Article

Bacteriological Profile and Antimicrobial Resistance Patterns of Pathogens Isolated from Patients with Infected Diabetic Foot Ulcers in Kisumu County, Kenya

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Abstract: Background: Diabetic foot ulcers (DFUs) are a significant complication of diabetes and are frequently associated with bacterial infections that hinder healing and increase the risk of antimicrobial resistance (AMR). However, local data on pathogen distribution and resistance patterns remain limited in many resource-constrained settings. Methods: A cross-sectional study was conducted from March to June 2025 across three referral hospitals in Kisumu County, Kenya. A total of 471 patients with DFUs were recruited using a stratified sampling approach. Specimens were processed in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2025 guidelines. Bacterial identification was performed using standard biochemical methods, and antimicrobial susceptibility testing was conducted using the Kirby–Bauer disk diffusion technique. Results: Out of 471 participants, 202 had culture-positive infections, yielding 221 bacterial isolates. Gram-negative organisms predominated, accounting for the majority of isolates. *Escherichia coli* and *Acinetobacter baumannii* were the most frequently isolated Gram-negative pathogens. Among Gram-positive bacteria, *Staphylococcus aureus* was the leading isolate and exhibited multidrug resistance (MDR). Conclusion: The predominance of Gram-negative pathogens and the presence of multidrug resistance highlight the limitations of empirical therapy in DFU management. These findings underscore the importance of routine microbiological testing and targeted antimicrobial stewardship strategies.

Keywords: diabetic foot ulcers; antimicrobial resistance; multidrug resistance; ESKAPE pathogens; clinical microbiology

1. Introduction

Antimicrobial resistance (AMR) has emerged as one of the most pressing challenges in contemporary medicine, threatening the effectiveness of current therapeutic options. Projections from the World Health Organization (WHO) indicate that, in the absence of coordinated global action, deaths attributable to drug-resistant infections could reach 10 million annually by 2050 [1]. This growing threat has prompted calls for comprehensive and coordinated strategies to mitigate what is increasingly described as a looming global health crisis. Surveillance reports from the United States Centers for Disease Control and Prevention (CDC) have identified 18 major AMR threats, while the European Union and European Economic Area have reported 16 priority threats associated with a limited group of high-burden pathogens [2]. Central to this challenge is the rise of multidrug resistance among clinically significant organisms prioritized by the WHO, including *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter species*, collectively referred to as the ESKAPE pathogens [2–4]. These organisms are consistently highlighted in global AMR frameworks, including the WHO Global Action Plan, the United Nations Interagency Coordination Group, and the One Health Global Leaders Group, due to their critical role in driving resistance worldwide [5–7].

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The global burden of diabetes continues to rise substantially, with an estimated 537 million adults aged 20–79 years affected in 2021, a figure projected to increase to 643 million by 2030 and 784 million by 2045 [8]. Among individuals with diabetes, complications such as diabetic foot ulcers (DFUs) contribute significantly to hospital admissions. For instance, studies from China indicate that approximately one-quarter of diabetes-related hospitalizations are linked to infected or ischemic diabetic foot conditions, with infections being the predominant cause. Clinically, diabetic foot infections present with localized inflammatory features, including erythema, edema, warmth, pain, and purulent exudate, and may also progress to systemic manifestations such as fever, circulatory instability, and metabolic derangements [8,9].

DFUs are associated with severe outcomes, accounting for nearly 60% of non-traumatic lower-limb amputations, and patients affected have an estimated 2.5-fold higher mortality risk compared to those without ulcers [10,11]. Beyond clinical consequences, these complications impose substantial psychological, social, and economic burdens on patients, families, and healthcare systems. Current management approaches primarily involve wound care, antimicrobial therapy, and, in advanced cases, surgical intervention including amputation [12,13]. However, treatment outcomes remain suboptimal, partly because empirical antibiotic regimens are less effective in the absence of targeted microbiological guidance [9].

Diagnosis of diabetic foot ulcers (DFUs) relies on clinical assessment, including evaluation of wound and infection markers [5]. Timely identification of infection-related markers is critical for appropriate management. Classification systems such as the Wagner grading scale and the IWGDF framework stratify disease severity and are associated with differences in microbial and antimicrobial susceptibility [6,7]. However, current evidence remains inconclusive as to whether infection markers vary significantly among specific bacterial pathogens. In this context, the present study aimed to characterize the bacterial pathogens isolated from infected diabetic foot ulcers, determine their antimicrobial susceptibility profiles and multidrug resistance patterns, and molecularly characterize selected resistance genes and biofilm formation among multidrug-resistant isolates.

