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Antibiotic Susceptibility Profile of Diabetic Foot Infection Pathogens in Enugu, Nigeria

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Abstract: Diabetic foot infection is a serious complication associated with diabetes. The condition affects the quality of life in patients of all classes, races, and ages. If left untreated, may lead to gangrene, osteomyelitis, and amputation. This study was conducted to ascertain the antibiotic susceptibility profiles of bacteria from diabetic foot infections (DFIs) in Enugu. Demographical and clinical history data were gathered using structured questionnaire. Diabetic wound samples were collected and analysed using standard microbiological procedures. Isolation and identification of bacterial species were based on cultural morphology, microscopy, and biochemical tests. Antibiotic susceptibility was assessed on eight antibiotic agents: amoxycillin/clavulanic acid, nitrofurantoin, cefoxitin, imipenem, gentamicin, ciprofloxacin, colistin sulphate, and cotrimoxazole following the Kirby-Bauer disc diffusion technique and CLSI guidelines. The majority were male (63%), aged 51-60 (26%), with diabetes of more than 10 years (43%). Positive polymicrobial bacterial growth was recorded in 100% (51/51) of the samples. A total of 126 bacteria colonies were isolated and identified as *Staphylococcus aureus* 30% (38/126), *Staphylococcus epidermidis* 19% (13/125), *Pseudomonas* spp. 25% (31/126), *Escherichia coli* 21% (27/126), *Klebsiella* spp. 9% (11/126) and *Proteus vulgaris* 5% (6/126). *Pseudomonas* records remarkable resistance (94%) to amoxycillin/clavulanic acid and (68%) cotrimoxazole. Virtually all bacteria species were resistant to colistin sulphate but showed considerable sensitivity to imipenem. Overall, amoxycillin/clavulanic acid had the highest resistance (82.5%), followed by colistin sulphate (73.8%), given a MAR index range of 0.1 – 0.7. The results revealed an increasing risk of antibiotic resistance and emphasized the importance of antimicrobial stewardship and culture-guided antibiotic prescription practices in the management of DFIs.

Keyworks: *Diabetes, Diabetic Foot Infection, Antibiotic Resistance, Foot Ulcer, Polymicrobial.*

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I. INTRODUCTION

Diabetes Mellitus (DM) has emerged as one of the most significant global health emergencies in recent times, whose prevalence is estimated to reach 850 million by 2045, with middle- and low-income countries, particularly in Sub-Saharan Africa, being more at risk (Genitsaridi et al., 2026). It hinders the achievement of Sustainable Development Goal 3, good health and well-being, as the burden is worsened by changing lifestyles, urbanization, and a high rate of undiagnosed cases. Poorly attended DM gives rise to complications, of which diabetic foot infections (DFIs) remain the most challenging. DFIs represent a significant complication of diabetes mellitus, often leading to prolonged hospitalization, increased healthcare costs, and a higher risk of lower limb amputations if not managed effectively (Govindaswamy et al., 2025).

DFIs typically originate from a foot ulcer, which is a consequence of peripheral neuropathy and peripheral arterial disease, including poor circulation and impaired immunity. Often associated with unnoticed trauma, poor healing, and then infection (Li et al., 2025; Zambelli et al., 2025). The diabetic foot ulcers are estimated to affect 10–15% of individuals with diabetes, and about half of the ulcers are found to be clinically infected when initially diagnosed (Genitsaridi et al., 2026). The ulcers provide a portal of entry for various microorganisms (from other parts of the body and the environment), giving a multifaceted polymicrobial synergy of Gram-positive and Gram-negative, aerobic and anaerobic organisms. There exist some slight regional variations in pathogen distribution among isolates from DFI. Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* were more prevalent in low-resource settings in tropical and subtropical regions, while Gram-positive bacteria like *Staphylococcus aureus* remain predominant in other geographical settings (Goh et al., 2020; Tarigan et al., 2025).

The polymicrobial nature of the DFIs complicates treatment plans and demands accurate microbiological identification and antibiotic susceptibility profiling to guide appropriate antimicrobial therapy (Aamer et al., 2025; Coskun et al., 2024), especially in the face of growing antimicrobial resistance. The increasing incidence of multidrug-resistant (MDR) strains among isolates from DFIs has further complicated therapeutic choices by reducing the effectiveness of standard antibiotics and limiting treatment options. For instance, a meta-analysis study involving 4,885 patients with DFIs revealed a high prevalence (51%) of MDR bacteria isolates, with a prevalence of 33% and 20% among Gram-negative bacteria and Gram-positive bacteria, respectively (Yang et al., 2024). In the study, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp. *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Enterococcus* spp. were the prevalent bacteria species found to be resistant against more than three classes of antibiotics. The identified predisposing factors to acquiring multiple drug resistance include prior antibiotic use, previous hospitalization, higher Wagner grade, osteomyelitis, peripheral vascular disease/neuropathy, and neuro-ischemic wounds (Guo et al., 2023; Yan et al., 2022). Once bacteria from DFI become MDR, treatment becomes complicated with longer hospital stays and increased risk of amputation and death.

