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V	Rod	Spreading	Red	Gram-negative

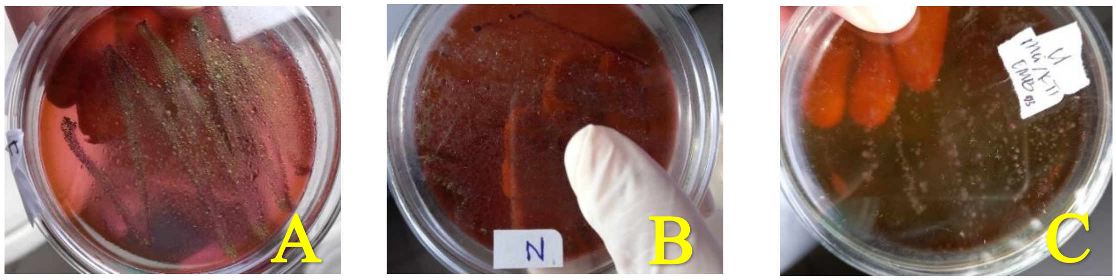

Figure 1. Colonies that Grow on EMB Agar, (a) *Escherichia coli* Isolate (b) *Klebsiella* sp. Isolate, and (c) *Proteus* sp. Isolate

Tabel 4. Biochemical Reaction Test Results

Sample Code	**Indole**	**MR /VP**	**Citrate**	**TSIA**				**Result**
				Slant	**Butt**	**Gas**	**H_2S**	
C, D, J, K, L, N, S	-	-/+	+	Acid	Acid	+	-	*Klebsiella* sp.
A, B, E, F, G, H, I, M, O, P, Q, R, S, T	+	+/-	-	Acid	Acid	+	-	*Escherichia coli*
U, V	-	-/+	+	Alkali	Acid	+	+	*Proteus* sp.

The final identification of the biochemical reaction medium revealed three types of bacteria present in the diabetic ulcer, one of which is *Klebsiella* sp. In addition to identifying Klebsiella sp. bacteria, two other types of Gram-negative bacteria, namely *Escherichia coli* and *Proteus* sp., were also found in diabetic ulcer wounds. The results obtained from 22 samples identified *Klebsiella* sp. bacteria in 7 samples with a percentage of 31.8%. Then, *Escherichia coli* bacteria were identified in 13 samples with a percentage of 59.1%, and *Proteus* sp. bacteria in 2 samples with a percentage of 9.1%.

The presence of several types of bacteria found in the pus of diabetic ulcers can occur due to the abundance of nutrients in the pus, which provides a great opportunity for bacteria to thrive and then infect the wound. The high blood sugar levels in DM patients with diabetic ulcers create a favorable environment for the growth of anaerobic bacteria. The presence of this condition is caused by uncontrolled blood plasma with high viscosity, resulting in disrupted blood flow and leading to insufficient oxygen supply to the body. This condition can lead to the worsening of diabetic ulcer degrees (Dewi & Asman, 2021).

The dominance of *Escherichia coli* in grade 3 and 4 ulcers may be attributed to its strong ability to invade deep tissues and produce toxins that accelerate tissue necrosis (Mashaly, et al., 2021). Similarly, Klebsiella sp., found in 31.8% of samples, is known to produce Extended Spectrum Beta-Lactamase (ESBL), leading to antibiotic resistance and delayed ulcer healing (Coşkun et al., 2024; Neog et al., 2021). The presence of *Klebsiella* sp. is often associated with severe ulcers (grade 4–5), where necrotic tissue and anaerobic conditions support bacterial proliferation (Baral et al., 2024; Rinaldo & Farhanah, 2017). Meanwhile, *Proteus* sp. was detected primarily in grade 3–4 ulcers, consistent with its motility and urease production that enhance its ability to colonize deeper tissue and contribute to osteomyelitis in chronic ulcers (Appapalam et al., 2021; Rizqiyah et al., 2020).

A clear trend emerged showing that as ulcer grade increased, the bacterial load and diversity also rose. This finding is in line with the results of Zambelli et al. (2025) and Gonçalves et al. (2024), who reported that severe diabetic ulcers (grade 3–5) were dominated by Gram-negative pathogens.. Environmental factors such as humidity, poor oxygenation, and high blood glucose levels promote the colonization of Gram-negative rods, resulting in persistent infections that are difficult to treat (Nikola et al., 2025; Wu et al., 2024).

