



Identification and Antibiotic Susceptibility of Bacterial Causes from Diabetic Ulcers in Hospital Kediri, Indonesia

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ABSTRACT:

Introduction: Diabetes is a clinical metabolic disease that, if not treated properly, can cause complications, namely diabetic ulcers. Diabetic ulcer infection in DM patients is generally characterized by pus with an open wound size that can increase bacterial infection. Gram-positive coccus bacteria usually cause bacterial infection in wounds of early acute DM patients, and there is only one type (monomicrobial). Furthermore, in the acute stage, several types of microbes will be found (polymicrobial).

Objectives: This study aims to investigate the bacterial profile and antibiotic susceptibility patterns of diabetic ulcer infections in patients with DM

Methods: Samples of diabetic ulcers were taken from DM patients treated at hospitals in Kediri, Indonesia. Sampling was carried out on pus in diabetic wounds aseptically. Furthermore, the identification of gram-positive and gram-negative bacteria was carried out. All successfully identified bacteria were tested for antibiotic susceptibility.

Results: The bacteria identified were *Staphylococcus aureus* (50%), *Pseudomonas aeruginosa* (25%), *Escherichia coli* (14.58%), *Salmonella paratyphi B* (6.25%) and *Klebsiella* spp (4.17%). The identified bacteria consisted of 4 gram-negative bacteria, the most commonly found being *Pseudomonas aeruginosa* and *Escherichia coli*. In comparison, the one most commonly found gram-positive bacteria was *Staphylococcus aureus*. *Staphylococcus aureus* bacteria (100%) are sensitive to kanamycin and ampicillin. At the same time, Chloramphenicol has the best performance in sensitivity to various gram-negative bacteria, namely *Pseudomonas aeruginosa* (58%), *Salmonella paratyphi B* (66.7%) and *Klebsiella* (50%).

Conclusions: The most common gram-negative bacteria found are *Pseudomonas aeruginosa* and *Escherichia coli*, while the most common gram-positive bacteria found are *Staphylococcus aureus*. Kanamycin and ampicillin are sensitive to *Staphylococcus aureus*, and Chloramphenicol is sensitive to *Pseudomonas aeruginosa*.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disease due to disorders in insulin production, insulin function, or both, so blood sugar levels increase. In general, DM is divided into 2 types, namely Type 1 Diabetes, which is a type of diabetes caused by damage to pancreatic

beta cells that requires lifelong insulin therapy, and Type 2 Diabetes, which is diabetes caused by insulin resistance and decreased insulin production due to unhealthy lifestyle mistakes (1). DM that is not treated correctly can cause complications, both acute and chronic (2). One of the complications that often occurs



in DM patients is pain and the emergence of wounds due to a combination of diabetic neuropathy, circulatory disorders, mechanical pressure, and impaired wound healing (3,4). In Indonesia, an estimated 15-30% of DM patients die from wounds and amputations due to DM complications (5).

Wound complications in DM patients, often called diabetic ulcers, are open wounds that appear on the skin of DM patients due to diabetic neuropathy and peripheral arterial disease (6,7). Diabetic ulcer infections in DM patients are generally characterized by pus with an irregular open wound size and red to brown. The presence of open wounds in DM patients increases the level of bacterial infection. This is due to the high blood sugar levels, so it is suitable as a medium for bacterial growth, both aerobic and anaerobic bacteria (8).

Bacterial infections in wounds of patients with DM in the early acute stage are usually caused by gram-positive cocci, and there is only one type (monomicrobial). In the chronic stage, the infection spreads rapidly so that a combination of gram-negative and positive bacterial infections occurs, both aerobic and anaerobic (9,10). These results are by the research of Poyil et al. (11), who succeeded in identifying bacteria in foot and hand ulcers of DM patients in a Saudi Arabian hospital with the results that there were various gram-positive and gram-negative pathogenic bacteria such as *E.Coli*, *Pseudomonas* spp, *Proteus* spp, *klebsiella* spp, *streptococcus* spp, and *staphylococcus aureus*.