2. Materials and Methods

2.1. Study Site

Kisumu County spans approximately 2085.9 km^2 and, according to the 2019 national census, has a population of 968,909 residents. The county borders Kericho to the east, Siaya to the west, Vihiga to the north, Nyamira to the south, Nandi to the northeast, and Homa Bay to the southwest, making it a strategically located region within western Kenya. Key healthcare facilities within the county capital include Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH), Kisumu County Referral Hospital (KCRH), and Lumumba Sub-County Hospital (LSCH). These institutions serve as major referral centers for the county's seven sub-counties and also provide healthcare services to neighboring counties within the region.

2.2. Study Design

This laboratory-based cross-sectional study investigated bacterial pathogens and antimicrobial resistance patterns in diabetic foot ulcers (DFUs). Clinical specimens were processed using standard microbiological procedures to isolate, identify, and characterize ESKAPE pathogens, followed by antimicrobial susceptibility testing. To select participants at each site, the sampling formula described below was applied.

2.3. Sample Size

The sample size is calculated using Yamane's formula [13]:

$$n = \frac{N}{1+N(e2)} = \frac{258}{1 + 258(0.05^2)} = \frac{258}{1.6345} = 157$$

where:

n =sample size

N = Total population (258 quarterly registered DFU patients)

e = Precision level (0.05)

Consequently, 157 participants were recruited from each of the three hospitals, for a total of 471.

2.4. Specimen Collection and Primary Culture

Pus swab and aspirate specimens were aseptically collected from patients with diabetic foot ulcers (DFUs) and promptly transported to the microbiology laboratory for processing. Samples were inoculated onto Blood

Agar, Chocolate Agar, and MacConkey Agar (Oxoid™, Basingstoke, UK) and incubated aerobically at 37 °C for 24 h. Swab specimens were streaked directly onto culture media, whereas aspirates were evenly inoculated with sterile loops to ensure uniform distribution. Following incubation, distinct colonies were selected and sub-cultured on Nutrient Agar to obtain pure isolates. Confirmed isolates were subsequently preserved in nutrient broth supplemented with 20% glycerol and stored at −80 °C for long-term preservation and downstream analyses.

2.5. Phenotypic Characterization of Isolates

2.5.1. Colony Morphology

Primary identification was based on colony appearance, including size, pigmentation, elevation, margin, and surface characteristics [14].

2.5.2. Gram Staining and Preliminary Classification

Gram staining was performed using standard procedures. Bacterial smears were heat-fixed and sequentially stained with crystal violet (1 min), Gram's iodine (1 min), decolorized using 95% ethanol (10–20 s), and counterstained with safranin (1 min) [15,16]. Slides were examined under oil immersion microscopy (×100).

Gram-positive organisms appeared purple, while Gram-negative organisms appeared pink. Results were confirmed using a 3% potassium hydroxide (KOH) string test, where formation of a viscous string indicated Gram-negative bacteria.

2.6. Biochemical Identification of Isolates

Gram-positive bacteria

Gram-positive isolates were identified using:

1. Catalase test to differentiate *staphylococci* (positive) from *streptococci/enterococci* (negative)
2. Coagulase test for confirmation of *Staphylococcus aureus*
3. Bile esculin hydrolysis test for identification of Enterococcus species
4. Hemolysis pattern on blood agar to support streptococcal classification

Gram-negative bacteria

A stepwise biochemical algorithm was applied:

Oxidase test: For the identification of oxidase-positive organisms such as *Pseudomonas aeruginosa*

Indole test: differentiation of *Escherichia coli* (positive) from other Enterobacteriaceae

Citrate utilization test: identification of citrate-positive organisms such as *Klebsiella pneumoniae*

Methyl Red–Voges Proskauer (MR-VP) tests: for metabolic pathway differentiation

Lysine decarboxylase test: confirmatory test for *Klebsiella* spp.

Pigment production on selective media: used for the identification of *Pseudomonas* spp.

2.7. Confirmatory Identification Using API System

All Gram-negative isolates were further confirmed using the API 20E identification system (bioMérieux, Marcy-l'Étoile, France) according to manufacturer instructions. A standardized inoculum was incubated at 37 °C and read at 24 and 48 h. Biochemical reactions were interpreted using APIweb™ (11th edition).