The rising prevalence of MDR organisms in DFI emphasizes the critical need for continuous surveillance of local epidemiological data to inform empirical treatment regimens (Aamer et al., 2025; Zambelli et al., 2025). In Nigeria, specifically in urban areas like Enugu, the management of DFIs faces a dual crisis: the high virulence of infecting organisms and the rising phenomenon of antimicrobial resistance (AMR) (Agbo et al., 2025; Ugwu et al., 2024). Further, late presentation at tertiary health institutions, coupled with repeated attempts at self-medication or patronizing traditional healers, complicates the management of DFI. The delay, coupled with the indiscriminate use of over-the-counter prescribed antibiotics, has likely altered the susceptibility profiles of local bacterial isolates, making standard treatment protocols less effective and increasing the risk of treatment failure. In several recent studies from diverse geographical settings, the bacterial profile of DFI showed the prevalence of multiple drug-resistant bacteria species (Chaves et al., 2025; Govindaswamy et al., 2025; Namatovu et al., 2025; Zambelli et al., 2025), including methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae (Matta-Gutierrez et al., 2021). Thereby buttressing the need for continuous monitoring of regional microbiological profiles and resistance patterns of isolates. Which is essential to guide practical therapy options, alleviate the burden of treatment failure due to antibiotic resistance, and mitigate the incidence of avoidable amputation.

This study aims to bridge this knowledge gap by investigating the antibiotic susceptibility of current bacterial pathogens of DFIs among patients attending selected tertiary hospitals in Enugu, Nigeria. The primary objective is to isolate and identify the specific aerobic bacteria associated with these infections and to determine their antibiotic susceptibility profiles against commonly used antimicrobial agents. By providing a localized antibiogram, this research seeks to improve clinical outcomes, promote antibiotic stewardship, and provide baseline data on the trend of bacterial resistance in the region.

II. MATERIAL AND METHODS

- *Study Design and Study Area*

The study took a cross-sectional study design, conducted at the surgical outpatient clinic and endocrinology department of Enugu State University Teaching Hospital (ESUTH) Parklane, Enugu, from November 2022 to January 2023. ESUTH is a state-owned tertiary health institution located in the Government Reserved Area (GRA), Parklane, Enugu, Nigeria. It serves as a major teaching and referral hospital, offering a wide range of general and specialised medical services and clinical training to medical and health science students. The hospital has a long history of healthcare delivery, evolving over decades into a full-fledged teaching

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hospital that provides outpatient care, inpatient services, emergency care, specialist consultations, surgical procedures, and training for healthcare professionals. The hospital is equipped with qualified medical staff and modern facilities to serve the health needs of residents of Enugu State and beyond.

Laboratory analysis were conducted in the Pharmaceutical Microbiology Laboratory of the Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology.

- *Ethical Approval*

Ethical clearance for the study was obtained from the office of the Chairman, Medical Advisory Committee ESUTH, with reference number ESUTHP/C-MAC/RA/034/Vol 31166. In addition, a letter of approval and authorization to interact with and obtain samples from patients was issued before the study commenced.

- *Inclusion and Exclusion Criteria*

All confirmed diabetic patients (type 1 or type 2 DM) who presented at the hospital with a clinical sign of foot ulcer gave informed consent. Recruited patients who were more than eighteen years old were included in the study, irrespective of having started antibiotic therapy or not. Exempted categories include patients that were pregnant, less than eighteen years old, had serious traumatic ulcers, or were critically ill and were unable to communicate throughout the study period. In all, a total of fifty-one (51) patients were included in this study.

- *Data Collection*

Prior to data collection, the purpose of the study, including risks and benefits, was narrated to concerned stakeholders. The data collection process began after written informed consent was obtained from all the eligible patients. To ensure confidentiality, names and other identifiers of patients and health care professionals were not recorded on the data collection tools.