Several studies have shown that inappropriate use of antibiotics can cause resistance to bacteria that cause diabetic ulcers. Research by Rezazadel et al. (12) reported that there were two types of bacteria most often found in diabetic ulcer samples at Taleghani Abadan Hospital, namely *staphylococcus aureus* (34.5%) and *pseudomonas aeruginosa* (30.4%) where the highest frequency of antibiotic resistance occurred in cotrimoxazole (33%) and tetracycline (28.2%). Similar research results were also reported by Bouharkat et al. (13), which showed that the highest prevalence of gram-negative bacteria in diabetic ulcer samples in Algeria was *E. Coli* (36.2%). In comparison, the highest gram-positive bacteria was *streptococcus aureus* (39%). From the results of this study,

information was obtained that 93.3% of bacteria were resistant to penicillin, and 71.1% were resistant to tetracycline.

Based on the background above, it is necessary to identify bacteria that cause diabetic ulcers in DM patients at the hospital in Kediri, Indonesia and it is necessary to conduct resistance tests of bacteria that cause diabetic ulcers to antibiotics commonly consumed by DM patients. This study aimed to identify pathogenic bacteria that cause diabetic ulcers at the hospital in Kediri, Indonesia and to see the antibiotic resistance profile of these bacteria.

2. Objectives

This study aims to investigate the bacterial profile and antibiotic susceptibility patterns of diabetic ulcer infections in patients with diabetes mellitus (DM). Specifically, the research seeks to:

- Identify the types of gram-positive and gram-negative bacteria present in diabetic ulcer samples from patients in Kediri, Indonesia.
- Determine the prevalence of these bacterial species and their distribution in relation to gram-positive and gram-negative classifications.
- Assess the antibiotic susceptibility of the identified bacterial strains to commonly used antibiotics, including kanamycin, ampicillin, and chloramphenicol.
- Compare the sensitivity profiles of gram-positive and gram-negative bacteria to identify effective treatment options for diabetic ulcer infections.

3. Methods

Study design and participant subjects

This study involved 112 DM patients who received treatment at Hospital "X" Kediri, Indonesia. Of the 112 DM patients, patients who had festering wounds (PUS) were selected. In this study, 24 patients had festering wounds (pus) whose pus would be taken as a sample. All patients have signed a letter of consent for sample collection. Some data was also taken during this research, such as gender, age, type of diabetes, duration of diabetes, and the patient's sugar level.



Sample collection

Sample collection was carried out from pus in diabetic wounds aseptically. First, the wound is cleaned with sterile distilled water; then, a cotton swab is moistened with sterile distilled water. The ulcer patient's festering wound is swabbed slowly in the direction of the cotton roll, and then the swab results are put into a sterile tube containing PZ. The tube was closed using cotton wool and aluminum foil and labelled according to the DM patient's data. The sample was taken based on ethical clearance from the Institute of Health Sciences Bhakti Wiyata Kediri No. 16/FTMK/EP/VI/2024.

Bacterial Identification

The gram-positive bacteria identification stage is carried out by inoculating samples in the transport media on BAP (Blood Agar Plate) media, then incubating them at 37° C for 24 hours. The results of the colonies formed on BAP media are then observed for shape, size, colour, edge, surface, consistency, and hemolysis, and then Gram staining is performed. The results are observed under a microscope, including the staining's shape, colour, colony arrangement, and Gram-positive properties. Colonies from BAP media are then inoculated into selective media, namely MSA (Mannitol Salt Agar) and NAS (Nutrient Agar Salt), then incubated at 37° C for 24 hours. Observations on MSA media include shape, size, colour, edge, surface, consistency, and mannitol fermentation, while observations on NAS media include shape, size, colour, edge, surface, consistency, and pigment. Identification is continued with a catalase test using 3% H₂O₂ reagent and coagulase using PZ and citrate plasma.