Reference strains used for quality control included: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 17978, and *Enterococcus faecalis* ATCC 29212

Antimicrobial susceptibility testing (AST)

Antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar following Clinical and Laboratory Standards Institute (CLSI) 2025 guidelines (M100, 35th edition). Zone diameters were interpreted according to CLSI breakpoints.

Antimicrobial agents tested, and the following antibiotic discs were used:

a. β-lactams: ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), piperacillin/tazobactam (100/10 µg)
b. Cephalosporins: Cefixime (30 µg), ceftazidime (30 µg), cefepime (30 µg)
c. Carbapenems: imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg)
d. Aminoglycosides: gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg)
e. Fluoroquinolones: ciprofloxacin (5 µg)
f. Macrolides: azithromycin (15 µg), erythromycin (15 µg)

g. Lincosamides: clindamycin (2 μg)
h. Sulfonamides: cotrimoxazole (1.25/23.75 μg)
i. Glycopeptides: vancomycin (30 μg)
j. Oxazolidinones: linezolid (30 μg)
k. Tetracyclines: tigecycline (15 μg)
l. Staphylococcal markers: cefoxitin (30 μg), oxacillin (1 μg)

Detection of ESBL and MRSA production

Extended-spectrum β-lactamase (ESBL) production in *E. coli* was screened using cefotaxime (30 μg), ceftazidime (30 μg), and cefpodoxime (10 μg). Phenotypic confirmation was performed using combination discs with clavulanic acid: cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, and cefpodoxime/clavulanic acid. An increase in inhibition zone diameter of ≥5 mm in the presence of clavulanic acid confirmed ESBL production. Molecular detection of the *mecA* gene was performed exclusively on phenotypically MDR *S. aureus* isolates because of financial and laboratory resource limitations. Consequently, the findings represent molecular characterization of the MDR subset rather than the entire *S. aureus* isolate population. Quality control strains such as *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as positive and negative controls.

2.8. Ethics Approval and Consent to Participate

Confidentiality and participant privacy were strictly maintained throughout the study, and no personal identifiers were recorded or disclosed during data collection or reporting. Written informed consent was obtained from all participants before enrolment.

Ethical approval for the study was granted by the Institutional Scientific and Ethical Review Committee (ISERC) of Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) (Ref. No. ISERC/JOOTRH/096/2024), the National Commission for Science, Technology and Innovation (NACOSTI) (Ref. No. NACOSTI/P/25/16381), and the Kisumu County Ministries of Health and Education (Ref. No. GN 133 VOL. XIX/168). The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

2.9. Data Management and Analysis

All laboratory assays were performed in triplicate and independently to ensure reproducibility and reliability of results. Data were initially recorded in laboratory notebooks and then entered into Microsoft Excel for cleaning and organization. The finalized dataset was exported to GraphPad Prism (version 8; GraphPad Software, La Jolla, CA, USA) for analysis and visualization.

Results were summarized and presented using tables, figures, and graphs where appropriate. Quantitative data were expressed as mean ± standard error of the mean (SEM), based on triplicate measurements for each test.

3. Results

3.1. Bacterial Isolation

Out of 471 specimens analyzed, 202 (42.9%) demonstrated positive microbial growth. A total of 221 bacterial isolates were recovered, with polymicrobial infections observed in a minority of cases. Gram-negative organisms predominated among the isolates at 67.4% (149/221), while Gram-positive bacteria comprised 32.6% (72/221). Among the Gram-negative isolates, *Escherichia coli* was the most frequently identified organism 18.5% (41/221), followed by *Acinetobacter baumannii* 14.5% (32/221), *Klebsiella pneumoniae* 12.7% (28/221), and *Pseudomonas aeruginosa* 9.5% (21/221). Within the Gram-positive group, *Staphylococcus aureus* was the predominant pathogen 15.8% (35/221), followed by *Enterococcus faecalis* 5.0% (11/221) and *Staphylococcus epidermidis* 4.5% (10/221) (Table 1).

Table 1. Bacterial isolates among diabetic foot ulcer study participants.