Relevant data pertaining to demographic characteristics (age, sex, ethnicity, and marital status) and clinical history, such as duration of diabetes, antibiotic regime, and past history of diabetic foot infections, were collected through the use of a well-structure questionnaire. Care was taken to ensure that data was collected from a patient only once during the study duration.

- *Sample Collection and Bacterial Isolation*

Wound swab samples were collected following standard procedures, after cleaning and debridement. Collected samples were promptly transported to the laboratory and processed within 4 hours of collection. Bacterial isolation was achieved by inoculation on Blood Agar (BA), Mannitol Salt Agar (MSA) (Oxoid, Hampshire, UK), and MacConkey Agar (MA) (Oxoid, Hampshire, UK), respectively. Inoculated culture plates were incubated at 37 °C for 24 hours, after which colony morphology was observed and recorded. Pure cultures were obtained by repeated streaking on respective media.

- *Bacterial Characterization and Identification*

Identification was based on conventional microbiological characterization, according to descriptions in the Bergey's Manual of Determinative Bacteriology.

Pure cultures were characterized based on morphological cultural characteristics and reaction to Gram staining and microscopy. Biochemical tests, including catalase, coagulase, indole, oxidase, and citrate test, were determined as described in Ozochi et al., (2025) to aid the identification of the isolates. In addition, presumptive colonies were grown on specific selective and differential medium to support further identification. Mannitol agar for *Staphylococcus*, cetrimide agar for *Pseudomonas*, and eosine methyl blue agar for *Escherichia coli*.

- *Catalase Test*

Fresh bacterial colony was picked using a sterile glass rod and immersed into hydrogen peroxide solution in a test tube. The presence of effervescence indicated a positive reaction, whereas no effervescence showed a negative reaction.

- *Coagulase Test*

The slide test procedure was used to detect bound coagulase (clumping factor). Firstly, a drop of plasma was placed on a clean microscopic slide. Then, a loop of the bacterial colony was picked and emulsified on a drop of plasma. The presence of visible clumping within 10 to 15 seconds indicates a positive result, while the absence of clumping after 20 seconds indicates a negative result.

- *Indole Test*

A 5 ml sterile tryptophan broth (Merck-KGaA, Germany) was prepared in a test tube according to the manufacturer's instructions. The bacterial colony was aseptically inoculated into the sterile medium and incubated for 24 hours at 35 °C. After incubation, 0.5 ml of Kovac's reagent was added and allowed to stand for 4 minutes. The formation of a red or pink ring layer on the medium within 10 minutes indicates a positive result. While the absence of a red or pink ring layer on the medium after 10 minutes signifies a negative result.

- *Oxidase Test*

The oxidase test followed the filter paper technique. Using a sterile pin, a bacterial colony was picked and smeared on filter paper, previously moistened with a few drops of oxidase reagent (Merck, Germany). A colour change to dark purple or blue within 15 seconds signifies a positive result, while no colour change indicates a negative result.

- *Citrate Test*

Bacterial colony was inoculated on the surface of a sterile Simon Citrate Agar slant and incubated at room temperature for 24 hours. Visible bacterial growth, as well as a change in the colour of the medium to blue indicates a positive result. The absence of visible growth and no change in the colour of the medium indicate negative results.

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➢ *Antibiotic Susceptibility Testing*

Antibiotic susceptibility profiling of bacterial isolates was determined in triplicate using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) (Oxoid, Hampshire, UK), following the principles of the Clinical Laboratory Standard Institute (CLSI, 2024). Eight broad- and narrow-spectrum antibiotics (amoxycillin/clavulanic acid (20 µg), nitrofurantoin (300 µg), cefoxitin (30 µg), imipenem (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), colistin sulphate (10 µg), and cotrimoxazole (25 µg)) representing eight classes (CLSI, 2024), were selected for the study based on frequently recommended and prescribed and easy-to-purchase over the-counter antibiotics.

Plates of MHA were prepared based on the manufacturer's instructions and dried in an oven to remove surface moisture. A standardized inoculum (bacteria suspension adjusted to 0.5 McFarland standards) was prepared using sterile normal saline and a fresh, 18-hours-old culture of each bacterial colony. Thereafter, the MHA plates were seeded with the standardized inoculum using a sterile swab stick (CLSI, 2024). The inoculum was allowed to stand for about 5 minutes before the antibiotic discs were impregnated firmly onto the inoculum surface using sterile forceps. The plates were incubated at 37 °C for 24 hours. Zones of growth inhibition around each antibiotic disc were then measured using a metric ruler and approximated to the nearest whole millimetre (mm). Bacterial classification as sensitive (S), intermediate (I), or resistant (R) was based on the interpretative criteria for the bacterial species and antibiotic type (CLSI, 2024). Bacterial isolates that fall under the "resistant" and "intermediate" classifications are categorized as being resistant to the antibiotic, and isolates that fall into the "sensitive" classification are regarded as susceptible to the antibiotic (CLIS, 2024; Ozochi et al., 2025). *E. coli* ATCC 25922 was used as the quality control strain.

