Journal homepage : https://ojs3.unpatti.ac.id/index.php/biopendix

B I O P E N D I X P-ISSN: 2407-4969
Volume 12, Number 2, October 2025 E-ISSN:2684-8341
Pages: 116-122 DOI:https://doi.org/10.30598/biopendixvol12issue2page116-122

Identification and Sensivity Test of *Pseudomonas aeruginosa* to Ciprofloxacin and Ceftazidime in Diabetic Ulcers

Safitri Nur Istiqomah[1], Didik Wahyudi[2*]

Department of Medical Laboratory Technology, National College of Health Sciences, Sukoharjo, Indonesia
*email: didik.wahyudi@stikesnas.ac.id

Submitted: June 10, 2025; Revised: June 20, 2025; Accepted: June 23, 2025; Published: October 31, 2025

Abstract. Diabetic ulcers are a serious complication in diabetic patients, often becoming infected with various pathogenic bacteria, including *Pseudomonas aeruginosa*, which is known for its high resistance to multiple antibiotics. The purpose of this research to identify the presence of *Pseudomonas aeruginosa* in diabetic ulcers and evaluate its sensitivity to the antibiotics ciprofloxacin and ceftazidime at the Diabetic Wound Care Center (RUMAT) in Surakarta. The research used a descriptive observational design with the Kirby-Bauer disk diffusion method for antibiotic susceptibility testing, along with bacterial isolation, Gram staining, and biochemical tests for identification. The results showed that out of 8 specimens examined, 4 specimens (50%) were identified as *Pseudomonas aeruginosa*, all of which demonstrated sensitivity to ciprofloxacin and ceftazidime, with inhibition zones above the sensitive standard according to CLSI M100 2023.

Keywords: *Pseudomonas aeruginosa*; Diabetic Ulcers; Antibiotic Sensivity; Ciprofloxacin; Ceftazidime

INTRODUCTION

Diabetic ulcers are chronic wounds that occur in people with diabetes mellitus, and are difficult to heal, especially in conditions of high blood sugar levels (Arisandi et al., 2023). Diabetic ulcer sufferers usually experience symptoms of sensory and motor neuropathy and disorders of the blood vessel system which cause wounds to be difficult to heal (Budiyanto et al., 2021). The presence of a superficial infection on the skin is an early symptom of diabetic ulcers, which then becomes an advanced infection that is difficult to heal, and in certain conditions can cause amputation (Novelni R, 2019; Waworuntu P.J., 2016).

Several studies have found several types of bacteria that cause diabetic ulcer infections (Azahra F, 2021). (Anggriawan et al., 2014) reported the presence of aerobic and anaerobic bacteria in pus cultures of diabetic patients, including *Salmonella* sp. (82,15%), *Staphylococcus aureus* (17,85%), *Enterobacter sp.* (10,71%), dan *Pseudomonas sp*. (17,86%). (Waworuntu P.J., 2016) found six types of bacteria in diabetic ulcers, namely *Staphylococcus* sp. (29,8%), *Pseudomonas* sp. (14,2%), *Bacillus subtilis* sp. (17,4%), *Streptococcus* sp. (15,8%), *Proteus* sp. (10,6%), dan *Enterobacter* sp. (10,1%). (Meilalita I., 2023) show that *Staphylococcus aureus* (77,3%), *Staphylococcus epidermidis* (19,3%), *Pseudomonas aeruginosa* (15,9%), dan *Escherichia coli* (13,6%) is the main pathogenic bacteria in diabetic ulcers. *Pseudomonas aeruginosa* is an opportunistic pathogen that often causes infections in diabetic ulcers and has the ability to form biofilms, thereby compromising the healing process and increasing the risk of serious complications, including sepsis and amputation (Rosyidah K.A., 2021; Nagaya et al., 2024)This bacteria is often associated with nosocomial infections and is known to be resistant to many types of antibiotics, which makes treatment more difficult (Wahyudi et al., 2019; Budiyanto et al., 2021).

