IRAQI Academic Scientific Journals
العراقية المجلات الأكاديمية العلمية
ISSN:1813-1638
The Medical Journal of Tikrit University
Journal Homepage: http://mjtu.tu.edu.iq
MJTU
The Medical Journal of Tikrit University

The Prevalence of *Pseudomonas aeruginosa* and antibiotic resistance in diabetic foot ulcer patients in Erbil\Iraq

Khader Abd Khader
University of Tikrit, College of Medicine
Department of Medical Microbiology
kheder.a@st.tu.edu.iq
Alaa Zanzal Raad
University of Tikrit
College of Medicine
Department of Medical Microbiology
dr.alaa.zanzal@tu.edu.iq

Received: 02/03/2025
Revising: 03/03/2025
Proofreading: 06/05/2025
Accepted: 23/06/2025

KEY WORDS:
DFU, DFI, *Pseudomonas aeruginosa*, antibiotic susceptibility

ABSTRACT

Background: Diabetic foot ulcers (DFUs) lead to significant hospitalization costs and are a primary contributor to medical amputations of the lower limb. *Pseudomonas aeruginosa* is notably common in DFUs. The identification of the most effective antibiotics for DFUs continues to be an evolving challenge.

Methodology: Cross-sectional study was carried out on 150 patients with DFU of both sexes aged 25-85 years. From each patient, wound swab was taken and cultured on blood agar and MacConkey agar. Then positive cultures were subcultured on cetrimide agar for preliminary identification by biochemical tests. Definitive identification was done by using VITEK-2 compact system and then tested for their sensitivity toward various antimicrobials.

Results: From 150, only 53 (35.33%) were *P. aeruginosa*. The results showed high prevalence of DFUs among males than females (70% and 30%, respectively). Antibiotic susceptibility testing of *P. aeruginosa* against 11 distinct antibiotics showed that the resistance rate of carbenicillin was (100%), levofloxacin was (79.24%), piperacillin was (77.36%), ciprofloxacin was (73.58%), cefepime was (69.81%), gentamicin was (66.04%), norfloxacin was (64.15%), meropenem was (56.6%), imipenem was (45.3%), netilmicin was (37.73%) and ceftazidime was (13.2%).

Conclusion: *P. aeruginosa* was found to be prevalent in DFUs. The antibiotic ceftazidime was found to be most effective antibiotic against *P. aeruginosa* in contrast to carbenicillin, which was found to be the less effective.

Keywords: DFU, DFI, *Pseudomonas aeruginosa*, antibiotic susceptibility

DOI: http://doi.org/10.25130/mjotu.30.1.1

INTRODUCTION

Diabetes mellitus: DM stands as the most common chronic condition globally, representing a significant risk to human health[1]. Diabetes is categorized into two distinct clinical types: type I diabetes mellitus (T1DM) and type II diabetes mellitus (T2DM). The prevalence of T2DM rises with age across all regions and income levels. Nonetheless, this concern is becoming more common among youth due to unhealthy lifestyle habits that have been established since their early years. The presence of high blood sugar levels is a hallmark of diabetes, which can be caused by either inadequate insulin production (T1DM) or ineffective insulin use (T2DM). Typical symptoms include increase urination, fatigue, and wounds that are slow to heal or do not heal at all [2].

Diabetic foot ulcer (DFU): is characterized as a disturbance in the epidermis and a piece of the dermis in persons with diabetes[3]. Diabetic foot syndrome encompasses a range of conditions that are directly associated with the complications arising from chronic DM. The composition primarily includes various pathological conditions that define the diabetic foot. The conditions include foot infections, foot ulceration, diabetic peripheral neuropathy, and diabetic peripheral vasculopathy or peripheral artery disease (PAD). The progression of events leading to DFU in individuals with DM begins with lower limb peripheral neuropathy[4]. It is among the most severe and expensive consequences of DM. Ultimately, 25% of patients with diabetes will develop a foot ulcer over their lives. Predictions indicate that almost 50% of individuals with DFU experience foot infections[5].

Diabetic foot infections (DFIs): Infection plays a crucial role in driving emergency department visits and hospitalizations for individuals with diabetes, especially in cases involving DFUs. DFUs serve as a significant precursor to the majority of DFIs. The risk of infection escalates with the presence of recurrent wounds, chronic wounds, and those that reach the bone, particularly in patients who have a recent history of non-foot infections (infections in other body sites eg. Urinary tract infections)[6]. Pathogenic microorganisms are frequently isolated from these wound sites, including *Staphylococcus, Pseudomonas, Klebsiella, and Escherichia coli*. An increased predominance of *Pseudomonas* species in the deeper strata of untreated chronic wounds may result in gangrene[7]. *P. aeruginosa* is consistently associated with DFUs[8].