The identification stage of gram-negative bacteria is carried out by inoculating samples in transport media on MCA (Mac Conkey Agar) media and incubating them for 24 hours at 37°C. The results of the colonies formed on MCA media are then observed for shape, size, colour, edge, surface, consistency, and lactose fermentation; then, gram staining is performed. The results are observed under a microscope, including the staining's shape, colour, arrangement, and nature. Bacterial colonies from MCA media are then inoculated on selective media, namely TSIA, urea, indole, MR, VP, and Citrate media, and incubated in an incubator at 37°C for 24 hours. Each selective media is then observed for its changes. On TSIA media, the slope,

base, H₂S, and gas formation are observed. On indole media, Novak reagent is added, MR media is added with MR reagent, and VP media is added with KOH and alpha naphthol. All media are then observed for changes and ring formation.

Bacterial Rejuvenation

Before Antibacterial Testing, it is necessary to rejuvenate the test bacteria first. All bacteria that have been successfully identified are then rejuvenated by taking a single colony and then inoculating it on selective media. The results are incubated at 37° C for 18-24 hours. Rejuvenation is carried out every two weeks.

Bacterial Sensitivity

A single colony of bacteria was taken from the rejuvenation culture and suspended in 10 ml of BHIB. The results were homogenized and then incubated at 37°C for 3 hours. After 3 hours, the turbidity formed was equalized with the Mc. Farlan standard. The preparation of the Mc. Farlan standard was carried out by filling a test tube with 0.05 ml of 1% BaCl₂, adding 9.95 ml of 1% H₂SO₄, and then homogenizing. The turbidity formed was used as the standard for bacterial turbidity.

Antibiotic susceptibility test

Antibiotic susceptibility test is carried out by inserting sterile cotton swabs into each bacterial suspension that has been prepared beforehand. The cotton swab is then fully swabbed on the surface of the MHA media until it is evenly distributed on the surface of the agar media. The media is left for 5 minutes to absorb the bacterial suspension. Antibiotic discs are placed on the media swabbed with bacterial suspension according to the label and then incubated at 37°C for 24 hours. After 24 hours, the inhibition zone is measured using a calliper. Data interpretation is carried out by comparing research data with the Clinical Laboratory Standards Institute (CLSL) (14).

4. Results

This study involved 112 DM patients in several hospitals in Kediri, Indonesia. Of the 112 patients, 88 did not have ulcers, while the rest (24 DM patients) had diabetic ulcers. All of them were examined for pus (PUS) to identify gram-positive and gram-negative



bacteria. All successfully identified bacteria were then tested for bacterial susceptibility to several antibiotics commonly used by DM patients. The flow of this study

is shown in Figure 1. The characteristics of ulcer patients at Hospital Kediri Indonesia are shown in Table 1.