Appropriate antibiotic use is essential in the management of these infections. Ciprofloxacin, a fluoroquinolone antibiotic, is often the first choice for treating skin and soft tissue infections, including diabetic ulcers. Ciprofloxacin has a broad spectrum for treating gram-negative bacterial infections such as *P. aeruginosa* . Ceftazidime, an antibiotic from the cephalosporin group, is also often used to treat infections caused by *P. aeruginosa* (Mohamed et al., 2022). Inappropriate use of antibiotics can trigger bacterial resistance, worsen infections, and worsen patient prognosis (Wijesinghe et al., 2019). Antibiotic susceptibility testing is necessary to determine the most effective therapeutic options and reduce the risk of resistance (Garousi et al., 2023). This study aims to identify *P. aeruginosa* isolates from diabetic ulcers and conduct

sensitivity tests to the antibiotics ciprofloxacin and ceftazidime at the RUMAT (Care Home) for Diabetic Wound Specialists, Surakarta City, Central Java.

MATERIALS AND METHODS

This study design uses descriptive observational, identifying *P. aeruginosa* bacteria from diabetic ulcers. The study includes bacterial identification using biochemical test methods, followed by sensitivity tests to ciprofloxacin and ceftazidime antibiotics using the disk diffusion method (*Kirby-Bauer*). Place and Time of Research at the Diabetes Mellitus Wound Specialist Nursing Home (RUMAT), Surakarta City, sampling using the swab method. Bacterial identification and antibiotic sensitivity tests were carried out at the Bacteriology Laboratory of Sekolah Tinggi Ilmu Kesehatan Nasional. The number of samples was 8 samples, using the accidental sampling technique during the period 12 February-10 March 2025.

Sampling was carried out using sterile cotton swabs moistened with 0.9% NaCl, then inoculated into BHI (Brain Heart Infusion) media, incubated for 24 hours at a temperature of 37℃ (Novelni, 2019). Bacterial identification is carried out by Gram staining, using Gram A reagent (2% crystal violet) for 1-2 minutes, Gram B (Lugol's solution) for 30 seconds, staining with Gram C (alcohol acetone), and re-staining using Gram D (0.25% safranin) for 2 minutes. (Budiyanto, 2021). The preparation was rinsed with running water, dried, and observed under a microscope at 1000x magnification using immersion oil..

Bacterial selection was carried out by inoculating on MacConkey media with the strike plate method. Bacterial growth was observed macroscopically and microscopically. Biochemical tests include TSIA (Triple Sugar Iron Agar), SIM (Sulfur Indole Motility), urea, citrate, MR-VP (Methyl Red-Voges Proskauer), and PAD (Phenylalanine Deaminase), as well as carbohydrate fermentation tests (glucose, lactose, mannitol, maltose, sucrose) (Anggriawan et al., 2014)

Antibiotic susceptibility test using the Kirby-Bauer method. The bacterial suspension was adjusted to the McFarland 0.5 turbidity standard, then inoculated onto MHA (Mueller Hinton Agar) media and leveled. Ciprofloxacin (5µg) and ceftazidime (30µg) antibiotic discs were placed on the media, incubated for 24 hours at 37℃, then the inhibition zone was measured according to the Clinical and Laboratory Quality Control guidelines (Table 1).

Table 1. Range of Antibiotic Inhibition Zones Against *P. aeruginosa* Bacteria.

Antibiotics	Interpretation of Inhibition Zone Diameter (mm)		
	Sensitive	Intermediate	Resistance
Ciprofloxacin 5µg	≤18	19-24	≥25
Ceftaxidime 30µg	≤14	15-17	≥18

(Gaur et al., 2023)

Quality control of antibiotic disks was carried out using the bacterial strain *Klebsiella oxytoca* ATCC 700324. The bacterial suspension was made using the Mc Farland 0.5 standard, and inoculated on MHA (Muller Hinton Agar) media with a flattening technique, then incubated for 15 minutes at 37℃. The antibiotic disk was placed on MHA (Muller Hinton Agar) media and incubated. The results were concluded in accordance with the Clinical and Laboratory Quality Control reference standard (Table 2).