Pseudomonas aeruginosa: *P. aeruginosa*, a rod-shaped, aerobic, Gram-negative bacteria, thrives in many environments. This bacterium is ubiquitous and can thrive in both biotic and abiotic environments. It can withstand temperatures ranging from 4 to 42 degrees Celsius[9]. *P. aeruginosa* infections are notoriously challenging to treat due to the bacteria's inherent resistance to medications and their ability to develop resistance to numerous antibiotic classes[10].

Diabetic foot ulcer treatment: DFU therapies must employ a multidisciplinary strategy that requires extensive experience in managing the condition, involves many specialists, and incorporates various diagnostic tools[11]. The treatment strategy includes debridement, wound care, management of peripheral artery disease(PAD), targeted antibiotic therapy (specifically for *P. aeruginosa, Staphylococcus, Streptococcus, and Klebsiella*), and arterial revascularization[12]. Innovative treatments are currently in development to enhance the healing process of ulcers, providing alternatives to conventional DFU therapy. Instances of innovative treatment

approaches encompass the application of adjuvant growth factors, inflammatory modulators, herbal extracts, biological therapies, blood products, hyperbaric oxygen therapy, hazardous pressure injuries, and skin replacements. Additional treatments do not substitute for the necessity of consistent diabetic foot care[13-15].

Antibiotic resistant of *P. aeruginosa*: *P. aeruginosa* is demonstrating a growing resistance to numerous medications. Three types of antibiotics resistant exist, firstly, multidrug resistant (MDR) which exhibits resistance to three or more categories of antibiotics, including penicillins, monobactams, fluoroquinolones, cephalosporins, carbapenems, and aminoglycosides[16]. Secondly, extensive drug resistant(XDR) which exhibits resistance against all except one or two categories of antibiotics, and lastly, pan drug resistant(PDR) or totally drug-resistant(TDR) which exhibits resistance to all classes of antibiotics[17]. The mechanisms underlying antibiotic resistance in *P. aeruginosa* can be classified into two primary categories: intrinsic and acquired. "Acquired resistance" refers to the resistance that emerges through the integration of additional mechanisms or as a result of mutations that arise due to selection pressure. On the other hand, "intrinsic resistance" denotes resistance that is transmitted through a range of encoded mechanisms[18].

Aim of the study

Determine the prevalence and antibiotics susceptibility patterns of *Pseudomonas aeruginosa* isolated from diabetic foot ulcers

Objectives

1. Discuss some demographic characteristics of patients with diabetic foot ulcers, including age and sex.
2. Isolate and identify *P. aeruginosa* from diabetic foot ulcers.
3. Determine the antibiotic susceptibility of *P. aeruginosa* strains isolated from diabetic foot ulcers.
4. Assess the prevalence of *P. aeruginosa* in diabetic foot ulcers.

MATERIAL

1- Culture media and preliminary identification

This study had utilized various media and conducted several tests, including, blood agar, cetrimide agar, MacConkey agar, Muller Hinton agar, Simmon citrate agar, kligler iron agar, peptone water, indole production test, methyl red test, voges proskauer test, catalase, urease, oxidase tests and Gram stain. Media that were used were prepared in accordance with the manufacturers' specifications. Samples were taken by sterilize cotton swabs within amies transport media.

The swabs were streaked on blood agar and MacConkey agar and incubated at 37°C for 18–24 hours in an incubator, then cultures were sub-cultured on cetrimide agar and were incubated at 42°C, then Gram staining and biochemical assays were implemented to facilitate preliminary identification.

2- Definitive identification

Definitive diagnosis of *P. aeruginosa* was done by (GN-ID) with the VITEK-2 compact system. The test was conducted in accordance with the manufacturer's specifications.

3- Antimicrobial susceptibility test

In accordance with the guidelines established by the Central Laboratory Standards Institute (CLSI) in 2024, antimicrobial susceptibility tests were carried out on colonies of P. aeruginosa that had been isolated and identified. These tests were carried out using antibiotic disks that had been commercially prepared and

performed on Mueller Hinton agar plates using the disk diffusion method. Antibiotics used in our study were carbenicillin(100µg), piperacillin(100µg), levofloxacin(5µg), ciprofloxacin(10µg), norfloxacin(10µg), cefepime(30µg), ceftazidime(30µg), gentamicin(10µg), netilmicin(30µg), meropenem(10µg) and imipenem(10µg).

