ORIGINAL ARTICLE

Analysis of Risk Factors for Antimicrobial Resistance in Bacterial Infections among Diabetic Foot Ulcer Patients

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ABSTRACT

Introduction: Diabetic foot ulcer (DFU) is a chronic and progressive complication of diabetes mellitus resulting from macroangiopathy and microangiopathy disorders. Acknowledging the relationship between the Wagner diabetic foot ulcer classification system and infection severity may offer a promising instrument for guiding empirical antibiotic selections in clinical settings. This study aimed to assess the relationship between Wagner grades and the pathogen profiles of patients with DFU, along with their susceptibility to antibiotic therapy.
Methods: A cross-sectional study was conducted from January 2021 to August 2023, utilizing 33 secondary datasets obtained from electronic medical records. The data contained the patients' Wagner grades alongside the results of their complete microbiological analysis and antibiotic susceptibility test. The association between determinant factors and patients' pathogen profiles and antibiotic susceptibility patterns was examined using the Chi-square bivariate analysis ($p<0.05$).
Results: Positive culture results were observed in 32 patients (97%), with 59% exhibiting resistance to first-line antibiotics. The most commonly isolated pathogen was *Staphylococcus aureus*. The antibiotic susceptibility patterns indicated that gentamicin-syn demonstrated the highest activity against Gram-positive bacteria (GPB) isolates, while erythromycin was the most effective against Gram-negative bacteria (GNB) isolates. With escalating Wagner grades, there was an increased proportion of mixed infections, GNB infections (n=8, $X^2=23.28$, p=0.003), and antibiotic resistance (n=8, $X^2=39.97$, p=0.000). GNB isolates showed higher resistance compared to GPB isolates (n=18, $X^2=42.15$, p=0.001).
Conclusion: Our findings suggest that DFU patients with varying Wagner grades exhibit different bacterial profiles, infection patterns, and antibiotic sensitivities.

Keywords: Antibacterial susceptibility; bacteriological profile; diabetic foot ulcer; Wagner diabetic foot ulcer classification system; diabetes

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Highlights:
1. This is the first study conducted in Indonesia to analyze the relationship between the Wagner diabetic foot ulcer classification system and patients' pathogen profiles and antimicrobial susceptibility.
2. This study incorporated an in-depth analysis of several infection patterns and the occurrence of antimicrobial resistance, hence offering valuable information on the application of the Wagner classification system not only as a tool for grading infection severity but also for guiding clinicians in selecting the appropriate antibiotics for patients with diabetic foot ulcers.

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INTRODUCTION

Diabetic foot syndrome is a prevalent and severe complication of diabetes mellitus, affecting an estimated 15–25% to 19–34% of patients with diabetes mellitus (Armstrong et al., 2017). As defined by the International Working Group on the Diabetic Foot (IWGDF), diabetic foot syndrome refers to foot tissue infection, ulceration, or destruction associated with neuropathy and/or peripheral arterial disease in individuals with a history of diabetes mellitus (van Netten et al., 2020). Diabetic foot syndrome is associated with significant adverse outcomes, including increased rates of infection, amputation, and mortality (Edmonds et al., 2021).

Peripheral neuropathy, a key factor in diabetic foot ulceration, impairs autonomic, motor, and sensory nerve functions (Akkus & Sert, 2022). Sensory neuropathy disrupts the protective skin integrity of the foot, increasing susceptibility to damage from pressure, mechanical injury, or thermal exposure (Tuttolomondo, 2015). Diabetic foot infections result from a triad of neuropathy, trauma with secondary infection, and occlusive arterial disease (Jais, 2023). Peripheral neuropathy causes foot muscle atrophy, creating high-pressure zones on the plantar surface. Repeated trauma and reduced sensory feedback increase the risk of skin injury and fat pad displacement, leading to ulceration and infection that can spread to deeper tissues, subsequently affecting muscles, joints, and tendons (Bandyk, 2018).