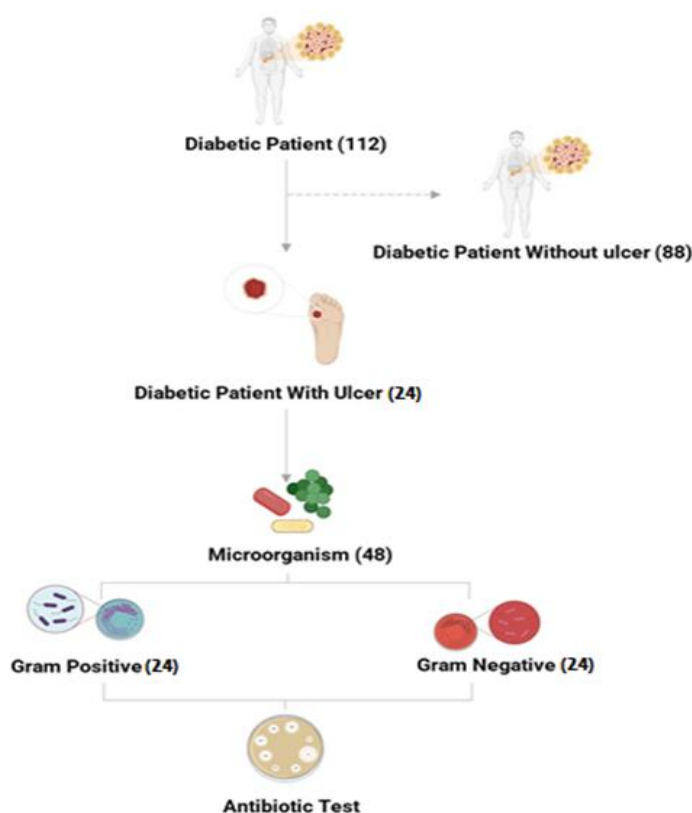


Figure 1. Summary Flow of This Study

Table 1. The Characteristics of Ulcer Patients at Hospital Kediri, Indonesia

Parameter	Value	Range or n (%)
Gender	Male	17 (70,8 %)
	Female	7 (29,2 %)
Age (years)	< 40	1 (4,2 %)
	40-55	9 (37,5 %)
	55-70	12 (50 %)
	>70	2 (8,3 %)
	Average	59,8 ± 12,6
Length of Diabetes (years)	< 10	11 (45,8 %)
	> 10	13 (54,2 %)
	Average	15,3 ± 4,4
Type of Diabetes	Type I	0 (0 %)
	Type II	24 (100 %)
Random blood sugar level (mg/dL)	< 200	3 (12,5 %)
	> 200	21 (87,5 %)
	Average	251,7 ± 72,57

Parameter	Value	Range or n (%)
Diabetes	Yes	15 (62,56 %)
complications	No	9 (37,5 %)

This study examined 24 DM patients who underwent examination at hospital in Kediri, Indonesia. Table 1 shows that 24 patients suffering from diabetic ulcers were 70.8% male and 29.2% were female, with an average age of 59,8 ± 12,6 years old. Based on the data, 100% of patients suffering from diabetic ulcers had type II diabetes. Table 1 also shows that 54.2% of patients with ulcers have had diabetes for more than ten years, with an average random blood sugar of 251.7 ± 72.57 mg/dL.

Identification of Bacterial



The results of bacterial identification are shown in the figure 2. From 24 diabetic ulcer patients whose pus samples were examined, 48 bacterial isolates were found. Based on biochemical characterization and selective media cultivation, four types of gram-negative bacteria and 1 type of gram-negative bacteria were found. These bacteria will then be tested for bacterial susceptibility to several types of antibiotics using the disc method. Tables 2 and 3 show the results of the characterization of gram-negative and gram-positive bacterial isolates from diabetic ulcer patients at Hospital Kediri, Indonesia.

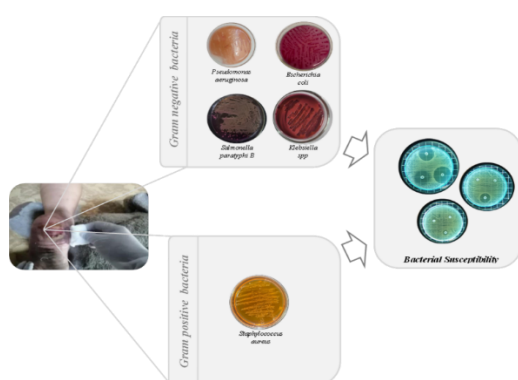


Figure 2. The results of bacterial identification from Diabetic Ulcer Patients at Hospital Kediri, Indonesia

Table 2 shows that in this study, 48 bacterial isolates comprised 24 rod-shaped bacteria (50%) and 24 coccus-shaped bacteria (50%). The results of biochemical characterization obtained several types of gram-negative bacteria that were successfully identified, namely *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella paratyphi B*, and *Klebsiella*. Table 3 shows that the results of biochemical characterization obtained 1 type of gram-positive bacteria, namely *Staphylococcus aureus*. Figure 3 shows that overall, the bacteria identified in this study were *Staphylococcus aureus* (50%), *Pseudomonas aeruginosa* (25%), *Escherichia coli* (14.58%), *Salmonella paratyphi B* (6.25%) and *Klebsiella spp* (4.17%).