Bacterial Isolates	**Distribution (n = 221)**
	n (%)
Gram-positive bacteria (n = 72)	
Staphylococcus aureus	35 (15.8)
Staphylococcus epidermidis	10 (4.5)
Staphylococcus spp.	6 (2.7)
Enterococcus faecalis	11 (5.0)
Enterococcus faecium	6 (2.7)
Streptococcus pyogenes	4 (1.8)
Gram-negative bacteria (n = 149)	

Table 1. *Cont.*

Bacterial Isolates	Distribution (n = 221)
Proteus mirabilis	1 (0.5)
Proteus spp.	2 (0.9)
Pseudomonas aeruginosa	21 (9.5)
Pseudomonas spp.	4 (1.8)
E. coli	41 (18.5)
Klebsiella pneumoniae	28 (12.7)
Klebsiella spp.	5 (2.3)
Proteus vulgaris	3 (1.40
Citrobacter diversus	7 (3.2)
Citrobacter spp.	5 (2.3)
Acinetobacter baumannii	32 (14.5)

3.2. Bacterial Profile between Locations

Nyando sub-county recorded the highest number of isolates, contributing 32.56% (72/221) of all positive samples. Overall, Gram-positive bacteria accounted for 32.6% (72/221) of isolates, with *Staphylococcus aureus* being the most frequently isolated species 48.6% (35/72). Nyando sub-county contributed the largest proportion of *S. aureus* isolates, 37.1% (13/35).

Gram-negative bacteria were predominant, accounting for 67.4% (149/221) of isolates. *Escherichia coli* was the most common Gram-negative organism 27.5% (41/149), with the highest contribution from Muhoroni sub-county 36.6% (15/41). *Acinetobacter baumannii* was the second most prevalent Gram-negative isolate 21.5% (32/149) and was also most frequently recovered in Muhoroni sub-county 37.5% (12/32).

Overall, Nyando and Muhoroni sub-counties demonstrated the highest pathogen burden, with a predominance of *S. aureus* in Nyando and Gram-negative organisms, particularly *E. coli* and *A. baumannii*, in Muhoroni (Table 2).

Table 2. Bacterial profile on infected, DFU patients from the different sub-counties.

Bacterial Isolates	Sub Counties						
	Muhoroni S.C	**Nyando S.C**	**Nyakach S.C**	**Kisumu C.S.C**	**Kisumu E.S.C**	**Kisumu W.S.C**	**Seme S.C**
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Gram-positive bacteria (n = 72)	19 (26.4)	24 (33.3)	11 (15.3)	3 (4.2)	5 (6.9)	4 (5.6)	6 (8.3)
Staphylococcus aureus (n = 35)	10 (28.6)	13 (37.1)	5 (14.3)	2 (5.7)	2 (5.7)	1 (2.9)	2 (5.7)
Staphylococcus epidermidis (n = 10)	2 (20)	4 (40)	0 (0)	1 (10)	2 (20)	0 (0)	1 (10)
Staphylococcus spp. (n = 6)	1(16.7)	2 (33.3)	1 (16.7)	0 (0)	0 (0)	2 (33.3)	0 (0)
Enterococcus faecalis (n = 11)	5 (45.5)	3 (27.3)	2 (18.2)	0 (0)	1 (9.0)	0 (0)	0 (0)
Enterococcus faecium (n = 6)	1(16.7)	1(16.7)	1(16.7)	0 (0)	0 (0)	0 (0)	3 (50)
Streptococcus pyogenes (n = 4)	0 (0)	1(25)	2 (50)	0 (0)	0 (0)	1 (25)	0 (0)
Gram-negative bacteria (n = 149)	47 (31.5)	48 (32.2)	15 (10.1)	16 (10.7)	12 (8.1)	7 (4.7)	4 (2.7)
Proteus mirabilis (n = 1)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Proteus spp. (n = 2)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
Pseudomonas aeruginosa (n = 21)	5 (23.8)	10 (47.6)	3 (14.3)	0 (0)	2 (9.5)	0 (0)	1 (4.8)
Pseudomonas spp. (n = 4)	1 (25)	1 (25)	0 (0)	1 (25)	0 (0)	1 (25)	0 (0)
E. coli (n = 41)	15 (36.6)	10 (24.4)	4 (9.8)	5 (12.2)	2 (4.9)	3 (7.2)	2 (4.9)
Klebsiella pneumoniae (n = 28)	8 (28.6)	11 (39.3)	3 (10.7)	2 (7.1)	3 (10.7)	0 (0)	1 (3.6)
Klebsiella spp. (n = 5)	1 (20)	2 (40)	0 (0)	0 (0)	2 (40)	0 (0)	0 (0)
Proteus vulgaris (n = 3)	0 (0)	0 (0)	1(33.3)	0 (0)	2 (66.7)	0 (0)	0 (0)
Citrobacter diversus (n = 7)	2 (28.6)	3 (42.8)	0 (0)	2 (28.6)	0 (0)	0 (0)	0 (0)
Citrobacter spp. (n = 5)	3 (60)	1 (20)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)
Acinetobacter baumannii (n = 32)	12 (37.5)	9 (28.1)	3 (9.4)	5 (15.6)	1 (3.1)	2 (6.3)	0 (0)
Total isolates (n = 221)	66 (29.9)	72 (32.5)	26 (11.8)	19 (8.6)	17 (7.7)	11 (5.0)	10 (4.5)