Bacterial involvement crucially contributes to diabetic foot infections (Murphy-Lavoie et al., 2024). As the grade evaluated by the Wagner diabetic foot ulcer classification system increases, the prevalence of Gram-negative bacteria rises (Xie et al., 2017). *Staphylococcus aureus* and *Enterococcus* are common Gram-positive bacteria found across all grades. *Enterobacteriaceae* (e.g., *Escherichia coli*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*) are typically associated with mild infections, while *Proteus* spp. are commonly present in moderate cases. In severe grade 4 ulcers, *Pseudomonas* and *Acinetobacter* are predominant (Xie et al., 2017). Recent research, including a study conducted by Selvarajan et al. (2021), has indicated significant antibiotic resistance issues. Production rates of 27.5% for methicillin-resistant *Staphylococcus aureus* (MRSA) and 22.7% for extended-spectrum beta-lactamase (ESBL) were observed in *Klebsiella pneumoniae* isolates, alongside a 20% ESBL production rate in *Escherichia coli* isolates. Additionally, Gayathri and Rani (2018) identified a 75% prevalence rate of multidrug-resistant organisms (MDROs) among 68 isolates, including a 6% rate of MRSA isolates and a 51.5% rate of ESBL producers. These findings have indicated that diabetic foot infections caused by MDROs result in significantly worsened patient outcomes (Saltoglu et al., 2018). The current first-line antibiotic therapy for diabetic foot infections consists of ciprofloxacin, levofloxacin, and moxifloxacin (Embil et al., 2018).

The Wagner classification system has been widely used to evaluate diabetic foot ulcers. It categorizes lesions based on ulcer depth, gangrene presence, and tissue necrosis, aiding in severity assessment and treatment guidance (Shah et al., 2022). This system is crucial for the effective management of diabetic foot syndrome (Pitocco et al., 2019). The aim of this study was to evaluate the profiles of bacterial isolates among diabetic foot infection cases at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. Furthermore, we aimed to analyze the risk factors contributing to antimicrobial resistance in bacterial infections among patients with diabetic foot ulcers.

METHODS

Research methodology and ethical clearance for this study

This study was cross-sectional, utilizing secondary data collected from patient medical records (Ranganathan & Aggarwal, 2019). The research subjects were patients with diabetic foot ulcers receiving treatment in the Inpatient Unit of Dr. Soetomo General Academic Hospital, a tertiary referral hospital located in Surabaya, Indonesia. This study was carried out from January 2021 to August 2023. The Health Research Ethics Committee of Dr. Soetomo General Academic Hospital issued the ethical approval for this study under protocol number 1488/LOE/301.4.2/X/2023 on October 21, 2023.

Patients with diabetic foot ulcers as the research population

The research population comprised patients who were clinically diagnosed with diabetic foot syndrome and received treatment in the Inpatient Unit of Dr. Soetomo General Academic Hospital during the period of this study. The study sample consisted of 33 medical records of patients diagnosed with diabetic foot syndrome that met the inclusion criteria. The following inclusion criteria were established for this study: adult patients aged 18 years or older with a confirmed diagnosis of diabetes mellitus and a clinical diagnosis of diabetic foot syndrome or diabetic foot ulcers, who were admitted to the Inpatient Unit of Dr. Soetomo General Academic Hospital specifically for diabetic foot ulcer management and had the capacity to understand and provide written informed consent. The exclusion criteria encompassed patients with non-diabetic foot ulcers or severe comorbid conditions (such as advanced cardiovascular disease, renal failure requiring dialysis, or advanced liver disease), pregnant or breastfeeding women, patients with severe mental health conditions that impede comprehension or adherence to the study protocol, those deemed unlikely to comply with study procedures, and individuals who had participated in another clinical trial within the preceding 30 days (Patino & Ferreira, 2018).

Data collection from patient medical records

The medical records provided complete data, including a clear description of the patients' diabetic foot ulcers evaluated by the Wagner classification system. In addition, the data encompassed the results of a complete microbiological analysis of ulcer culture, specifically from pus samples obtained from the foot ulcers, together with the results of antibiotic susceptibility tests. Data pertaining to patient characteristics, such as age, length of stay, and sex, were also recorded (Kelly et al., 2024).

Statistical analysis of the relationship between variables

The statistical analysis was performed using IBM SPSS Statistics for MacOS, version 26.0 (IBM Corp., Armonk, NY, USA, 2019). The Chi-square test was employed to determine the relationships between variables, including the Wagner diabetic foot ulcer classification system (grades 1–5), patients' sex, age, length of stay, and culture results (monomicrobial or polymicrobial) in relation to antibiotic resistance and bacteriological profiles. A statistical significance was noted by $p<0.05$ (McHugh, 2013).

RESULTS

Among 418 recorded cases of various diabetic complications at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, 33 patients were found to have diabetic foot ulcers, as indicated by the complete data in their medical record. Figure 1 illustrates the processes required for selecting the research population to be included in this study. Meanwhile, Table 1 presents the demographic and clinical characteristics of the research subjects.