Bacterial Susceptibility

The results of the susceptibility test of bacteria causing diabetic ulcers in patients at the hospital in Kediri, Indonesia are shown in the Table 4. Based on the Table, 100% of *Staphylococcus aureus* bacteria are sensitive to

kanamycin and ampicillin, while 54.2% of *Staphylococcus aureus* bacteria are resistant to penicillin. *Pseudomonas* bacteria are 100% resistant to several antibiotics, such as kanamycin, rifampicin, ampicillin, amoxicillin, bacitracin, erythromycin, and cotrimoxazole. 100% of *E. coli* bacteria also resisted several antibiotics such as rifampicin, ampicillin, amoxicillin, bacitracin, erythromycin, and cotrimoxazole. All *Salmonella paratyphi B* bacteria also showed resistance to rifampicin, ampicillin, amoxicillin, bacitracin, and amilacin, while all *klebsiella* bacteria were resistant to all types of antibiotics except chloramphenicol.

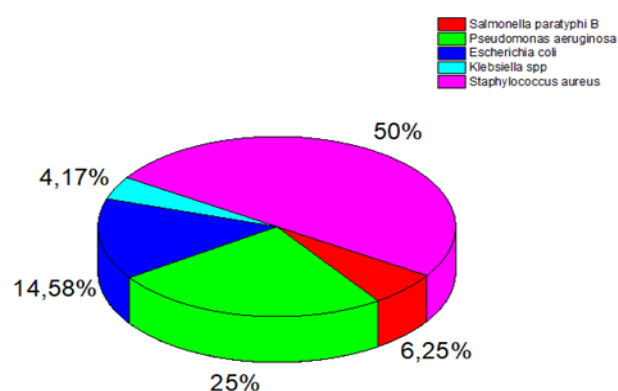


Figure 3. The results of bacterial identification from Diabetic the bacteria identified from Diabetic Ulcer Patients at Hospital Kediri, Indonesia

Bacterial Susceptibility

The results of the susceptibility test of bacteria causing diabetic ulcers in patients at the hospital in Kediri, Indonesia are shown in the Table 4. Based on the Table, 100% of *Staphylococcus aureus* bacteria are sensitive to kanamycin and ampicillin, while 54.2% of *Staphylococcus aureus* bacteria are resistant to penicillin. *Pseudomonas* bacteria are 100% resistant to several antibiotics, such as kanamycin, rifampicin, ampicillin, amoxicillin, bacitracin, erythromycin, and cotrimoxazole. 100% of *E. coli* bacteria also resisted several antibiotics such as rifampicin, ampicillin, amoxicillin, bacitracin, erythromycin, and cotrimoxazole. All *Salmonella paratyphi B* bacteria also showed resistance to rifampicin, ampicillin, amoxicillin, bacitracin, and amilacin, while all *klebsiella* bacteria were resistant to all types of antibiotics except chloramphenicol.

**Table 2.** Characterization of Gram-Negative Bacterial Isolates from Diabetic Ulcer Patients at Hospital Kediri, Indonesia

Bacterial Isolates (n)	Colony colour in MCA	Staining	Biochemical Parameters							Fermentation of Sugars					Identified Gram Negative Bacteria		
			GS	CS	MT	TSIA	ID	MR	VP	CIT	UR	G	L	Mn		MI	S
3	Clear	Bacilli	+	+	+	-	-	+	-	+	-	+	-	+	+	-	<i>Salmonella paratyphi B</i>
12	Clear	Bacilli	-	+	+	-	-	-	+	-	-	-	-	-	-	-	<i>Pseudomonas aeruginosa</i>
7	Red	Bacilli	+	+	-	+	+	-	-	-	+	-	-	-	-	-	<i>Escherichia coli</i>
2	Red	Bacilli	+	-	-	-	-	-	+	+	+	+	+	+	+	+	<i>Klebsiella spp</i>