Multiple antibiotic resistance (MAR) index was evaluated for each isolate using the formula,

$$MAR\ index = {}^{a}/_{b}$$

where "a" represents the number of antibiotics to which the test isolate depicted resistance and "b" represents the total number of antibiotics to which the test isolate has been assessed for susceptibility (Ozochi et al., 2025).

➢ *Data Analysis*

All data analysis were performed using IBM SPSS Version 23. Descriptive statistical analysis was performed for variables obtained from questionnaire, such as socio-demographic characteristics, clinical factors, neuropathy, and foot self-care practice. A linear logistic regression was performed to identify factors associated with the outcome variable. A crude and adjusted odds ratio with their 95% CI was estimated to determine the strength of association. A p-value less than 0.05 in the analysis was considered to declare statistical significance.

III. RESULTS

The antibiotic susceptibility profile of bacteria isolated from diabetic foot infection in Enugu, South East Nigeria, was assessed to provide current baseline data on essential therapeutic options in the management of DFI. Important data on demographical and diabetic clinical history were obtained. While isolated bacterial species were screened for susceptibility to eight commonly used antibiotic agents.

➢ *Demographical Characteristics*

Demographic information of the diabetic foot infection patient, including sex, age range, marital status, ethnicity, educational attainment, and occupation, was analysed and presented in Table 1. The study recorded the participation of 51 patients, belonging to the male (63%) and female (37%) genders. The age range 18 to 30 had zero respondents, while the age range 51 to 60 recorded the highest number (25.5%) of respondents. Closely followed was the age range 61 to 70 (21.6%) and 40 to 50 (19.6%). There was seemingly no difference in the number of respondents that attained the major educational levels: primary, secondary, and tertiary education.

➢ *Diabetic Clinical History*

The diabetic clinical history of patients with DFI was obtained using a structured questionnaire, analysed, and presented in Table 2. The results indicate that prevalence of those who had DM for more than 10 years was the highest (43.1%), followed by those who had DM for between 5 and 10 years (35.3%). The least prevalent were respondents with a DM of less than 5 years (21.6%). Eight-eight per cent (88.2%) of the patients with DFI responded "yes" to having a family history of diabetes. All respondents (100%) had their blood sugar regulated and currently are undergoing antibiotic therapy, with ciprofloxacin (76.5%) serving as the most prescribed and used antibiotic agent. Despite being on antibiotic therapy, 66.7% reported "no" to improvement in the wound since therapy. Fortunately, only 23.5% had a previous history of amputation (Table 2).

Table 1: Demographical Characteristics of Diabetic Foot Infection Patient.

Item Questions	**Response Distribution (N = 51)**	
Response options	**Number**	**Percentage**
Sex		
Male	32	62.7
Female	19	37.3
Age range		
18 – 30	0	0
31 – 40	7	13.7

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41 – 50	10	19.6
51 – 60	13	25.5
61 - 70	11	21.6
71 – 80	8	15.7
Above 80	2	3.9
Marital status		
Single	4	7.8
Married	29	56.9
Divorced	3	5.9
Separated	7	13.7
Widowed	8	15.7
Ethnic group		
Igbo	48	94.2
Hausa	0	0
Yoruba	1	1.9
Other	2	3.9
Highest educational status		
None	3	5.9
Primary school	11	21.6
Junior secondary school	2	3.9
Senior secondary school	17	33.3
College of education	2	3.9
University	12	23.6
Polytechnic	4	7.8
Others	0	0
Occupational status		
Unemployed	11	21.6
Self employed	11	21.6
Private employed	8	15.7
Government worker	14	27.4
Artisan	7	13.7

Table 2: Diabetic Clinical History of DFI Patients

Item Questions	**Response Distribution (N = 51)**	
Response options	**Number**	**Percentage**
Duration of Diabetes		
Less than 5years	11	21.6
5 - 10 years	18	35.3
Above 10 years	22	43.1
Diabetics in family history		
Yes	45	88.2
No	6	11.8
Blood sugar regulation		
Yes	51	100
No	0	0
“Yes” to signs of neuropathy		
Does your feet get hot?	43	84.3
Do you have tingling sensations in your feet?	50	98.2
Do you notice foul smell in the wound?	42	82.4
Do you clean the surface of the wound?	51	100
Has the wound gotten worse since it started?	16	31.3
Duration of DFI		
Less than 6 months	10	19.6
6 - 12 months	9	17.6
12 - 18 months	6	11.8
18 - 24 months	8	15.7
24 - 36 months	8	15.7
Above 36 months	10	19.6