Risk factors and patient profiles

Table 1 presents the overall risk factors for antimicrobial resistance among patients with diabetic foot ulcers treated in the Inpatient Unit of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from January 2021 to August 2023. Of the 33 patients with diabetic foot ulcers included in this study, the majority were female (55%). Fifteen patients (45%) were classified as grade 4 in the Wagner diabetic foot ulcer classification system, signifying severe ulceration accompanied by local gangrene in the forefoot or heel. The data on patient distribution by age indicated that those hospitalized for diabetic foot complications were predominantly within the age range of 60–69 years (42%). The majority of patients with diabetic foot ulcers were hospitalized for no more than seven days (48%) and were mostly discharged in a recovered state (79%). All included patients underwent pus specimen collection for culture and antimicrobial susceptibility testing. Positive culture results were observed in 32 patients (97%), with 18 patients (55%) experiencing monomicrobial infections. The infections were primarily caused by the Gram-positive bacterium *Staphylococcus aureus* (33%), while the predominant Gram-negative bacterium was *Acinetobacter baumannii* (18%).

The culture of pus specimens collected from the 33 patients revealed that 18 samples indicated the occurrence of monomicrobial infections. The isolates identified in the monomicrobial infections consisted of *Staphylococcus aureus* in seven samples, *Acinetobacter baumannii* in four samples, *Klebsiella pneumoniae* in two samples, *Morganella morganii* in two samples, *Pseudomonas aeruginosa* in one sample, *Proteus mirabilis* in one sample, and *Escherichia coli* in one sample. Moreover, the culture of pus specimens indicated polymicrobial infections, with the antimicrobial susceptibility testing panel revealing the presence of at least one additional bacterial species apart from the main pathogen identified in the culture.

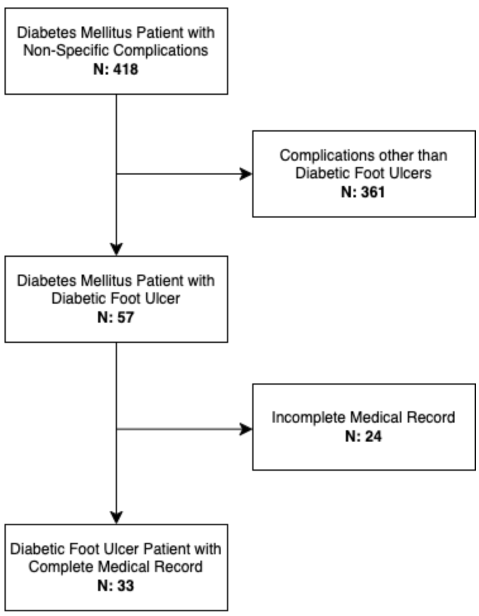

Figure 1. Flowchart for the inclusion of research subjects in this study

Table 1. Demographic and clinical characteristics of the patients

Parameters	n (%)
Age (years)	
30–39	1 (3)
40–49	7 (21)
50–59	11 (33)
60–69	14 (42)
Sex	
Male	15 (45)
Female	18 (55)
Length of stay (days)	
≤7	16 (48)
8–14	8 (24)
15–21	8 (24)
22–28	0 (0)
29–35	1 (3)
Outcomes	
Recovery	26 (79)
Mortality	5 (15)
Forced discharge before recovery	2 (6)
Wagner classification	
Grade 1	2 (6)
Grade 2	9 (27)
Grade 3	4 (12)
Grade 4	15 (45)
Grade 5	3 (9)
Culture results	
No infection	1 (3)
Monomicrobial infection	18 (55)
Polymicrobial infection	14 (42)
Bacteriological profiles	
Gram-positive isolates	13 (41)
Staphylococcus aureus	11 (33)
Corynebacterium striatum	1 (3)
Enterococcus faecalis	1 (3)
Gram-negative isolates	19 (59)
Klebsiella pneumoniae	5 (15)
Acinetobacter baumannii	6 (18)
Pseudomonas aeruginosa	1 (3)
Morganella morganii	2 (6)
Proteus mirabilis	2 (6)
Escherichia coli	3 (9)

Antibiotic susceptibility of the Gram-negative bacterial isolates

Table 2 lists the antibiotics to which the Gram-negative isolates identified in the pus specimen culture exhibited susceptibility and resistance. It was found that the Gram-negative bacterial isolates demonstrated the highest sensitivity rates to the following antibiotics: erythromycin (100%), linezolid (100%), quinopristin-dalfopristin (100%), and vancomycin (100%). Meanwhile, the highest resistance rates of the Gram-negative bacterial isolates were observed for several antibiotics as follows: colistin (100%), gentamicin (100%), tobramycin (100%), and ampicillin (93%).

