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Original Article

Identification of Bacteria in Foot Wound Swabs from Patients Diabetes Mellitus

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia that predisposes patients to impaired wound healing and chronic infections, including diabetic foot ulcers. Bacterial colonization of these wounds plays a critical role in delayed healing, increased morbidity, and risk of amputation. This study aimed to identify the bacterial profiles isolated from foot wound swabs of patients with diabetes mellitus. A descriptive observational study was conducted using foot wound swab samples collected from patients with diabetes mellitus receiving wound care at Mandalika Care, Mataram City. Bacterial identification was performed through macroscopic and microscopic examination, Gram staining, and standard biochemical tests following culture on Blood Agar Plate (BAP) and MacConkey Agar (MCA). Data were analyzed descriptively and presented as frequencies and percentages. Six samples met the inclusion criteria and were analyzed. All isolates were Gram-negative bacteria. The bacterial species identified were *Pseudomonas aeruginosa* (33,36%), *Morganella morganii* (16,6%), *Pseudomonas putrefaciens* (16,6%), *Enterobacter agglomerans* (16,6%), and *Pseudomonas mallei* (16,6%). The conclusion is that Gram-negative bacteria predominated in diabetic foot wound infections in this study, with *Pseudomonas aeruginosa* being the most frequently isolated species. These findings highlight the importance of early microbiological examination to guide appropriate wound management and antimicrobial therapy in diabetic patients.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from insulin resistance and inadequate insulin secretion (Kementerian Kesehatan Republik Indonesia, 2024). One of the most common and serious complications of DM is diabetic ulceration, a condition that arises when pathogenic bacteria penetrate damaged skin tissue, with elevated blood glucose levels creating an environment conducive to bacterial growth (American Diabetes Association, 2020). Diabetic ulcers are typically polymicrobial, involving both aerobic and anaerobic bacteria (Novelni, Irwandi, & Pratiwi, 2019). According to the International Diabetes Federation, Indonesia remains among the top ten countries with the highest number of diabetes cases globally, with an estimated more than 19 million adults living with diabetes based on the latest estimates up to 2024–2025 (International Diabetes Federation, 2024). At the regional level, densely populated urban areas such as DKI Jakarta, particularly East Jakarta, which accommodates nearly one-third of the province's

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population, are recognized as high zones for diabetes-related complications (Resti & Cahyati, 2022; Bria, Missa, & Sombo, 2022). Meanwhile, reports from the West Nusa Tenggara (NTB) provincial Health Office indicate a continuing upward trend in diabetes prevalence in recent years (2023–2024), including in Mataram City, highlighting the growing public health challenge posed by DM and its complications at both national and regional levels. In 2021, NTB had 63.488 diabetic patients, including 8.546 in Mataram City. By 2022, these numbers rose to 64.544 in NTB and 8.741 in Mataram City (Central Statistics Agency of West Nusa Tenggara Province, 2022).

Diabetic foot Ulcer is a serious and debilitating complication of uncontrolled diabetes, often leading to chronic infections, prolonged hospitalization, and in severe cases, amputations (Hardianto, 2020). Physically, they may cause foot deformities, pain, infections, and deeper tissue damage due to nerve and blood vessel problems associated with diabetes. People with diabetes are more likely to get these ulcers. Ulcers start when bacteria enter the foot, and high blood sugar helps bacteria grow. Both aerobic and anaerobic bacteria can be involved. To avoid infection, people with diabetes should keep their feet clean, especially if they have wounds (Anggraini et al., 2020). Mulyati (2024) found that Gram-negative bacteria are often present in diabetic foot wounds, including *Staphylococcus aureus* (22,5%), *Staphylococcus epidermidis* (22,5%), *Proteus mirabilis* (7,5%), *Pseudomonas aeruginosa* (7,5%), *Klebsiella pneumoniae* (15%), *Enterobacter agglomerans* (5%), *Escherichia coli* (12,5%), and *Citrobacter freundii* (5%). Patricia et al. (2023) reported that diabetic patients receiving home care for wounds had 82,35% Gram-negative and 17,65% Gram-positive bacteria in their ulcers. The Gram-negative bacteria included *Proteus mirabilis* (17,65%), *Proteus morgana* (5,88%), *Citrobacter diversus* (23,53%), *Pseudomonas aeruginosa* (11,7%), *Escherichia coli* (5,8%), *Enterobacter aglomerans* (5,88%), and *Enterobacter cloacae* (11,76%) (Zuliana, Suliati, & Endarini, 2023; Hayati et al., 2019).