Key: S.C-Sub County; C.S.C-Central Sub County; E.S.C-East Sub County; W.S.C-West Sub County.

Bacterial distribution among the genders. Overall, bacterial isolates were more frequently recovered from male participants (n = 141) than female participants (n = 80), with a higher burden observed for both Gram-positive and Gram-negative organisms. Among Gram-positive bacteria, *Staphylococcus aureus* was the most common isolate in both genders, with a markedly higher proportion in males, 19.1% (27/141), compared to females, 10.0% (8/80). Similarly, *Staphylococcus epidermidis* and *Streptococcus pyogenes* were predominantly isolated from males, while females showed relatively higher proportions of *Staphylococcus* spp., *Enterococcus faecalis*, and *Enterococcus faecium*, though at lower overall frequencies.

A similar pattern was observed for Gram-negative bacteria, which were more prevalent among male participants. *Escherichia coli* was the leading isolate in males at 22.0% (31/141), followed by *Klebsiella pneumoniae at* 12.8% (18/141) and *Pseudomonas aeruginosa* at 10.6% (15/141). In females, the most frequently

isolated organisms were *Acinetobacter baumannii* 22.5%, (18/80), followed by *E. coli* and *K. pneumoniae,* both 12.5%, (10/80). Notably, *Proteus vulgaris* and *Pseudomonas* spp. were more represented among females than males, although at lower absolute counts. Overall, males exhibited a higher overall bacterial burden, while females showed a relatively more even distribution of Gram-negative pathogens, particularly *A. baumannii* (Table 3).

Table 3. Bacterial profile of infected DFU patients across genders.

Bacterial Isolates	**Male (n = 141)**	**Female (n = 80)**
	n (%)	**n (%)**
Gram-positive bacteria		
Staphylococcus aureus (n = 35)	27 (19.1)	8 (10.0)
Staphylococcus epidermidis (n = 10)	7 (5.0)	3 (3.8)
Staphylococcus spp. (n = 6)	2 (1.4)	4 (5.0)
Enterococcus faecalis (n = 11)	6 (4.3)	5 (6.3)
Enterococcus faecium (n = 6)	2 (1.4)	4 (5.0)
Streptococcus pyogenes (n = 4)	4 (2.8)	0 (0.0)
Gram-negative bacteria		
Proteus mirabilis (n = 1)	0 (0.0)	1 (1.3)
Proteus spp. (n = 2)	1 (0.7)	1 (1.3)
Pseudomonas aeruginosa (n = 21)	15 (10.6)	6 (7.5)
Pseudomonas spp.(n = 4)	1 (0.7)	3 (3.8)
E. coli (n = 41)	31 (22.0)	10 (12.5)
Klebsiella pneumoniae (n = 28)	18 (12.8)	10 (12.5)
Klebsiella spp. (n = 5)	3 (2.1)	2 (2.5)
Proteus vulgaris (n = 3)	0 (0.0)	3 (3.8)
Citrobacter diversus (n = 7)	6 (4.3)	1 (1.3)
Citrobacter spp. (n = 5)	4 (2.8)	1 (1.3)
Acinetobacter baumannii (n = 32)	14 (9.9)	18 (22.5)

3.3. Antibiotic Resistance

Antimicrobial susceptibility testing demonstrated variable resistance profiles among the isolates, as in Table S1. Among Gram-positive bacteria, *Staphylococcus aureus* exhibited the highest rate of multidrug resistance (MDR) at 25.7% (9/35) with resistance observed across antibiotic classes R3–R7 (Table 4). In contrast, *Streptococcus pyogenes* showed complete susceptibility to all antibiotics tested.