Table 2. Range of Antibiotic Inhibition Zones Against *Klebsiella oxytoca* ATCC 700324

Antibiotic	**Inhibition Zone Diameter (mm)**
Ciprofloxacin 5µg	≥31 mm
Ceftaxidime 30µg	≥21 mm

(Gaur et al., 2023)

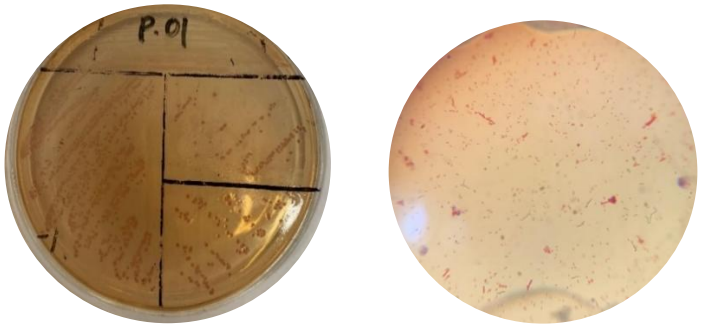
RESULTS AND DISCUSSION

The results of the identification and sensitivity test of *P. aeruginosa* to Ciprofloxacin and Ceftazidime in diabetic ulcers of 8 respondents obtained the results as shown in Table 3.

Table 3. Results of Identification of *P. aeruginosa* Bacteria in Diabetic Ulcers

Specimen Code	Identification Results
P.a.001	*P. aeruginosa*
P.a.002	Not identified
P.a.003	*P. aeruginosa*
P.a.004	*P. aeruginosa*
P.a.005	Not identified
P.a.006	*P. aeruginosa*
P.a.007	Not identified
P.a.008	Not identified

Table 3 shows that the examined ulcer swab samples found 4 isolate *P. aeruginosa* bacteria that had been identified by Gram staining, colony observation on McConkey media and biochemical tests (Figure 1). The results of biochemical tests on several media showed positive results in the motile test on SIM media, and citrate, on TSIA (Triple Sugar Iron Agar) media showed alkali/alkali results, other media showed negative results, including on carbohydrate fermentation media (glucose, lactose, mannitol, maltose, sucrose) (Figure 2).



(A) (B)

Figure 1. *P. aeruginosa* bacteria. (A) on McConkey Media (B) Microscopic Observation with Gram Staining.

Figure 2. Biochemical Test Results of *P. aeruginosa* bacteria. (Note: from left TSIA, SIM, Urea, Citrate, MR, VP, PAD, and Carbohydrate Fermentation media.

The antibiotic disk quality control test was conducted simultaneously with the sensitivity test of both antibiotics using the disk diffusion method (Kirby-Bauer), aimed at ensuring that the condition of the antibiotic disk used was still in a stable and good condition. The results of the quality control test showed the in control criteria (meeting quality control standards) because the inhibition zone formed was still included in the reference range required for ciprofloxacin and ceftazidime antibiotics (Table 4).

Table 4. Quality control results of Ciprofloxacin and Ceftazidime antibiotics

Antibiotic	Inhibition Zone Diameter (mm)	CLSI	Result
Ciprofloxacin (5µg)	31 mm	≥ 31 mm	*in control*
Ceftazidime (30µg)	26,5 mm	≥ 21 mm	*in control*

The results of the sensitivity test of *P. aeruginosa* to the antibiotics ciprofloxacin and ceftazidime on MHA media are shown in Figure 3, the bacterial colonies show bluish green pigmentation, this is one of the characteristics of *P. aeruginosa*, which has pyocyanin pigment. Table 5 shows the results of the sensitivity test

of *P. aeruginosa* to the antibiotics ciprofloxacin and ceftazidime, showing sensitive results in all isolates examined P.a.001, P.a.003, P.a.004, and P.a006 (100%).