Abbreviations:

GS : Gram staining; CS : Capsule staining; MT : Motility test; TSIA : Triple Sugar Iron Agar; ID : Indole test; MR : Methyl red test; VP : Voges-Proskauer test; CIT : Citrate test; UR : Urea test; G : Glucose; L : Lactose; Mn : Mannose; MI : Maltose; S : Sucrose

Table 3. Characterization of Positif-Negative Bacterial Isolates from Diabetic Ulcer Patients at Hospital Kediri, Indonesia

Bacterial Isolates (n)	NAS Pigmentation	Staining	Biochemical Parameters							Fermentation of Sugars					Identified Gram Positif Bacteria		
			GS	CS	MT	ESS	CAT	COA	MR	VP	NR	G	L	Mn		MI	S
24	golden yellow	Coccus	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>Staphylococcus aureus</i>

Abbreviations:

GS : Gram staining; CS : Capsule staining; MT : Motility test; ESS : Endospores staining; CAT : Catalase test; COA : Coagulase test; MR : Methyl red test; VP : Voges-Proskauer test; NR: Nitrate reductase test; G : Glucose; L : Lactose; Mn : Mannose; MI : Maltose; S : Sucrose

Table 4. Susceptibility Test of Bacteria Causing Diabetic Ulcers in Patients at the Hospital in Kediri, Indonesia

Anti-biotic	<i>Staphylococcus aureus</i> (24)				<i>Pseudomonas aeruginosa</i> (12)				<i>Escherichia coli</i> (7)				<i>Salmonella paratyphi B</i> (3)				<i>Klebsiella spp</i> (2)			
	S	%	R	%	S	%	R	%	S	%	R	%	S	%	R	%	S	%	R	%
Kan	24	100	0	0	0	0	12	100	5	71,4	2	28,6	2	66,7	1	33,3	0	0	2	100
Rif	21	87,5	3	12,5	0	0	12	100	0	0	7	100	0	0	3	100	0	0	2	100
Amph	24	100	0	0	0	0	12	100	0	0	7	100	0	0	3	100	0	0	2	100
Amox	22	91,7	2	8,3	0	0	12	100	0	0	7	100	0	0	3	100	0	0	2	100
Pen	11	45,8	13	54,2	0	0	12	100	0	0	7	100	0	0	3	100	0	0	2	100
Bac	18	75,0	6	25,0	0	0	12	100	0	0	7	100	0	0	3	100	0	0	2	100
Ery	16	66,7	8	33,3	0	0	12	100	0	0	7	100	1	33,3	2	66,7	0	0	2	100
Chlo	22	91,7	2	8,3	7	58	5	41,67	3	42,9	4	57,1	2	66,7	1	33,3	1	50	1	50
Cot	19	79,2	5	20,8	0	0	12	100	0	0	7	100	1	33,3	2	66,7	0	0	2	100
Amyl	18	75,0	6	25,0	3	25	9	75	2	28,6	5	71,4	0	0	3	100	0	0	2	100

Abbreviations

S : sensitivity; R : Resistance; Kan : Kanamycin; Rif : Rifampicin; Amph : Ampicillin; Amox : Amoxicillin; Pen : Penicillin; Bac : Bacitracin; Ery : Erythromycin; Chlo : Chloramphenicol; Cot : Cotrimaxazole; Amyl : Amylacin



5. Discussion

Diabetic ulcers are wounds that occur in DM patients and are one of the most common complications of diabetes (6,15). In this study, the majority of patients who experienced ulcers were men. This is due to the research of Shi et al. (15), which showed that 62.9% of DM patients suffering from diabetic ulcers in China were male. Similar results were reported by Atlaw et al. (16), who stated that 67.69% of diabetic ulcer patients in Ethiopia were male. Most DM patients who have diabetic ulcers are male because men tend to do more activities outside, so the possibility of ulcers is greater than in women. In this study, 50% of diabetic ulcer patients were aged 55-70, averaging 59.8 years. These results are by research by Thanganadaran et al., (17) which states that diabetic ulcers are generally experienced by middle-aged people (late 50s) due to complications of diabetes such as vasculopathy and neuropathy, as well as decreased immunity.