Table 2. Antibiotic susceptibility of Gram-negative isolates identified in the culture of pus specimens

Antibiotics	S (%)	R (%)
Amikacin	14 (74)	5 (26)
Amoxicillin-clavulanic acid	3 (19)	13 (82)
Ampicilin	1 (7)	14 (93)
Ampicilin-sulbactam	4 (24)	13 (76)
Astreonam	7 (41)	10 (59)
Cefepime	5 (36)	9 (64)
Cefoperazone-sulbactam	9 (60)	6 (40)
Cefotaxime	5 (29)	12 (71)
Cefoxitine	0 (0)	0 (0)
Ceftazidime	6 (35)	11 (65)
Ceftriaxone	5 (29)	12 (71)
Cefazolin	3 (19)	13 (81)
Chloramphenicol	5 (33)	10 (67)
Ciprofloxacin	4 (25)	12 (75)
Clindamycin	1 (50)	1 (50)
Collistin	0 (0)	2 (100)
Cotrimoxazole (SXT)	5 (29)	12 (71)
Erythromycin	1 (100)	0 (0)
Fosfomycin	5 (45)	6 (55)
Fusidic acid	0 (0)	1 (100)
Gentamicin	6 (35)	11 (65)
Gentamicin-syn	0 (0)	0 (0)
Imipenem	5 (33)	10 (67)
Levofloxacin	2 (14)	12 (86)
Linezolid	1 (100)	0 (0)
Meropenem	10 (59)	7 (41)
Moxifloxacin	4 (36)	7 (64)
High-level mupirocin	0 (0)	0 (0)
Oxacillin	0 (0)	0 (0)
Penicillin G	1 (50)	1 (50)
Piperacilin	1 (10)	9 (90)
Piperacillin-tazobactam	8 (50)	8 (50)
Quinopristin-dalfopristin	1 (100)	0 (0)
Rifampin	0 (0)	0 (0)
Teicoplanin	0 (0)	0 (0)
Tetracycline	2 (14)	12 (86)
Tigecycline	4 (33)	8 (67)
Tobramycin	0 (0)	1 (100)
Trimethoprim	0 (0)	0 (0)
Vancomycin	1 (100)	0 (0)

Notes: S=susceptible; R=resistant.

Antibiotic susceptibility of the Gram-positive bacterial isolates

Table 3 presents the list of antibiotics to which the Gram-positive bacterial isolates identified in the pus specimen culture demonstrated susceptibility and resistance. The test revealed that the Gram-positive bacterial isolates responsible for diabetic foot infections exhibited the highest sensitivity rates to the following six antibiotics: gentamicin-syn

(100%), meropenem (100%), high-level mupirocin (100%), rifampin (100%), teicoplanin (100%), and vancomycin (100%). Simultaneously, the highest resistance rates of the Gram-positive bacterial isolates were observed for the following antibiotics: cefepime (100%), cefazolin (100%), colistin (100%), fusidic acid (100%), tigecycline (100%), tobramycin (100%), trimethoprim (100%), penicillin G (91%), and ampicillin (90%).

Table 3. Antibiotic susceptibility of Gram-positive isolates identified in the culture of pus specimens

Antibiotics	S (%)	R (%)
Amikacin	3 (43)	4 (57)
Amoxicillin-clavulanic acid	5 (63)	3 (38)
Ampicilin	1 (10)	9 (90)
Ampicilin-sulbactam	2 (50)	2 (50)
Astreonam	1 (33)	2 (67)
Cefepime	0 (0)	3 (100)
Cefoperazone-sulbactam	2 (67)	1 (33)
Cefotaxime	1 (20)	4 (80)
Cefoxitine	1 (50)	1 (50)
Ceftazidime	1 (33)	2 (67)
Ceftriaxone	2 (33)	4 (67)
Cefazolin	0 (0)	3 (100)
Chloramphenicol	6 (60)	4 (40)
Ciprofloxacin	4 (44)	5 (56)
Clindamycin	7 (78)	2 (22)
Colistin	0 (0)	1 (100)
Cotrimoxazole (SXT)	6 (46)	7 (54)
Erythromycin	7 (64)	4 (36)
Fosfomycin	6 (75)	2 (25)
Fusidic acid	0 (0)	1 (100)
Gentamicin	5 (38)	8 (62)
Gentamicin-syn	1 (100)	0 (0)
Imipenem	1 (50)	1 (50)
Levofloxacin	5 (71)	2 (29)
Linezolid	5 (83)	1 (17)
Meropenem	3 (100)	0 (0)
Moxifloxacin	5 (56)	4 (44)
High-level mupirocin	3 (100)	0 (0)
Oxacillin	4 (57)	3 (43)
Penicillin G	1 (9)	10 (91)
Piperacilin	1 (33)	2 (67)
Piperacillin-tazobactam	3 (75)	1 (25)
Quinopristin-dalfopristin	6 (86)	1 (14)
Rifampin	3 (100)	0 (0)
Teicoplanin	7 (100)	0 (0)
Tetracycline	4 (31)	9 (69)
Tigecycline	0 (0)	1 (100)
Tobramycin	0 (0)	2 (100)
Trimethoprim	0 (0)	1 (100)
Vancomycin	8 (100)	0 (0)

Notes: S=susceptible; R=resistant.