Inclusion and exclusion Criteria

1- **Inclusion**: Focuses on diabetic patients with clinically significant ulcers, standardized sampling, and reliable data to ensure valid isolation of *P. aeruginosa*.
2- **Exclusion**: Eliminates confounding factors (e.g., non-diabetic ulcers, recent use of antibiotics, patients with immunosuppression unrelated to diabetes) that could distort prevalence estimates.

Statistical Analysis

Statistical analysis was assessed using SPSS software version 25.

RESULTS

1-Preliminary identification: A total of 150 specimens were obtained from DFUs, including superficial and deep-seated infections of all patients, only 53 swabs showed growth on cetrimide agar and diagnosed as *P. aeruginosa* by the preliminary identification (complete hemolysis on blood agar, non-lactose fermenter on MacConkey agar, gram -ve, indole -ve, methyl red -ve, Voges Proskauer -ve, citrate +ve, catalase +ve, kligler alkaline\alkaline, H_2S -ve, urease -ve, oxidase +ve, growth at 42°C +ve and growth on cetrimide agar +ve).

(Figure 1) presents the frequency of *P. aeruginosa* isolation according to age groups. The highest frequency of *P. aeruginosa* isolation occurred in the 46-65-year age group (67.92%), followed by the 66-85-year group (26.42%), and those 25-45year group (5.66%). The study identified a correlation between DFU infections and sex. The prevalence of DFUs was greater in male patients (70%) than in female patients (30%), see (**figure 2)**.

2- Definitive identification: The 53(35.33%) isolates were diagnosed definitively as being *P. aeruginosa* by VITEK-2 compact system. See **(figure 3).**

3- Susceptibility: *P. aeruginosa* isolates were resistant to carbenicillin (100%), levofloxacin (79.24%), piperacillin (77.36%), ciprofloxacin (73.58%), cefepime (69.81%), gentamicin (66.04%), norfloxacin (64.15%), meropenem (56.6%), imipenem (45.3%), netilmicin (37.73%) and ceftazidime (13.2%); see **(figure 4)**.

DISCUSSION

Foot ulceration represents the most severe complication of DM. Uncontrolled diabetes or inadequate health self-care can lead to DFU[19]. DFIs frequently occur in individuals with DFUs and represent the leading cause of nontraumatic amputation, hospitalization, and a decline in quality of life for those affected by DFU. "*P. aeruginosa*" is recognized as a frequently isolated bacterium from clinical specimens, presenting a considerable challenge in the treatment of both nosocomial and community-acquired infections. Identifying and selecting an appropriate antimicrobial agent to initiate therapy is essential for optimizing clinical outcomes[20]. The significant and widespread antibiotic resistance of *P. aeruginosa* has limited treatment options[18]. The purpose of this cross-sectional study is to explore the prevalence and antibiotic susceptibility patterns of *P. aeruginosa* isolated from DFU patients. In this study, from 150 DFUs, 53 isolates of *P. aeruginosa* were collected; the prevalence of *P. aeruginosa* was 35.33%. The closest prevalence of *P. aeruginosa* to our study, to our knowledge, was found in Al-Najaf City, Iraq (34.4%)[21], Abadan City, Iran 30.4%[22]. Less percentage of prevalence were recorded in Hila City, Iraq

24%[23], in Sulaimani City, Iraq 8.5%[24], and in Syria 13%[25].

In our present study, the prevalence of DFUs was found to be high in the male patients, more than females (70% and 30%; respectively). Nearly similar percentage were documented in various studies carried out in countries neighboring Iraq, including Kuwait (66.9% male, 33.1% female)[26] and Syria (63% male, 37% female). In Sulaimani City, Iraq (56.5% male, 43.5%female). Men are more likely than women to develop DFUs due to a higher prevalence of peripheral neuropathy, peripheral artery disease (PAD), and cardiovascular disease among men with diabetes. Also, women exhibit a higher likelihood of engaging in recommended self-care and foot care practices[27].

In this study a high prevalence of *P. aeruginosa* was found in the 46-65 age group (67.92%), followed by the 66-85 age group (26.42%) and the 25-45 age group (5.66%). A study in Al-Najaf, Iraq, had showed the highest prevalence was recorded in the (50-59 year) age group (39.2%)[21].