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Wagner classification of ulcer		
Wagner Grade 0	0	0
Wagner Grade 1	28	54.9
Wagner Grade 2	20	39.2
Wagner Grade 3	2	3.9
Wagner Grade 4	1	2.0
Wagner Grade 5	0	0
Already on antibiotic therapy		
Yes	51	100
No	0	0
Antibiotic prescription/administration advance		
Ciprofloxacin	39	76.5
Metronidazole	7	13.7
Erythromycin	3	5.9
Gentamicin	2	3.9
Improvement in wound since therapy		
Yes	17	33.3
No	34	66.7
Any previous amputation		
Yes	12	23.5
No	39	76.5

➢ *Bacterial Characterization*

All 51 (100%) of the DFI wound samples collected in this study were culture positive and showed the polymicrobial nature of bacterial growth. Both Gram-positive and Gram-negative bacterial were detected in this study. The identification of the isolated colony followed the conventional microbiological procedure: cultural, microscopic, and biochemical characteristics as shown in Table 3, below. The identified bacterial species include *Staphylococcus aureus, Pseudomonas* spp., *Escherichia coli, Klebsiella* spp., *Proteus vulgaris* and *Staphylococcus epidermidis.* A total of 126 colonies from the 51 studied samples were randomly isolated and used for antibiotic susceptibility profiling. The bacterial species and frequency of occurrence are presented in Table 4. *Staphylococcus aureus* had the highest percentage frequency occurrence, followed by *Pseudomonas* spp. (Table 4).

Table 4: Percentage Occurrence of Bacterial Species Isolated from DFI

Bacterial Specie	**Number of Isolates**	**Percentage Frequency (%)**
Staphylococcus aureus	38	30.2
Staphylococcus epidermidis	13	10.3
Gram positive _Total	**51**	**40.5**
Pseudomonas spp.	31	24.6
Escherichia coli	27	21.4
Klebsiella spp.	11	8.7
Proteus vulgaris	6	4.8
Gram negative _Total	**75**	**59.5**

➢ *Antibiotic Susceptibility Profiling*

The assessment of the antibiotic susceptibility pattern of the 126 bacterial isolates was investigated against eight commonly prescribed antibiotics. The result as presented in Table 5 showed that the bacterial species differ in the class of susceptibility (sensitive, intermediate, and resistant) to each antibiotic. For instance, more than 90% of *Staphylococcus (*both *S. aureus* and *S. epidermidis)* in this study were resistant to colistin sulphate, but more than 70% were sensitive to imipenem, gentamicin, cotrimoxazole, ciprofloxacin, cefoxitin, and nitrofurantoin. In the same vein, 100% of *Proteus vulgaris* in this study were resistant to colistin sulphate and cefoxitin, respectively, but 100% were sensitive to imipenem and gentamicin (Table 5).

An evaluation for drug resistance and susceptibility indicates that 82% (104/126) of the isolates in this study were resistant to amoxycillin/clavulanic acid, while 86% (109/126) of the isolates were susceptible to imipenem (Table 6). The MAR index ranged from 0.1 to 0.7, with the most common resistance pattern shown in Table 7.

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Table 3: Bacterial Characterization and Identification of Bacterial Isolates from DFI

Isolates type ID	Cultural characteristics	Microscopic characteristics/Gram reaction	Biochemical Test					Bacterial specie
			Cat	Coa	Ind	Oxi	Cit	
WSI_1	Golden-yellow, large and raised colonies	Gram +ve irregular cocci in clusters	+ve	+ve	-ve	-ve	-ve	*Staphylococcus aureus*
WSI_2	Golden-yellow, large and raised colonies	Gram +ve irregular cocci in clusters	+ve	-ve	-ve	-ve	-ve	*Staphylococcus epidermidis*
WSI_3	Large mucoid, dark pink colonies on MA	Gram -ve short rod in pairs	+ve	-ve	-ve	-ve	+ve	*Klebsiella spp.*
WSI_4	Bright pink, smooth colonies on MA, with metallic green sheen on EMB	Gram -ve short rod in pairs	+ve	+ve	+ve	-ve	-ve	*Escherichia coli*
WSI_5	Pigmented flat-edged smooth colonies on agar	Gram -ve slender single rods, often straight or curved	+ve	-ve	-ve	+ve	-ve	*Pseudomonas spp.*
WSI_6	Small circular, pale opaque colonies on MA	Gram -ve rod	+ve	-ve	+ve	-ve	+ve	*Proteus vulgaris*