This study aims to provide insight into the bacterial profile and antimicrobial resistance in diabetic foot infections. Research by Arjan et al. (2025) showed a high prevalence of Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*, as well as *Staphylococcus aureus* among Gram-positive isolates. According to Macdonald et al. (2021), the most commonly identified bacteria are *Staphylococcus aureus*, which is MRSA. Other common bacteria are *Pseudomonas spp*., *E. coli*, and *Enterococcus spp*. Understanding the microbiological profile in diabetic ulcers can improve monitoring and appropriate antibiotic selection in an effort to reduce high amputation rates (Husna, 2018; Anshar, 2017). Research on bacterial infections in diabetic foot ulcer patients in NTB has not been widely conducted, so this study aims to identify the types of bacteria found in diabetic ulcers.

Methods

This study is a descriptive, observational research project that identifies bacteria in foot-wound swabs from patients with diabetes mellitus. The Researchers examined cultures both macroscopically and microscopically, along with biochemical tests, and selected samples by chance. The study was conducted at the Health Testing and Calibration Center (BLKPK) laboratory and at Mandalika Care, a wound care center in Mataram City, from January to June 2025. The study population consisted of patients diagnosed with diabetes mellitus who had foot wounds and received treatment at Mandalika Care. Samples were selected using accidental sampling. The sample size was determined based on the availability of eligible patients during the study period. The participants were patients with diabetes mellitus and foot wounds who received care at Mandalika Care and provided up to 6 ulcer wound swab samples. Data were analyzed descriptively and shown in tables as percentages.

The materials used were ulcer swabs from diabetic patients. The bacterial identification process was carried out by inoculating them onto Blood Agar Plate (BAP) and Mac Conkey Agar (MCA) media, then incubating the media at 37 °C for 24 hours (Kosasi, Lolo, & Sudewi, 2019; Lisnawati & Prayoga, 2020).

Results

The research sample was collected at Mandalika Care wound care center in Mataram City. Out of 10 patients, 6 met the criteria, so 6 samples were tested at the Health Laboratory Testing and Calibration Center from February to June 2025. The results are shown in the following table:

Table 1. Macroscopic Research Results on Diabetic Foot Wound Swab Samples on Mac Conkey Agar Media

Sample Code	Colony Color	Size	Shape	Edge
00396,1	Red	0,7–2,1 mm	Round	Convex
00396,2	Yellowish	1,5–2,0 mm	Round	Convex
00396,3	Brownish	0,5–1,7 mm	Round	Convex
00421	Pink	2,0–3,0 mm	Round	Convex
00461	Yellowish Brown	0,5–1,0 mm	Round	Convex

00498	White	0,5–1,0 mm	Round	Convex

After incubating the sample for 1x24 hours, macroscopic observations showed colony growth on MacConkey agar, whereas no colonies grew on blood agar. Based on Table 1, the results obtained were colony color: red for 2 samples (0,7–3,0 mm), white for 2 samples, 1,5–2,0 brown for 2 samples (0,5–1,7 mm), round with convex edges, and a smooth surface.

Table 2. Results of Microscopic Research on Diabetic Foot Wound Swab Samples

Sample Code	Shape	Structure	Gram
00396,1	Bacil	Spreading	Gram -
00396,2	Bacil	Spreading	Gram -
00396,3	Bacil	Spreading	Gram -
00421	Bacil	Spreading	Gram -
00461	Bacil	Spreading	Gram -
00498	Bacil	Spreading	Gram -

In Table 2, the study collected 6 samples. The results were obtained by Gram staining and viewed under a microscope at 100x magnification, showing red-stained bacilli in a spread arrangement, indicating Gram-negative bacteria.

Table 3. Sugar and Biochemical Test Results

Kode Sampel	00396,1	00396,2	00396,3	00421	00461	00498
Glu	+	+	+	+	+	-
Gas			+		+	
LK	-	-	-	-	-	-
MN	+/-	+/-	-	+	-	-
ML	-	-	-	+	-	-
SK	-	-	-	+	-	+
IND	+	-	-	-	-	+
MR	+	-	-	+	-	-
VP	-	-	-	-	-	-
SC	-	+	+	-	+	-
MT	+	+	+	+	+	-
TSIA	K/K	M/M	K/K	K/K	K/K	K/K
Gas						
H_2S		+				
UREA	+	+	-	-	-	-

Description: Description: GL (Glucose), LK (Lactose), MN (Mannitol), ML (Maltose), SK (Sucrose), IND (Indole), MR (Methyl Red), VP (Voges Proskauer), SC (Simmon Citrate), MT (Motility), TSIA (Triple Sugar Iron), k/k (Yellow/Yellow), M/M (Red/Red).