In the current study, *P. aeruginosa* had showed sensitivity to ceftazidime (83%), netilmicin (60%), imipenem (43%), and meropenem (42%). A previous study had carried out in Coimbatore, India showed sensitivity to imipenem (33.3%)[28], A study carried in Sulaimani City, Iraq, had showed the sensitivity patterns was imipenem (100%), meropenem (90.2%), and 52.9 % for ceftazidime[24]. In Syria a previous study had showed sensitivity of imipenem (100%)[25]. The variability in results from numerous studies suggests that microbial infection patterns in patients with DFUs are inconsistent. Therefore, repeated assessments of microbial characteristics and antibiotic sensitivity are crucial for the proper selection of antibiotics.

CONCLUSION

P. aeruginosa was found to be prevalent in DFUs. The antibiotic ceftazidime was found to be most effective antibiotic against *P. aeruginosa* in contrast to carbenicillin, levofloxacin, piperacillin, ciprofloxacin, cefepime, gentamicin and norfloxacin antibiotics which were found to be the less effective.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by the author.

ACKNOWLEDGEMENTS

American medical complex \ Erbil

REFERENCES

1. Al-Khashmani AJK, Salman AA, Raad AZ. Epidemiology of asymptomatic bacteriuria among diabetic patients in Tikrit City, Iraq. Iraq Medical Journal. 2021;5(3):94-7.
2. Yameny AA. Diabetes Mellitus Overview 2024. Journal of Bioscience and Applied Research. 2024;10(3):641-5.
3. Van Netten JJ, Bus SA, Apelqvist J, Lipsky BA, Hinchliffe RJ, Game F, et al. Definitions and criteria for diabetic foot disease. Diabetes/metabolism research and reviews. 2020;36:e3268.
4. Tuttolomondo A, Maida C, Pinto A. Diabetic foot syndrome: Immune-inflammatory features as possible cardiovascular markers in diabetes. World journal of orthopedics. 2015;6(1):62.
5. Hurlow JJ, Humphreys GJ, Bowling FL, McBain AJ. Diabetic foot infection: A critical complication. International wound journal. 2018;15(5):814-21.
6. Fang M, Hu J, Matsushita K, Selvin E, Hicks CW. Abstract MP10: Risk factors for diabetic foot infection: the Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 2022;145(Suppl_1):AMP10-AMP.
7. Srivastava P, Sivashanmugam K. Combinatorial drug therapy for

controlling Pseudomonas aeruginosa and its association with chronic condition of diabetic foot ulcer. The International Journal of Lower Extremity Wounds. 2020;19(1):7-20.
8. Srivastava P, Sivashanmugam K. Efficacy of sub-MIC level of meropenem and ciprofloxacin against extensive drug-resistant (XDR) Pseudomonas aeruginosa isolates of diabetic foot ulcer patients. Infection, Genetics and Evolution. 2021;92:104824.
9. Behzadi P, Baráth Z, Gajdács M. It's not easy being green: a narrative review on the microbiology, virulence and therapeutic prospects of multidrug-resistant Pseudomonas aeruginosa. Antibiotics. 2021;10(1):42.
10. Mahgoub FM. Detection of Exotoxin a Gene in Pseudomonas aeruginosa Strains Isolated from Burn Infections in Tanta University Hospital. Egyptian Journal of Medical Microbiology. 2022;31(2):7-11.
11. Ruke M, Savai J. Diabetic foot infection, biofilm & new management strategy. Diabetes Research: Open Access. 2019;2019(1):7.
12. di Martino E, Nelson EA, Nixon JE, Russell DA, Goodman AL, Mehta SR, et al. Clinical perspectives on sampling and processing approaches for the management of infection in diabetic foot ulceration: A qualitative study. International Wound Journal. 2024;21(6):e14912.
13. Ahmed A, Getti G, Boateng J. Ciprofloxacin-loaded calcium alginate wafers prepared by freeze-drying technique for potential healing of chronic diabetic foot ulcers. Drug Delivery and Translational Research. 2018;8:1751-68.
14. Gasca-Lozano LE, Lucano-Landeros S, Ruiz-Mercado H, Salazar-Montes A, Sandoval-Rodríguez A, Garcia-Bañuelos J, et al. Pirfenidone accelerates wound healing in chronic diabetic foot ulcers: a randomized, double-blind controlled trial. Journal of diabetes research. 2017;2017(1):3159798.
15. Ram M, Singh V, Kumawat S, Kumar D, Lingaraju MC, Singh TU, et al. Deferoxamine modulates cytokines and growth factors to accelerate cutaneous wound healing in diabetic rats. European Journal of Pharmacology. 2015;764:9-21.
16. Aboelseoud H, Ismael E, Moustafa G, Badawy E. Hygienic studies on biofilms in drinking water systems in poultry farms: Isolation, molecular identification, and antibiotic sensitivity. J Anim Health Prod. 2021;9(4):443-54.
17. Pachori P, Gothalwal R, Gandhi P. Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes & diseases. 2019;6(2):109-19.
18. Pang Z, Raudonis R, Glick BR, Lin T-J, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnology advances. 2019;37(1):177-92.
19. Mendes J, Marques-Costa A, Vilela C, Neves J, Candeias N, Cavaco-Silva P, et al. Clinical and bacteriological survey of diabetic foot infections in Lisbon. Diabetes research and clinical practice. 2012;95(1):153-61.
20. Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH. Pseudomonas aeruginosa bloodstream infection: importance of appropriate initial antimicrobial treatment. Antimicrobial agents and chemotherapy. 2005;49(4):1306-11.
21. Alkhudhairy MK, Al-Shammari MMM. Prevalence of metallo-β-lactamase–producing Pseudomonas aeruginosa isolated from diabetic foot infections in Iraq. New microbes and new infections. 2020;35:100661.