Table 4. MDR pattern of selected Gram-positive bacteria isolated from infected DFU patients.

		Gram-Positive Bacteria					
Antimicrobial Class Used to Define MDR		***S. aureus* (n = 35)**	***S. epidermidis* (n = 10)**	***Staphylococcus* spp. (n = 6)**	***E. faecalis* (n = 11)**	***E. faecium* (n = 6)**	***S. pyogenes* (n = 4)**
Degree		**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Glycopeptide (Vancomycin)	R0	19 (54.3)	7 (70)	3 (50)	5 (45.5)	1 (16.7)	4 (100)
Aminoglycoside (Gentamycin)	R1	6 (17.1)	2 (20)	1 (16.7)	4 (36.4)	4 (66.6)	0 (0)
Cephalosporine (Cefixime)	R2	1 (2.9)	1 (10)	2 (33.3)	2 (18.1)	1 (16.7)	0 (0)
Quinolone (Ciprofloxacin)	R3	1 (2.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sulfonamides (Cotrimoxazole)	R4	1 (2.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Oxazolidinones (Linezolid)	R5	3 (8.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Macrolides (Azithromycin)	R6	2 (5.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
R7		2 (5.7)	0 (0)	0(0)	0 (0)	0 (0)	0 (0)
TOTAL MDR		9 (25.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Key: R0: Sensitive against all selected antibiotic class; R1: Resistant to at least one antibiotic class; R2: Resistant to two antibiotic class; R3: Resistant to three antibiotic class; R4: Resistant to four antibiotic class; R5: Resistant to five antibiotic class; R6: Resistant to six antibiotic class; R7: Resistant to all seven antibiotic class; MDR: Resistant to at least three antibiotic class.

Among Gram-negative organisms, *Acinetobacter baumannii* demonstrated the highest prevalence of multidrug resistance (MDR) at 15.6% (5/32), followed by *Klebsiella pneumoniae* at 7.1% (2/28). *Escherichia coli*

and *Pseudomonas aeruginosa* each exhibited lower MDR rates of 4.9% and 4.8%, respectively. All MDR isolates showed resistance across multiple antibiotic classes (R3–R6) (Table 5).

In terms of antimicrobial susceptibility, *Proteus mirabilis* exhibited complete sensitivity 100% to all tested antibiotics. This was followed by *Pseudomonas* spp. 75%, *Proteus vulgaris* 66.7%, *Klebsiella* spp. 60%, *Citrobacter diversus* 57.1%, and *Proteus* spp. 50%. Lower susceptibility rates were observed for *Pseudomonas aeruginosa* (42.9%), *Acinetobacter baumannii* (40.6%), and *Citrobacter* spp. 40%, while *Klebsiella pneumoniae* showed the lowest susceptibility, 7.1%.

Table 5. MDR pattern of selected Gram-Negative bacteria isolated from infected DFU patients.

Antimicrobial Class Used to Define MDR.	Degree	Gram-Negative Bacteria										
		***P. mirabilis* (n = 1)**	***Proteus* spp (n = 2)**	***P. aeruginosa* (n = 21)**	***Pseudomonas* spp (n = 4)**	***E. coli* (n = 41)**	***K. pneumoniae* (n = 28)**	***Klebsiella* spp (n = 5)**	***P. vulgaris* (n = 3)**	***C. diversus* (n = 7)**	***Citrobacter* spp (n = 5)**	***A. baumannii* (n = 32)**
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Penicillin	R0	1 (100.0)	1 (50.0)	9 (42.9)	3 (75)	6 (14.6)	2 (7.1)	3 (60)	2 (66.7)	4 (57.1)	2 (40)	13 (40.6)
Aminoglycosides	R1	0 (0)	0 (0)	7 (33.3)	1 (25)	30 (73.2)	4 (14.3)	1 (20)	0	2 (28.6)	3 (60)	4 (12.5)
Cephalosporin	R2	0 (0)	1 (50.0)	4(19.0)	0 (0)	3 (7.3)	20 (71.4)	1 (20)	1 (33.3)	1 (14.3)	0 (0)	10 (31.3)
Quinolone	R3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sulfonamides	R4	0 (0)	0 (0)	0 (0)	0 (0)	2 (4.9)	1 (3.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Carbapenem	R5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2(6.3)
	R6	0 (0)	0 (0)	1 (4.8)	0 (0)	0 (0)	1 (3.6)	0 (0)	0 (0)	0 (0)	0 (0)	3 (9.3)
TOTAL MDR (n= 10)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)	2 (4.9)	2 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5(15.6)

KEY: R0: Sensitive against all selected antibiotic class; R1: Resistant to at least one antibiotic class; R2: Resistant to two antibiotic class; R3: Resistant to three antibiotic class; R4: Resistant to four antibiotic class; R5: Resistant to five antibiotic class; R6: Resistant to six antibiotic class; MDR: Resistant to at least three antibiotic class.