Risk factors and infection patterns among the diabetic foot ulcer patients

Table 4 presents the results of the Chi-square analysis, displaying the differences in patients' risk factors for bacterial infections according to the infection patterns. Out of the 33 diabetic foot ulcer patients who underwent pus culture examination, 32 patients (97%) had bacterial growth validated by microbiological testing. It was revealed that only the Wagner classification and antibiotic resistance variables showed significant differences in relation to the culture results.

Patients with elevated Wagner grades exhibited a higher probability of acquiring polymicrobial infections, predominantly involving Gram-negative bacteria. Conversely, patients with lower Wagner scale grades were more likely to develop monomicrobial infections, primarily associated with Gram-positive bacteria (n=8, X^2=23.28, p=0.003). Additionally, a higher prevalence of antibiotic resistance was found in polymicrobial diabetic foot infections compared to monomicrobial infections (n=30, X^2=52.03, p=0.008).

Table 4. The Chi-square analysis of the differences in risk factors according to the culture results

Risk factors	Infection patterns				p
	No infection n (%)	Monomicrobial infection		Polymicrobial infection n (%)	
		Gram-positive n (%)	Gram-negative n (%)		
Isolates	1 (3)	8 (24)	10 (30)	14 (42)	
Sex					
Male	1 (7)	4 (27)	3 (20)	7 (47)	0.504
Female	0 (0)	4 (22)	7 (39)	7 (39)	
Age (years)					
30–39	0 (0)	1 (100)	0 (0)	0 (0)	
40–49	1 (14)	3 (43)	2 (29)	5 (71)	0.837
50–59	0 (0)	4 (36)	5 (45)	5 (45)	
60–69	0 (0)	0 (0)	0 (0)	1 (7)	
LoS (days)					
≤7	1 (6)	4 (25)	5 (31)	6 (38)	
8–14	0 (0)	0 (0)	3 (38)	1 (13)	
15–21	0 (0)	0 (0)	2 (25)	6 (75)	0.31
22–28	0 (-)	0 (-)	0 (-)	0 (-)	
29–35	0 (0)	0 (0)	0 (0)	1 (100)	
Outcomes					
Recov.	1 (4)	7 (27)	8 (31)	10 (38)	
Mort.	0 (0)	1 (20)	2 (40)	2 (40)	0.771
FD	0 (0)	0 (0)	0 (0)	2 (100)	
Wagner classification (grades)					
1	1 (50)	0 (0)	1 (50)	0 (0)	
2	0 (0)	6 (67)	2 (22)	1 (11)	
3	0 (0)	1 (25)	0 (0)	3 (75)	0.003*
4	0 (0)	1 (7)	6 (40)	8 (53)	
5	0 (0)	0 (0)	1 (33)	2 (67)	
Antibiotic resistance (n)					
None	1 (100)	0 (0)	0 (0)	0 (0)	
1	0 (0)	0 (0)	2 (67)	1 (33)	
2	0 (0)	2 (100)	0 (0)	0 (0)	
3	0 (0)	3 (100)	0 (0)	0 (0)	
5	0 (0)	1 (50)	1 (50)	0 (0)	
6	0 (0)	1 (100)	0 (0)	0 (0)	
8	0 (0)	1 (50)	0 (0)	1 (50)	
10	0 (0)	0 (0)	1 (33)	2 (67)	0.008*
11	0 (0)	0 (0)	0 (0)	2 (100)	
12	0 (0)	0 (0)	0 (0)	1 (100)	
13	0 (0)	0 (0)	1 (50)	1 (50)	
14	0 (0)	0 (0)	0 (0)	1 (100)	
16	0 (0)	0 (0)	3 (100)	0 (0)	
17	0 (0)	0 (0)	0 (0)	2 (100)	
19	0 (0)	0 (0)	2 (50)	2 (50)	
24	0 (0)	0 (0)	0 (0)	1 (100)	

Notes: LoS=length of hospital stay; recov.=recovery; mort.=mortality; FD=forced discharge before recovery. An asterisk (*) denotes any significant result from the Chi-square test.

Risk factors and susceptibility to first-line antibiotics among the diabetic foot ulcer patients

Table 5 displays the Chi-square analysis results regarding differences in the patients' risk factors for bacterial infections associated with antibiotic susceptibility patterns. Among 32 diabetic foot ulcer samples that exhibited bacterial growth, as microbiologically confirmed by pus cultures, 19 (59%) demonstrated resistance to several first-line antibiotics. These drugs included ciprofloxacin, levofloxacin, and/or moxifloxacin, commonly used in the management of diabetic foot infections.