Based on Table 3, the results of the sugar and biochemical tests for sample 00396,1 are shown in the table above: sugar media, glucose (+), lactose (-), mannitol (+/-), maltose (-), and sucrose (-). In the Indole (+), MR (+) VP (-), SC (-), MT (+), Urea (+), TSIA media, the results obtained were yellow slopes, yellow bases, H_2S (-), and gas (+). It can be concluded that the bacterial profile corresponds to *Morganella marganii* (16,6%). In sample 00396,2, namely in the sugar media, glucose (+), lactose (-), mannitol (+/-), maltose (-), and sucrose (-), with no color change from purple to yellow, were observed. In the media of Indole (-), MR (-), VP (-), SC (+), MT (+), Urea (+), TSIA, the results obtained were a red slope, a red base, H_2S (+), and gas (-). It can be concluded that the bacterial profile is *Pseudomonas putrefaciens* (1,6%). Samples 00396,3 and 00461, in sugar media, showed the following results, glucose (+), gas (+), can change the color from purple to yellow, positive gas, lactose (-), mannitol (-), maltose (-), sucrose (-). In the media of Indole (-), MR (-), VP (-), SC (+), MT (+), Urea (-), TSIA, the results obtained were a yellow slope, a yellow base, H_2S (-), and gas (+). It can be concluded that the bacterial profile corresponds to *Pseudomonas aeruginosa* (33,36%). Sample 00241 in sugar media yielded the following results: glucose (+), lactose (-), mannitol (+), maltose (+), sucrose (+). In Indole (-), MR (+), VP (-), SC (-), MT (+), Urea (-), TSIA media, the results obtained were yellow slope, yellow base, H_2S (-), gas (+). It can be concluded that the bacterial profile corresponds to *Enterobacter agglomerans* (16,6%). Sample 00498 in sugar media yielded the following results: glucose (-), lactose (-), mannitol (-), maltose (-), and only sucrose (+). In Indole (+), MR (-), VP (-), SC (-), MT (-), Urea (-), TSIA media, the results obtained were yellow slopes, yellow bases, capable of fermenting glucose, lactose, and sucrose, H_2S (-), gas (+). It can be concluded that the bacterial profile corresponds to *Enterobacter agglomerans* bacteria (16,6%).

Discussion

Diabetic foot wounds are often portals for bacterial entry, including aerobic Gram-positive and Gram-negative bacteria that spread rapidly and can cause severe tissue damage (Trisnawati, Anggraini, & Nurvinanda, 2023). This study showed that the dominant Gram-negative bacterial colonies in diabetic foot wounds were found in men and women over 45 years of age, with a duration of diabetes exceeding one year, presenting with infected pus wound samples, characterized by grade 5 gangrene with lesions on the entire surface of the foot, and uncontrolled blood sugar levels.

Based on the research results, there was no growth on the BAP, but there was colony growth on the MCA. This generally indicates the microbe's character and the nature of the media used (Zhou et al., 2023). First, MCA is a selective medium for Gram-negative bacteria, because it contains crystal violet and sodium salts that inhibit Gram-positive bacteria (Febriza, Adrian, & Sucipto, 2021). This medium is also differential because it allows lactose fermentation, with colonies appearing pink for fermenters and clear for non-fermenters. In contrast, BAP is enriched and non-selective, supporting the growth of many bacteria, including fastidious ones, and is used primarily to observe hemolysis patterns. Therefore, if microbes grow on MCA but not on BAP, it is most likely that Gram-negative bacteria are involved that do not require blood-derived growth factors, and that MCA provides sufficient nutrients, pH, and suitable conditions. However, if BAP does not grow at all, there are technical factors such as an incorrect inoculation procedure, expired or non-sterile media, an inappropriate pH (ideally 7,2–7,6), or media containing inhibitors that affect only BAP (Mutonga et al., 2019; Sianipar, Sartini, & Riyanto, 2020). Certain Gram-negative bacteria can also grow faster on the minimal MCA, whereas they grow slowly or not at all on BAP. Therefore, the combination of MCA growth and no BAP growth indicates that the bacteria present are Gram-negative and not fussy, and therefore suitable for growth on MCA spectrum media, but too slow or undetectable on BAP (Ratnasari et al., 2024; Nisak, 2021).