All patients examined in this study were type II diabetes sufferers. This result is from Rossboth study (18), which states that type II diabetic patients are more likely to suffer from diabetic ulcers than type I. This is because type II diabetes has a higher prevalence of additional risk factors such as obesity, high blood pressure, diabetic neuropathy, and vascular problems. Similar research results were also reported by Hamid et al. (19), who reported that 80% of diabetic ulcer patients in Qatar were Type II DM patients because type II DM patients had experienced complications, especially peripheral vascular disease and peripheral neuropathy. This is also by the results of our study, which showed that 62.56% of DM patients at the hospital in Kediri, Indonesia also had other complications, such as high blood pressure and diabetic neuropathy.

The results of our research show that patients who have suffered from DM for a long period and have uncontrolled blood sugar levels tend to be more susceptible to the risk of diabetic ulcers. These results are by the study of Reddy (20), which showed that DM patients who have had diabetes for a long time are more susceptible to various DM complications such as neuropathy, vascular disorders, and decreased immunity, so they are more susceptible to diabetic ulcers. In addition, according to Eltrikanawati et al.

(21), the duration of diabetes and uncontrolled blood sugar levels can increase the risk of diabetic ulcers. Shi's study (15) showed that the average random blood sugar level in diabetic ulcer patients in China was 256.7 ± 58.9 mg/dL.

Gram-negative and gram-positive bacteria easily infect diabetic ulcer wounds (9,10, 11). In this study, the most commonly found gram-positive bacteria were *Staphylococcus aureus*, while the most commonly identified gram-negative bacteria were *Pseudomonas aeruginosa* and *Escherichia coli*. This is to the research of Sannathimmappa et al. (22), which reported that the most gram-positive bacteria successfully identified in diabetic ulcer patients in Oman were *Staphylococcus aureus*, namely 109 bacterial isolates (34.5%), while the most commonly found gram-negative bacteria were *Pseudomonas aeruginosa*, namely 40 bacterial isolates (17%) and *Escherichia coli* as many as 37 bacterial isolates (17%). Similar results were also reported by Rezazadeh et al. (12), that the most gram-positive bacteria successfully identified in diabetic ulcer patients in Iran were *Staphylococcus aureus*, namely 109 bacterial isolates (34.5%), while the most gram-negative bacteria found were *Pseudomonas aeruginosa*, namely 96 bacterial isolates (30.4%) and *Escherichia coli* as many as 61 bacterial isolates (19.3%).

Staphylococcus aureus is a gram-positive pathogenic bacteria in the form of a coccus that has the pathogenic ability to produce enzymes that can damage tissue and contribute to infection and inflammation. This causes *Staphylococcus aureus* to infect diabetic wounds and is often identified in patients with diabetic ulcers (23,24). In this study, the most common gram-negative bacteria found in diabetic ulcers were *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* bacteria are gram-negative bacteria that are rod-shaped and pathogenic and play a role in many infectious diseases. This is because *Pseudomonas aeruginosa* can adapt to various environmental conditions, including diabetic wounds that may be moist and lack oxygen (25,26).

In addition to *Pseudomonas aeruginosa*, the type of gram-negative bacteria that is often found in *Escherichia coli*. This is because *Escherichia coli* can adhere to body tissue and wound surfaces through the formation of fimbriae (pilus) and adhesins. *Escherichia coli* can also colonize wounds that have experienced



primary infections by other bacteria due to non-sterile and contaminated wounds (27,28). This study also found several other gram-negative bacteria, namely *Salmonella paratyphi B* and *Klebsiella* spp. This is to the research of Shi et al. (15), which showed that in addition to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, several other types of bacteria were also found, such as *Klebsiella* species (20.2%), *Acinetobacter* species (13.5%), *Protease* species (2.2%) and other types of bacteria (2.2%). Diabetic wounds are generally moist and contain high sugar levels, making them susceptible to bacterial infections (8).