22. Rezazadeh M, Hajian F, Radmanesh E, Maghsoudi F, Hazbenejad A, Mobarak S. Investigation of the Most Common Bacterial Strains in Diabetic Foot Ulcer Patients. Avicenna Journal of Clinical Microbiology and Infection. 2023;10(2):70-4.
23. Abbas SK, Omran DG, Abdulazeem L. Bacterial Infections and Inflammatory Markers in Diabetic Foot Ulcers: Assessing the Roles of Staphylococcus aureus, Pseudomonas aeruginosa, Vascular Endothelial Growth Factor, and Interleukin-6. Medical Journal of Babylon. 2024;21(2):330-6.
24. Qadir AN, Mahmoud BM, Mahwi TO, Al-Attar DMRA, Mahmood SO. Prevalence of microorganisms and antibiotic sensitivity among patients with diabetic foot ulcer in Sulaimani City, Iraq. Hospital Practices and Research. 2020;5(2):56-63.
25. Al Abbas LA, Khaddam W, Shreibati F. INVESTIGATING BACTERIA ISOLATED FROM DIABETIC FOOT ULCERS AND STUDYING THEIR SENSITIVITY TO ANTIBIOTICS–SYRIA. Bulletin of Pharmaceutical Sciences Assiut. 2022;45(1):451-8.
26. Alhubail A, Sewify M, Messenger G, Masoetsa R, Hussain I, Nair S, et al. Microbiological profile of diabetic foot ulcers in Kuwait. Plos one. 2020;15(12):e0244306.
27. McDermott K, Fang M, Boulton AJ, Selvin E, Hicks CW. Etiology, epidemiology, and disparities in the burden of diabetic foot ulcers. Diabetes Care. 2023;46(1):209-21.
28. Sivanmaliappan TS, Sevanan M. Antimicrobial susceptibility patterns of Pseudomonas aeruginosa from diabetes patients with foot ulcers. International journal of microbiology. 2011;2011(1):605195.

Figure 2: The frequency of DFUS in relation to the sex

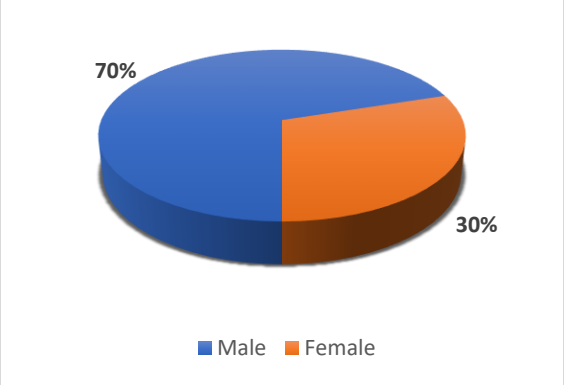

Figure 3: the prevalence of *P. aeruginosa* among DFU patients

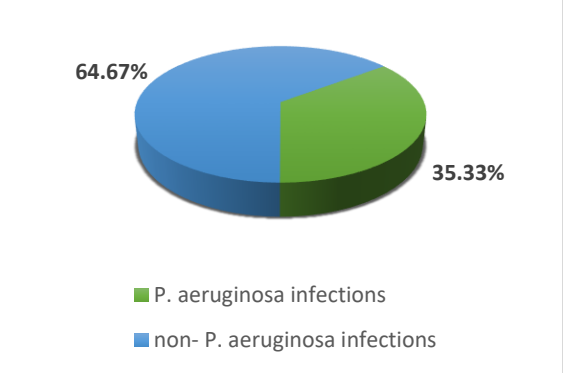

Figure 4: Susceptibility pattern of isolated *P. aeruginosa* from DFUs