Table 5. The Chi-square analysis of the differences in risk factors based on the sensitivity patterns of first-line antibiotics (i.e., ciprofloxacin, levofloxacin, and moxifloxacin)

Risk factors	Antibiotic susceptibility		p
	S n (%)	R n (%)	
Isolates	13 (41)	19 (59)	
Sex			
Male	5 (36)	9 (64)	0.476
Female	8 (47)	10 (59)	
Age (years)			
30–39	3 (43)	4 (57)	0.774
40–49	5 (50)	5 (50)	
50–59	5 (36)	9 (64)	
60–69	0 (0)	1 (100)	
Length of stay (days)			
≤7	5 (33)	10 (67)	0.705
8–14	5 (63)	3 (38)	
15–21	3 (38)	4 (50)	
22–28	0 (-)	0 (-)	
29–35	0 (0)	1 (100)	
Outcomes			
Recovery	12 (48)	13 (52)	0.532
Mortality	1 (20)	4 (80)	
Forced discharge	0 (0)	2 (100)	
Wagner classification			
Grade 1	1 (100)	0 (0)	0.000*
Grade 2	9 (100)	0 (0)	
Grade 3	2 (50)	2 (50)	
Grade 4	1 (7)	14 (93)	
Grade 5	0 (0)	3 (100)	
Infection patterns			
Gram-positive monomicrobial infection	8 (100)	0 (0)	0.000*
Gram-negative monomicrobial infection	3 (30)	7 (70)	
Mixed infection	2 (14)	12 (86)	
Isolated bacteria			
Staphylococcus aureus	7 (64)	4 (36)	0.001*
Klebsiella pneumoniae	2 (40)	3 (60)	
Acinetobacter baumannii	1 (17)	5 (83)	
Pseudomonas aeruginosa	0 (0)	1 (100)	
Morganella morganii	1 (50)	1 (50)	
Proteus mirabilis	1 (50)	1 (50)	
Escherichia coli	0 (0)	3 (100)	
Corynebacterium striatum	0 (0)	1 (100)	
Enterococcus faecalis	1 (100)	0 (0)	

Notes: S=susceptible; R=resistant. An asterisk (*) denotes any significant result from the Chi-square test.

The Chi-square analysis revealed that the Wagner classification, culture results, and types of infectious bacteria exhibited significant differences in relation to antibiotic resistance patterns against first-line antibiotics. Patients with escalated Wagner grades demonstrated a notable shift from non-resistance to resistance to first-line antibiotics (n=8, X^2=39.97, p=0.000). In terms of culture results, samples indicating monomicrobial infections associated with Gram-positive bacteria showed no resistance to first-line antibiotics. Conversely, monomicrobial infections associated with Gram-negative bacteria as well as polymicrobial infections demonstrated increased resistance patterns (n=4, X^2=40.38, p=0.000). Additionally, there was a significant difference in resistance patterns based on the type of infectious bacteria isolated, indicating that different bacterial species had distinct resistance profiles to first-line antibiotics (n=18, X^2=42.15, p=0.001).

DISCUSSION

Bacterial culture in diabetic foot disease

In this study, 97% of pus swab specimens from patients with diabetic foot ulcers were microbiologically confirmed to indicate infections. This is consistent with previous findings, including from a study conducted by Windriya et al. (2020) at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, in 2012. The study reported a 100% prevalence rate of infections in the culture of specimens from 30 patients. Similarly, a study carried out by Sánchez-Sánchez et al. (2017) in Mexico revealed that all 215 patients examined indicated bacterial growth. Atlaw et al. (2022) observed a comparable result in their research conducted in Ethiopia, where a 100% infection prevalence rate was noted among 120 patients subjected to microbiological testing. The high prevalence of infections marked by positive microbiological culture results is likely associated with the severity of the patients' foot ulcers. In the aforementioned studies, the patients typically presented to healthcare providers with ulcers classified as Wagner grades 3 and 4, similar to the findings of this research.

Bacteriological profiles in patients with diabetic foot disease

The microorganisms causing infections in diabetic foot disease vary based on the severity of the foot ulcers. Globally, *Staphylococcus aureus* is the most common bacterial pathogen in diabetic foot infections, accounting for 33% of total cases, followed by *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*. This is consistent with the findings of the 2012 study conducted at Dr.