KEY: Cat = Catalase test, Coa = Coagulase test, Ind = Indole test, Oxi = Oxidase test, Cit = Citrate Test

Table 5: Antibiotic susceptibility profile of bacteria isolates from DFI

Bacterial Isolate	Classification	Antibiotics Number of isolate and percentage (%)							
		AMC	F	FOX	IPM	CN	CIP	CT	COT
S. aureus (n=38)	S	9(24)	27(71)	27(71)	34(89)	29(76)	31(82)	2(5)	31(82)
	I	22(58)	8(21)	10(26)	4(11)	4(11)	7(18)	1(3)	7(18)
	R	7(18)	3(8)	1(3)	0(0)	5(13)	0(0)	35(92)	0(0)
Pseudomonas spp. (n=31)	S	1(3)	19(61)	19(61)	22(71)	22(71)	29(94)	28(90)	7(23)
	I	1(3)	7(23)	8(26)	8(26)	4(13)	1(3)	2(7)	3(9)
	R	29(94)	5(16)	4(13)	1(3)	5(16)	1(3)	1(3)	21(68)
Escherichia coli (n=27)	S	6(22)	2(7)	16(59)	23(85)	2(7)	4(15)	2(7)	2(7)
	I	7(26)	3(11)	8(30)	1(4)	6(22)	7(26)	3(11)	4(15)
	R	14(52)	22(82)	3(11)	3(11)	19(71)	16(59)	22(82)	21(78)
Klebsiella spp. (n=11)	S	0(0)	9(82)	11(100)	11(100)	10(91)	3(27)	1(9)	10(91)
	I	0(0)	2(18)	0(0)	0(0)	1(9)	1(9)	1(9)	1(9)
	R	11(100)	0(0)	0(0)	0(0)	0(0)	7(64)	9(82)	(0)
Proteus vulgaris (n=6)	S	3(50)	1(17)	0(0)	6(100)	6(100)	1(17)	0(0)	1(17)
	I	3(50)	0(0)	0(0)	0(0)	0(0)	1(17)	0(0)	3(50)
	R	0(0)	5(83)	6(100)	0(0)	0(0)	4(67)	6(100)	2(33)
S. epidermidis (n=13)	S	3(23)	12(92)	12(92)	13(100)	13(100)	10(77)	0(0)	11(85)
	I	1(8)	1(8)	0(0)	0(0)	0(0)	3(23)	0(0)	2(15)
	R	9(69)	0(0)	1(8)	0(0)	0(0)	0(0)	13(100)	0(0)

[**Key**: **S**-susceptible, **I**-Intermediate, **R**-resistant, **AMC**- Amoxycillin/Clavulanic acid, **F**- Nitrofurantoin, **FOX**- Cefoxitin, **IPM**- Imipenem, **CN**- Gentamicin, **CIP**- Ciprofloxacin, **CT**- Colistin Sulphate, and **COT**- Cotrimoxazole]

Table 6: Antibiotic Resistance and Susceptibility Appraisal of Isolates from DFI

Antibiotic Class	Antibiotic agent	Disc code (Potency)	No. (%) of resistant isolates (n=126)	No. (%) of susceptible isolates (n=126)
β-lactam	Amoxycillin/clavulanic	AMC (20 µg)	104(82.5)	22 (17.5)
Nitrofurans	Nitrofurantoin	F (300 µg)	56(44.4)	70 (55.6)
Cephems	Cefoxitin	FOX (30 µg)	41(32.5)	85 (67.5)
Carbapenems	Imipenem	IPM (10 µg)	17(13.5)	109 (86.5)
Aminoglycosides	Gentamicin	CN (10 µg)	44(34.9)	82 (65.1)
Quinolones	Ciprofloxacin	CIP (5 µg)	48(38.1)	78 (61.9)
Lipopeptides	Colistin sulphate	CT (10 µg)	93(73.8)	33 (26.2)

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Folate pathway inhibitors	Cotrimoxazole	COT (25 µg)	64(50.8)	62 (49.2)

Table 7: Multiple Antibiotic Resistance Index and Common Resistance Pattern of Isolates from DFI

MARI	Common Antibiotic Resistance Pattern
0.7	AMC, CT, CIP, F, CN, COT
0.5	FOX, CT, F, CIP
0.4	AMC, CT, CIP
0.2	AMC, CT
0.2	AMC, COT
0.1	CT

IV. DISCUSSION

Diabetic foot infection is an increasing problem worldwide. It occurs when microorganisms gain entry to the wound on the foot of a diabetic patient. DFI is one of the most serious and devastating complications of diabetes mellitus (DM), requiring hospitalization. DFI is found to be present in more than half of all new diabetic wounds at the time of hospital admission (Genitsaridi et al., 2026).