The results of the bacterial identification study obtained 6 samples of foot wound swabs from diabetes mellitus patients, which obtained bacterial profiles of *Pseudomonas aeruginosa* (33,36%), *Morganella marganii* (16,6%), *Pseudomonas putrefaciens* (16,6%), *Enterobacter agglomerans* (16,6%), and *Pseudomonas mallei* (16,6%). The results of the study showed that the dominant bacterial colonies found were Gram-negative bacteria, namely *Pseudomonas aeruginosa*, as much as (33,3%) with the characteristics of rotting brown colonies with a size of 0,5–1,7 mm, round, convex edges, and smooth surfaces. The results of this study are in accordance with research conducted by Jonker (2022) and Prasetia (2016), which stated that the bacterial patterns obtained were mostly Gram-negative (51%) than Gram-positive (49%), and one of the findings was *Pseudomonas aeruginosa* (24,3%), *Escherichia coli* (15,3%), *Citrobacter koseri* (2,7%), *Proteus vulgaris* (6,3%), and *Klebsiella pneumoniae* (9%) (Jonker, 2022; Oliver, 2010). The results of research conducted by Patricia et al. (2023) showed the prevalence of bacterial types, namely (82,35%) Gram-negative and (17,65%) Gram-positive. The Gram negatives include *Pseudomonas aeruginosa* (23,54), *Proteus morgani* (5,88%), *Citrobacter diversus* (17,65%), *Proteus mirabilis* (11,76%), *Escherichia coli* (5,88%), *Enterobacter agglomerans* (5,88%), and *Enterobacter cloacae* (11,76%) (Patricia et al., 2023; Novitasari, 2022).

A study by Garousi et al. (2023) also reported a similar percentage of bacterial infections among patients with diabetes mellitus. Furthermore, culture results showed that most isolates were Gram-negative bacteria (88%), and *Pseudomonas aeruginosa* was the dominant microorganism isolated (43%), followed by *Klebsiella pneumonia* (21%), *Proteus mirabilis* (11%), *Escherichia coli* (9%), and *S. marcescens* (4%) (Garousi et al., 2023; Rahmawati, 2022; Shintawati, Rina, & Ermaya, 2020). In this study, the results obtained from 10 samples of diabetic ulcer pus that had been examined included 6 bacteria, including *Pseudomonas aeruginosa* (20%), *Klebsiella pneumonia* (20%), *Burkholderia cepacia* (10%), *Providencia stuartii* (10%), *Enterobacter aerogenes* (10%), and *Proteus mirabilis* (10%). In Novelni, Irwandi, & Pratiwi's research (2019), it was reported that the cause of most ulcers in Gram-negative bacteria is *Pseudomonas aeruginosa*.

Pseudomonas sp. bacteria are one of the main causes of foot wound infections in people with *diabetes mellitus (*Ramadona, Rustam, & Syauqie, 2021; Rif'at, Hasneli, & Indriati, 2023; Robbia, Yahdi, & Dewi, 2021). This bacterium is a Gram-negative bacterium that thrives in moist environments and wounds containing exudate. In people with diabetes, foot wounds often heal slowly due to impaired blood circulation and a weakened immune system, creating an opportunity for *Pseudomonas* to multiply and cause infection (Sari, Soleha, & Carolia, 2019; Sari, Retnowati, & Setiawati, 2020). Infection by this bacterium can slow the wound-healing process due to toxins and enzymes that damage tissue and increase the risk of serious complications, such as the spread of infection to deeper tissues and bones, which can lead to amputation (Sathe et al., 2023). Therefore, treatment of Pseudomonas infections in diabetic foot wounds requires appropriate antibiotic therapy and optimal wound care to ensure proper healing and prevent complications.

Conclusions

This study demonstrated that diabetic foot wound infections in the observed population were predominantly caused by Gram-negative bacteria, with *Pseudomonas aeroginosa* being the most frequently isolated pathogen. Limitations in this study include the small sample size and the use of a descriptive design, which limit the generalizability of the findings. Furthermore, antibiotic susceptibility testing was not performed, which could provide more comprehensive clinical guidance.

Author contributions

NH, NNA, and BIH contributed to the study's concept and design. NH assisted with experimental studies and data acquisition. NH managed the literature search, data analysis, and statistical analysis. All authors participated in manuscript preparation, with NNA and BIH responsible for editing and review. All authors have read and agreed to the published version of the manuscript.

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Ethical approval statement

This research activity was approved by the Ethics Committee of the Kupang Health Polytechnic (Poltekkes Kemenkes Kupang) No.LB.02.03/1/0250/2024.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary materials

No supplementary material available.

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