In this study, gram-negative bacteria were more resistant to various antibiotics than gram-positive bacteria. These results are based on the study of Sannathimappa et al. (29), which showed that 36% of gram-negative bacteria isolated from diabetic ulcer samples in Oman experienced Multi-Drug Resistance (MDR). In addition, our research results show that gram-positive bacteria have good susceptibility to several antibiotics, such as kanamycin and ampicillin. Gram-positive bacteria have very thick cell walls but do not have an outer membrane. In contrast, gram-negative bacteria have more complex cell walls and an outer membrane that prevents many antibiotics from entering the cell wall (30).

Pseudomonas aeruginosa and *Escherichia coli* bacteria experienced resistance to almost all antibiotics used. These results are by the research of Atlaw et al. (16), which showed that *pseudomonas aeruginosa* bacteria isolated from diabetic ulcer patients in Ethiopia experienced resistance to various antibiotics such as doxycycline (95.8%), sulfamethoxazole (87.5%), Polymyxin (100%), cefotaxime (100%), cefepime (100%), ampicillin (100%) and augmentin (100%). Meanwhile, *Escherichia coli* bacteria were resistant to tobramycin (90.5%), sulfamethoxazole (90%), Polymyxin (100%), cefotaxime (95.5%), cefepime (100%), ampicillin (100%) and augmentin (100%). Similar results were also reported by Thanganadar et al. (17), who reported that *pseudomonas aeruginosa* bacteria isolated from diabetic ulcer patients in India experienced resistance to various antibiotics such as amoxicillin (100%), cotrimoxazole (100%), erythromycin (100%), tetracycline (92%) and ciprofloxacin (77%). Meanwhile, *Escherichia coli*

bacteria were resistant to amoxicillin (100%), cotrimoxazole (100%), erythromycin (100%), and ciprofloxacin (80%). In addition to having an outer membrane, *Pseudomonas aeruginosa* and *Escherichia coli* bacteria can form biofilms that can increase bacterial immunity and inhibit antibiotic penetration so that bacteria become resistant to antibiotics (31,32).

In this study, Chloramphenicol had the best performance in sensitivity to various gram-negative bacteria, namely *Pseudomonas aeruginosa* (58%), *Salmonella paratyphi B* (66.7%) and *Klebsiella* (50%). Meanwhile, Kanamycin had the best sensitivity to *Escherichia coli* (71.4%). Chloramphenicol has effective antibiotic activity against various gram-negative bacteria because Chloramphenicol has lipophilic properties so that it can penetrate the outer membrane of gram-negative bacteria and enter the bacterial cytoplasm (33,34). *Escherichia coli* bacteria generally have high sensitivity to Kanamycin because *Escherichia coli* has sensitive ribosomes and efficient active transport to Kanamycin (35).

6. Conclusion

The analysis of patients with diabetic ulcers in Patients at the Hospital in Kediri, Indonesia whose pus samples were examined showed 48 bacterial isolates. The bacterial isolates consisted of 24 bacilli bacteria; the rest were coccus. The identified bacteria consisted of 4 gram-negative bacteria, the most commonly found bacteria being *Pseudomonas aeruginosa* and *Escherichia coli*. The bacteria identified in this study were *Staphylococcus aureus* (50%), *Pseudomonas aeruginosa* (25%), *Escherichia coli* (14.58%), *Salmonella paratyphi B* (6.25%) and *Klebsiella* spp (4.17%). In comparison, the one most commonly found gram-positive bacteria was *Staphylococcus aureus*. In addition, the results of this study indicate that Chloramphenicol has the best performance in sensitivity to various gram-negative bacteria, namely *Pseudomonas aeruginosa* (58%), *Salmonella paratyphi B* (66.7%) and *Klebsiella* (50%). Kanamycin has the best sensitivity to *Escherichia coli* (71.4%).

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