In this study, the demographical characteristics of 51 DFI patients revealed a predominance of male patients (62.7%) compared to females (37.3%). The finding aligns with numerous current studies (Aamer et al., 2025; Govindaswamy et al., 2025; Ismayıl et al., 2025; Ugwu et al., 2024; Zambelli et al., 2025), suggesting that males are more likely to engage in activities that predispose them to foot ulcers, including delaying seeking medical attention and poor foot care practices. It is not surprising that the majority of the patients were aged 51–60 years (25.5%), followed by 61–70 years (21.6%) and then 41–50 years (19.6%). No cases were recorded among people aged 18–30 years, confirming that the risk of DFI increases with age and longer duration of diabetes. Contrary to other findings (Karimi et al., 2025; Tola et al., 2021), which reported stronger social support systems and lower risk of DFI among married people, this study observed that more than half of the DFI patients were married (56.9%). This finding is notable but may not be a true representation of a predisposing factor. Rather, it could mean the group that receives more support, care and assistance to seek proper medical help. An overwhelming majority of participants were Igbo (94.2%), with minimal representation from Yoruba (1.9%) and other ethnic groups (3.9%). This distribution likely reflects the geographical location of the study rather than ethnic susceptibility. Therefore, ethnicity in this context may not necessarily indicate genetic predisposition but rather regional population characteristics.

In this study, a large proportion of patients had lived with diabetes for more than 10 years (43.1%), while 35.3% had diabetes for 5–10 years. Only 21.6% had the disease for less than 5 years. This pattern supports established evidence that the risk of DFI increases with longer duration of diabetes (Du et al., 2022), including the likelihood of developing peripheral neuropathy. Our study recorded neuropathic symptoms such as tingling sensations, hot feet, and foul smells from wounds in 98.2%, 84.3%, and 82.4% of the respondents, respectively. Neuropathy reduces pain perception, leading to unnoticed trauma and delayed treatment (Li et al., 2025; Yan et al., 2022). The high prevalence of neuropathic symptoms aligns with clinical evidence that peripheral neuropathy is present in long-standing diabetic patients. A positive family history of diabetes, as shown in 88.2% of the patients, may have contributed to the prevalence of DFI in this study. Although all the respondents claimed to be regulating their blood sugar, the regulation may have started after the presentation at the hospital with DFI. Further, the presence of DFI despite reported positive blood sugar regulation suggests suboptimal regulation, inconsistent monitoring, or late presentation. All patients (100%) were already on antibiotic therapy, with ciprofloxacin being the most commonly prescribed (76.5%), followed by metronidazole (13.7%); yet, 66.7% observed no improvement, and 23.5% had undergone previous amputation. This finding raises concerns about the public health rise of antibiotic resistance.

This study recorded the positive and polymicrobial nature of bacterial growth in all (100%) of the samples. Although a few studies reported increased incidence of polymicrobial growth (Goh et al., 2020; Ugwu et al., 2024) as against other studies that recorded the prevalence of monomicrobial growth (Govindaswamy et al., 2025; Ismayıl et al., 2025). Our findings conform with reports that polymicrobial infections are more common in patients who have chronic infections or have been previously exposed to antibiotic therapy (Yang et al., 2024; Zambelli et al., 2025). The result for the all (100%) polymicrobial growth observed in our study could be attributed to the prolonged duration of DFI, previous antibiotic regime and Wagner grade of ulcers as was the case in the patients under investigation. Both Gram-positive and Gram-negative species, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas* spp., *Escherichia coli*, *Klebsiella* spp., and *Proteus vulgaris*, were isolated in this study. The percentage occurrence of Gram-negative bacteria (59.5%) was more than the percentage occurrence of Gram-positive bacteria (40.5%). This agrees with the established global pattern, buttressing the prevalence of Gram-negative bacteria in DFI. The isolation of similar bacterial species from DFI has been reported previously across countries from various continents: Africa (Aamer et al., 2025; Agbo et al., 2025; Atlaw et al., 2022; Namatovu et al., 2025), Asia (Goh et al., 2020; Govindaswamy et al., 2025; Tarigan et al., 2025), Europe (Saltoglu et al., 2018; Zambelli et al., 2025), the US (Winski et al., 2025), and North America (Das et al., 2025). Nevertheless, the diversity and range of bacteria isolated may be influenced by factors such as site of infection, prior