Soetomo General Academic Hospital, Surabaya, Indonesia, where *Staphylococcus aureus* was the most frequently isolated bacterium (Windriya et al., 2020). It is noteworthy that there has been little variation in the bacterial profile of diabetic foot infections at Dr. Soetomo General Academic Hospital from 2012 to 2023. In the study conducted in Mexico, *Staphylococcus aureus* was the most frequently identified Gram-positive pathogen (27%), whereas *Enterobacter* sp. and *Serratia* sp. were the most common Gram-negative pathogens (Sánchez-Sánchez et al., 2017). A study carried out in Southern India also identified *Staphylococcus aureus* as the causative pathogen in 29% of infections, with *Pseudomonas aeruginosa*, *Klebsiella* sp., and *Proteus* sp. being the predominant Gram-negative bacteria (Selvarajan et al., 2021).

From January 2021 to August 2023, *Staphylococcus aureus* was determined as the main causative pathogen in 11 cases (33%) of diabetic foot infections at Dr. Soetomo General Academic Hospital, followed by *Acinetobacter baumannii* (18%), *Klebsiella pneumoniae* (15%), and *Escherichia coli* (9%). Notably, *Pseudomonas aeruginosa*, responsible for approximately 18% of infections in the study by Windriya et al. (2020), was identified in only 3% of cases in this study. *Pseudomonas aeruginosa* is often recognized as a nosocomial agent, indicating that its reduction may be associated with improved healthcare standards that more effectively prevent nosocomial infections (Maki et al., 2008; Labovská, 2021). Nonetheless, further research is necessary to provide enhanced confirmation regarding these findings.

Antibiotic susceptibility and resistance patterns in patients with diabetic foot disease

In this study, 59.4% of isolates from the culture of diabetic foot disease patients' specimens exhibited resistance to first-line antibiotics, including ciprofloxacin, levofloxacin, and moxifloxacin. Among the 19 antibiotic-resistant isolates were *Staphylococcus aureus* (26.3%), *Acinetobacter baumannii* (26.3%), *Klebsiella pneumoniae* (15.8%), *Escherichia coli* (15.8%), *Proteus mirabilis* (5.3%), *Cornyebacterium striatum* (5.3%), and *Morganella morganii* (5.3%). Four out of 11 *Staphylococcus aureus* isolates exhibited methicillin resistance (MRSA). Among the *Escherichia coli* and *Klebsiella pneumoniae* isolates, four out of eight produced beta-lactamase (ESBL), and one was identified as carbapenem-resistant enterobacterales (CRE). Additionally, three *Acinetobacter baumannii* isolates demonstrated multidrug-resistant organism (MDRO) characteristics.

This study revealed higher rates of MRSA, ESBL, CRE, and MDRO compared to the findings reported by Selvarajan et al. (2021). Another study conducted in India indicated that MRSA was present in 6%, ESBL in 51.5%, and MDRO in 75% of a total of 68 isolates (Gayathri & Rani, 2018). The Gram-positive bacteria demonstrated the highest sensitivity to various antibiotics, including amikacin, amoxicillin-clavulanic acid, ampicillin, ampicillin-sulbactam, aztreonam, and cefepime. For the Gram-negative bacteria, the antibiotics with the highest sensitivity were cefotaxime, cefoxitin, ceftazidime, ceftriaxone, and cefazolin.

Staphylococcus aureus showed 100% resistance to penicillin-class antibiotics and high resistance rates to cephalosporins and other antibiotics. Similar resistance patterns were observed in the study conducted by Atlaw et al. (2022) in Ethiopia, where *Staphylococcus aureus* exhibited high resistance to penicillin G, cefoxitin, and doxycycline. Similarly, a study taking place in China reported high resistance of *Staphylococcus aureus* to penicillin G, ampicillin, and cefazolin (Du et al., 2022).

Klebsiella pneumoniae showed high resistance to beta-lactam antibiotics. This is consistent with the findings of a prior study, which indicated that *Enterobacter* species exhibited resistance to cephalosporins (Sánchez-Sánchez et al., 2017). The ability of *Klebsiella pneumoniae* and other *Enterobacteriaceae* to resist multiple classes of antibiotics is due to their production of ESBL and CRE (Effah et al., 2020). In addition, these species exhibit resistance to certain antibiotics owing to their ability to produce biofilm (Vuotto et al., 2014; Nirwati et al., 2019; Guerra et al., 2022; Shi et al., 2022). In this study, 60% of *Klebsiella pneumoniae* isolates were producers of ESBL or CRE.

Acinetobacter baumannii, the predominant Gram-negative pathogen in this study, demonstrated 100% resistance to penicillin-class antibiotics. Similar findings were reported in a separate study, where *Acinetobacter baumannii* showed high resistance to beta-lactam antibiotics (Atlaw et al., 2022). Furthermore, a study conducted at Ulin Regional General Hospital, Banjarmasin, Indonesia, also revealed high resistance rates in Gram-negative bacteria to ampicillin and cefazolin (Yani et al., 2021).