Figure 3. Results of the *P. aeruginosa* Bacteria Sensitivity Test

Table 5. Results of the *P. aeruginosa* Sensitivity Test to Ciprofloxacin and Ceftazidime Antibiotics

Specimen Code	Antibiotic Inhibition Zone Diameter (mm)		Result	
	CIP (5µg)	CAZ (30µg)	CIP	CAZ
P.01	36,9	30,7	S	S
P.03	37,0	27,0	S	S
P.04	38,7	29,3	S	S
P.06	35,6	24,65	S	S

Note: CIP = Ciprofloxacin, CAZ = Ceftazidime; S = Sensitive,

The results of *P. aeruginosa* identification of in 8 patients with the swab method on diabetic ulcers were found in 50% (n = 4) in samples with codes P.a.001, P.a.003, P.a.004, P.a.006. Other bacteria were also found, namely *Acinobacter sp*. sample code P.a.002, *Klebsiella pneumoniae* in samples codes P.a.005 and P.a.007, and *Proteus sp.* in sample code P.a.008. These findings are in line with the results of the study (Meilalita & Susanti, 2023), who reported that half of the samples studied identified *P. aeruginosa* and the remainder showed the presence of other gram-negative bacteria.

Identification of *P.aeruginosa* in this study was carried out through several stages, starting from sample culture on BHI (Brain Heart Infusion) media, Gram staining, planting on McConkey media, and biochemical tests. Diabetic ulcer swabs obtained were first inoculated into BHI (Brain Heart Infusion) media to ensure optimal bacterial growth for 24 hours at a temperature of 37℃. All samples showed turbidity, which means that all specimens were overgrown with bacteria (Wahyudi et al., 2019; Arivo & Dwiningtyas, 2017) Gram staining is performed to determine the type of bacteria based on the characteristics of its cell wall. This Gram staining is used as a basis for seeing the growth of Gram-negative bacteria which are then selected on McConkey media to observe the characteristics of their colonies. The results of Gram staining in this study showed the presence of Gram-negative in almost all samples (P.a.001, P.a.003, P.a.004, P.a.005, Pa..006, P.a.007, and P.a.008), although Gram-positive in the form of cocci and Gram-positive in the form of rods were also found.

The results of inoculation on McConkey media show olive green bacterial colonies with a distinctive aroma which is one of the characteristics of *P. aeruginosa* isolates on McConkey media. The green color is produced from the production of pigments such as pyocyanin (blue-green) and pyroverdin (yellow-green), which are water-soluble and fluoresce, especially under UV light. *P. aeruginosa* bacteria do not ferment lactose, so the colonies remain transparent on McConkey media, this is what distinguishes these bacteria from Escherichia coli or Klebsiella pneumoniae bacteria which form pink colonies due to lactose fermentation (Aryzki S., 2020; Rehman et al., 2019).

Bacterial colonies on McConkey media were further identified using biochemical tests. The results of the biochemical tests showed that 4 isolates were *P. aeruginosa*. The isolates produced positive results in the citrate test, indicating oxidative metabolism and their ability to use various carbon sources. In the TSIA (Triple Sugar Iron Agar) test, isolates with sample codes P.a.001, P.a.003, P.a.004, and P.a.006 showed an alkali/alkali (AL/AL) reaction without gas or H2S production, because they were unable to ferment glucose or lactose fermentatively. The test results on the SIM (Sulfur Indole Motility) media test, were seen in sample codes

P.a.001, P.a.003, P.a.004, and P.a.006, the bacteria were motile but did not produce indole. The urea and PAD (Phenylalanine Deaminase) tests on isolates with sample codes P.a.001, P.a.003, P.a.004, and P.a.006 were negative, confirming the difference with Proteus bacteria which are usually positive in this test (Setianingsih et al., 2016; Septiana, 2024).