The results of this study are in line with the research conducted by Rinaldo & Farhanah (2017) at RSUP Dr. Kariyadi Semarang, which found that the bacteria present in diabetic ulcer pus included *Klebsiella* sp. (15.3%), *Escherichia coli* (21.2%), *Proteus* sp. (9.5%), and *Staphylococcus* sp. (7.1%). Additionally, the research conducted by Fahmi (2015) at Cengkareng Regional General Hospital found the bacteria *Klebsiella* sp. (13.3%), *Escherichia coli* (26.6%), *Proteus* sp. (6.6%), and *Pseudomonas* sp. (6.6%). The bacteria *Proteus mirabilis* (19.6%), *Proteus vulgaris* (17.6%), *Klebsiella* Sp (13.7%), and *Escherichia coli* (13.7%) were also discovered in the study by Najihah (2021). This demonstrates that bacteria are frequently found in ulcers in DM patients, which is in line with the findings of the study.

Klebsiella sp. bacteria are one of the pathogens causing nosocomial infections in patients undergoing treatment in hospitals (Neog et al., 2021). *Klebsiella* sp. infections also manifest as asymptomatic carriers, which means the body tests positive for the disease but shows no clinical symptoms, and opportunistic infections, which are infections caused by bacteria, viruses, or parasites that attack individuals with weakened immune systems (Justiz-Vaillant et al., 2023). The *Klebsiella* sp. bacteria cause infections of soft tissue, the central nervous system, bronchopneumonia (chronic destructive lesions and multiple abscesses), and bacteremia (Olivia, 2020). Wounds with *Klebsiella* sp. infections can experience longer recovery times and hospital stays. The case is due to *Klebsiella* sp. producing the ESBL (Extended Spectrum Beta Lactamase) enzyme, which can inhibit the effectiveness of various types of antibiotics (Salim et al., 2020). *Klebsiella* sp. bacteria are often found in ulcers with degrees 3 and 4, with the highest prevalence in degree 4 (Rinaldo & Farhanah, 2017).

The presence of *Escherichia coli* bacteria in the pus of diabetic ulcers indicates the most frequently encountered pathogenicity in diabetic ulcers, causing rapid and extensive tissue damage (Mashaly, Ibrahim, et al., 2021). *Escherichia coli* bacteria are commonly found in almost all degrees of ulcer wounds with abscesses (Rizqiyah et al., 2020). *Escherichia coli* is also capable of producing higher levels of ESBL enzymes, which can lead to stronger resistance to various types of drugs and increase the risk of a higher number of these bacteria infecting diabetic ulcer wounds and prolonging wound healing if treatment is incorrect (Anggraini et al., 2020).

The *Proteus* sp. bacteria are one of the infectious agents that are also frequently found in diabetic ulcers (Appapalam et al., 2021). The *Proteus* sp. bacteria are capable of producing toxins that are effective in pathogenicity. *Proteus* sp. is capable of producing toxins that can lead to chronic osteomyelitis (bone inflammation) in patients with diabetes mellitus. Diabetic ulcers with osteomyelitis start from ulcers that have reached grade 3. *Proteus* sp. is a bacteria commonly found in patients undergoing amputation (Rizqiyah et al., 2020). *Proteus* sp. bacteria are commonly found in grade 3 and 4 ulcer wounds (Rinaldo & Farhanah, 2017).

Based on the microbiological review of DM patients with diabetic ulcers, Gram-negative bacteria, both aerobic and anaerobic, can be found. The differences in the bacteria that dominate diabetic ulcers are influenced by various factors such as the demographic characteristics of the patients, patient hygiene, the area where the patients live, the severity of the wounds, blood sugar levels, and the antibiotics used (Salim et al., 2020). Various studies conducted in places with hot and humid weather have shown that Gram-negative rod-shaped bacteria more frequently cause diabetic ulcers (Rizqiyah et al., 2020).

CONCLUSION

This study identified three dominant Gram-negative bacteria from diabetic ulcer pus samples: *Escherichia coli* (59.1%), *Klebsiella* sp. (31.8%), and *Proteus* sp. (9.1%), with E. coli being the most prevalent. The predominance of *Escherichia coli* and *Klebsiella* sp. suggests that Gram-negative pathogens play a major role in moderate to severe diabetic ulcer infections (grade 3–4).

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