Escherichia coli exhibited 100% resistance to ampicillin, ciprofloxacin, cotrimoxazole, levofloxacin, moxifloxacin, piperacillin, and tetracycline. Similar patterns were observed in earlier research conducted in Tabriz, Iran, where *Escherichia coli* exhibited resistance to ciprofloxacin, tetracycline, and ampicillin (Akhi et al., 2015). In this study, 66.6% of *Escherichia coli* isolates were ESBL producers, comparable to the findings of a study carried out in China, which reported that 68% of *Escherichia coli* isolates expressed ESBL genes (Shi et al., 2022).

Wagner diabetic foot ulcer classification system and microorganism profiles

This study revealed that the severity of diabetic foot ulcers, as assessed by the Wagner classification system, was associated with the type of infectious bacteria, infection patterns, and antibiotic resistance. Lower Wagner grades, namely grade 3 or below, were associated with Gram-positive bacteria and monomicrobial infections. Simultaneously, higher Wagner grades, specifically grade 4 or above, were linked to Gram-negative bacteria and polymicrobial infections. Similar findings were reported in the Ethiopian study, where lower Wagner grades (≤ 3) were mainly associated with Gram-positive bacteria, while higher scores (≥ 4) were linked to Gram-negative bacteria (Atlaw et al., 2022). Prior research conducted by Xie et al. (2017) in China showed an increase in the proportion of Gram-negative bacterial isolates with higher Wagner grades. In addition, the study indicated that *Staphylococcus aureus* was the most common pathogen in diabetic foot disease.

Culture results and microorganism profiles

This study determined that there was a significant relationship between culture results and first-line antibiotic resistance. Isolates identified in polymicrobial infections exhibited higher resistance levels to antibiotics compared to those found in monomicrobial infections. No resistance to first-line antibiotics was observed among Gram-positive bacterial isolates. However, Gram-negative bacterial isolates showed a 76% resistance rate. These findings slightly differ from those of prior research conducted by Du et al. (2022) in China, which revealed first-line antibiotic resistance in Gram-positive bacterial isolates, with resistance rates ranging from 5.4% to 59.4%. The research further revealed that Gram-negative bacterial isolates demonstrated resistance rates ranging from 25% to 66.7%. A separate study also supports these findings, noting that polymicrobial cultures, particularly those involving *Staphylococcus aureus*, increase antibiotic tolerance due to biofilm formation (Nabb et al., 2019).

This study provides important data as the first investigation in Indonesia to analyze the relationship between the Wagner diabetic foot ulcer classification system and patients' pathogen profiles and antimicrobial susceptibility. Furthermore, this study incorporated an in-depth analysis of varying infection patterns and the occurrence of antimicrobial resistance. However, due to the small number of samples acquired in this study, further studies in Indonesia involving extensive data collection may be necessary to provide a more detailed look at the association between antimicrobial resistance and escalating Wagner grades. Moreover, the discrepancy in antibiotics tested for each culture sample might affect the results of this study.

CONCLUSION

The investigation of diabetic foot disease at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, uncovers critical findings about bacterial profiles and antibiotic resistance patterns in infected ulcers. Bacterial isolates cultured from diabetic foot ulcers demonstrate proliferation, with most exhibiting resistance to first-line antibiotics. Our findings suggest a clear relationship between ulcer severity and infection patterns alongside culture results and antibiotic resistance. Specifically, severe ulcers are associated with Gram-negative bacteria and elevated antibiotic resistance, while less severe ulcers are linked to Gram-positive bacteria and lower resistance levels. Additionally, there is a significant association between the isolated bacteria and antibiotic resistance. The findings underscore the critical need for precise microbial identification and understanding of susceptibility patterns to guide the effective management of diabetic foot disease.

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CONFLICT OF INTEREST

The authors declare that there are no potential conflicts of interest regarding the publication of this article.

ETHICS CONSIDERATION

This study received ethical approval from the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, under protocol number 1488/LOE/301.4.2/X/2023 on October 21, 2023. The East Java Regional Government Office authorized the execution of this study.

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AUTHOR CONTRIBUTION

For this work, AW contributed to the conception and design of the study, analyzed and interpreted the data, drafted the article, provided funding, and collected and assembled the data. Furthermore, YES provided critical revisions of the article for important intellectual content and supported the study administratively, technically, and logistically. ADWW contributed by providing study materials and patients, as well as offering statistical expertise on suitable analysis methods. Lastly, HN gave final approval of the article.

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