There were variations in the presence of other bacteria found in this study, such as *Acinobacter sp*., *Klebsiella pneumoniae*, and *Proteus sp*., all of which are opportunistic Gram-negative bacteria that are often found in chronic wound infections and nosocomial infections. This variation is in line with the findings (Anggriawan et al., 2014) which also found similar bacteria. One sample (code P.a.002) did not show growth on McConkey media, the staining results on the sample showed clustered coccus bacteria indicating the possible presence of Gram-positive bacteria such as *Staphylococcus spp*. This is in line with the findings, which states that certain Gram-positive bacteria, including the genus Staphylococcus, have certain resistances to environmental conditions and may have special nutritional requirements that are not met on selective media such as McConkey.

The results of the sensitivity test carried out showed that all *P. aeruginosa* isolates were sensitive to the antibiotics ciprofloxacin and ceftazidime (Table 5), this is in line with research (Wahyudi et al., 2019) conducted at Dr. Moewardi Hospital, Surakarta, which showed that *P. aeruginosa* isolates from diabetic ulcer patients examined were sensitive to ciprofloxacin and ceftazidime. Other findings in Samarinda (Setianingsih et al., 2016; Amalia F F., 2020) reported that *P. aeruginosa* causing diabetic ulcers remains sensitive to ciprofloxacin and ceftazidime, although there is a tendency for increasing resistance over time due to uncontrolled antibiotic use.

All respondents had been diagnosed with diabetes mellitus and had diabetic ulcers (Garousi et al., 2023) explained that infection in diabetic ulcers can continue even though the patient's blood glucose levels are within the normal range. All respondents in this study stated that they had received antibiotic therapy even though no ciprofloxacin or ceftazidime was given. Respondents also stated that they had carried out routine wound care and followed the advice of the officers. The results of the researcher's observations showed that good wound care practices had not been able to eliminate infection, even though the wounds appeared to be well cared for.

Diabetic ulcers are serious complications in patients with diabetes mellitus who are often infected with pathogenic bacteria, one of which is *P. aeruginosa*. Risk factors such as gender, age, blood glucose control, and wound care can affect the severity of infection and response to antibiotic therapy (Nagaya et al., 2024). The results of this study indicate that all *P. aeruginosa* isolates from diabetic ulcers in respondents were sensitive to ciprofloxacin and ceftazidime (Mohamed et al., 2022). stated that there are several clinical factors and patient behavior that can affect bacterial sensitivity tests in diabetic ulcers. All respondents in this study were male, under 50 years of age, with blood glucose levels within normal limits at the time of data collection. Respondents stated that they were always compliant with routine wound care, including the use of antibiotics, although not ciprofloxacin and ceftazidime. This factor has the potential to reduce the level of bacterial colonization and resistance.

Research result (Mohamed et al., 2022) dan (Garousi et al., 2023) found that a good level of knowledge about wound care is correlated with a decreased risk of infection in diabetic ulcers. Public education regarding early detection of diabetic ulcers significantly increases knowledge and preventive behavior. These findings indicate the importance of education and compliance with wound care in suppressing the level of colonization of resistant bacteria in patients with diabetes mellitus. Another factor that may need to be considered is the ability of bacteria to form biofilms. Biofilms can increase bacterial resistance to antibiotics. Although biofilms do not always develop in all cases of diabetic ulcers, especially in patients with good blood sugar control and disciplined and adequate wound care, as explained in the study (Septiana, 2024; Wahyudi et al., 2019).

CONCLUSION

The results of the study on the identification of *P. aeruginosa* in diabetic ulcers, found that 4 out of 8 specimens (50%) were identified as *P. aeruginosa*. The sensitivity test of *P. aeruginosa* to antibiotics showed that all isolates were still sensitive to ciprofloxacin and ceftazidime (100%).

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