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exposure to antibiotic drugs, source of sample and collection method, geographical variation, and severity of the infections. Notably, *Staphylococcus aureus* accounted for the highest proportion (30.2%), concurring with reports identifying *Staphylococcus* as one of the most common causative agents of DFI. *S. aureus* is known for its virulence factors, toxin production, and ability to develop antibiotic resistance (like methicillin-resistant strains) that makes management challenging (Agbo et al., 2025; Glen and Lamont, 2021). *Pseudomonas* spp. was the most prevalent Gram-negative organism (24.6%). It is responsible for most hospital-acquired infections and commonly associated with chronic and non-healing wounds. The high incidence of *E. coli* suggests possible contamination from environmental or gastrointestinal sources.

The antibiotic susceptibility results (Tables 5–7) reveal significant resistance patterns among bacterial isolates recovered from DFI, highlighting a serious antimicrobial resistance burden. *Pseudomonas* resistance to amoxicillin/clavulanic acid (94%) and cotrimoxazole (68%) supports the data on *Pseudomonas* intrinsic resistance to many β-lactam antibiotics and folate pathway inhibitors (Glen and Lamont, 2021). With the exception of *Pseudomonas*, all other bacterial species demonstrated marked resistance to colistin in this study (Table 5). *Staphylococcus* resistance to colistin is understandable, but the marked resistance observed in *E. coli* and *Klebsiella* is quite worrisome because colistin is one of the last-resort antibiotics reserved for treating infections caused by Gram-negative organisms (O'Neil, 2015). On the other hand, virtually all test organisms showed high susceptibility to Imipenem, suggesting that carbapenems remain effective against most bacteria in this setting. The increased susceptibility is probably associated with its limited prescription in the study region. From our findings, none of the patients were on imipenem as part of their antibiotic treatment regimen. Similarly, most organisms in this study showed considerable susceptibility to cefoxitin, suggesting that the cephems remain a viable treatment option for DFI within the study location. Ciprofloxacin, the most frequently prescribed drug in the study area, remains effective against *Pseudomonas* (94%), *S. aureus* (82%) and *S. epidermidis* (77%), but is fast becoming inefficient in the treatment of infections involving *E. coli*, *Klebsiella*, and *Proteus vulgaris*. This calls for a more rational, timely, and adequate use of the drug in order to avert selective pressure that will foster the development of antibiotic resistance.

Overall, the antibiotic resistance trend of all 126 isolates in this study collectively showed the highest resistance to amoxicillin/clavulanic acid (82.5%), colistin (73.8%), and cotrimoxazole (50.8%) and the highest susceptibility to imipenem (86.5%), cefoxitin (67.5%), and gentamicin (65.1%). The high resistance to amoxicillin/clavulanic acid indicates widespread β-lactam resistance, possibly due to overuse or misuse of this antibiotic, a growing concern in chronic wound infections globally (Glen and Lamont, 2021). The MAR index values (0.1-0.7) with the highest resistance pattern (AMC, CT, CIP, F, CN, COT) in this study support a possible abuse or misuse of antibiotics. Studies have it that MAR index values greater than 0.2 are indicative of high-risk contamination sources where antibiotics are frequently used (Ozochi et al., 2025). The proportion of isolates in this study with a MAR index above 0.2 was 32% (40/126), while a MAR index below 0.2 was 68% (86/126). This is a clear indication of threatening multidrug resistance (MDR), a significant challenge in the management of DFIs, including increased morbidity and mortality.

V. CONCLUSION

A polymicrobial growth of potential pathogens and a significant number of multiple antibiotic-resistant species was detected in the diabetic foot infection. The antibiotic susceptibility profile demonstrates a high burden of multidrug-resistant organisms among DFI isolates, with particularly high resistance to β-lactams and folate inhibitors. Carbapenems and cephems remain the most effective therapeutic option in the setting. There is an urgent need for enhanced antimicrobial stewardship, routine susceptibility testing, and culture-guided antibiotic prescription practices to curb the growing resistance trend in diabetic foot infections.

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