Extended-spectrum β-lactamase (ESBL) production in *E. coli* was screened using cefotaxime (30 µg), ceftazidime (30 µg), and cefpodoxime (10 µg). Phenotypic confirmation was performed using combination discs with clavulanic acid: cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, and cefpodoxime/clavulanic acid. An increase in inhibition zone diameter of ≥5 mm in the presence of clavulanic acid confirmed ESBL production. Quality control strains such as E. coli ATCC 25922 and S. aureus ATCC 25923 were used as positive and negative controls.

3.4. Detection of ESBL-Producing Escherichia Coli

A total of 41 *Escherichia coli* isolates were recovered and screened for extended-spectrum β-lactamase (ESBL) production using cefotaxime (30 µg), ceftazidime (30 µg), and cefpodoxime (10 µg) discs. Isolates that demonstrated reduced susceptibility to one or more of the screening agents were subjected to phenotypic confirmation using combination discs containing clavulanic acid.

Of the 41 *E. coli* isolates tested, 2 isolates (4.9%) were confirmed as ESBL producers. ESBL production was confirmed by comparing the inhibition zone diameters of cefotaxime (CTX), ceftazidime (CAZ), and cefpodoxime (CPD) discs with their corresponding clavulanic acid-containing combination discs (CTX/CLA, CAZ/CLA, and CPD/CLA). An increase of ≥5 mm in inhibition zone diameter in the presence of clavulanic acid was interpreted as positive for ESBL production. In the ESBL-positive isolate, increases of 8 mm, 7 mm, and 7 mm were observed for CTX, CAZ, and CPD, respectively. In contrast, the ESBL-negative isolate showed increases of less than 5 mm for all antimicrobial agents. Overall, 2 of 41 (4.9%) *E. coli* isolates were confirmed as ESBL producers, while 39 (95.1%) were classified as non-ESBL producers as in Figure 1. The remaining 39 isolates (95.1%) did not demonstrate a significant increase in zone diameter and were therefore classified as non-ESBL producers as in Table 6.

Table 6. Prevalence of ESBL-producing *E. coli* isolates.

ESBL Status	Number of Isolates (n = 41)	Percentage (%)
ESBL Positive	2	4.9
ESBL Negative	39	95.1
Total	41	100

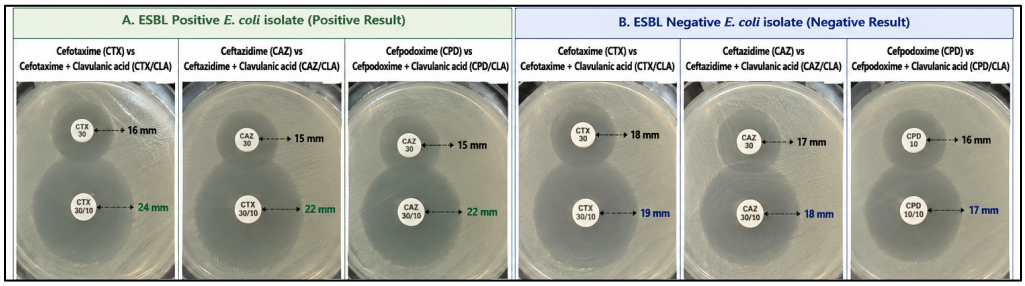

Figure 1. Phenotypic confirmation of ESBL-producing *Escherichia coli* isolates using the Combined Disc Synergy Test (CDST).

A total of 35 *Staphylococcus aureus* isolates were recovered. Due to limited resources, molecular characterization was performed only on the nine phenotypically multidrug-resistant (MDR) *S. aureus* isolates. Among these, four (44.4%) were positive for the Methicillin-resistant *Staphylococcus aureus* (MRSA), confirming methicillin resistance. This proportion refers only to the MDR subset and should not be interpreted as the prevalence of MRSA among all *S. aureus* isolates as show in Figures 2 and 3.

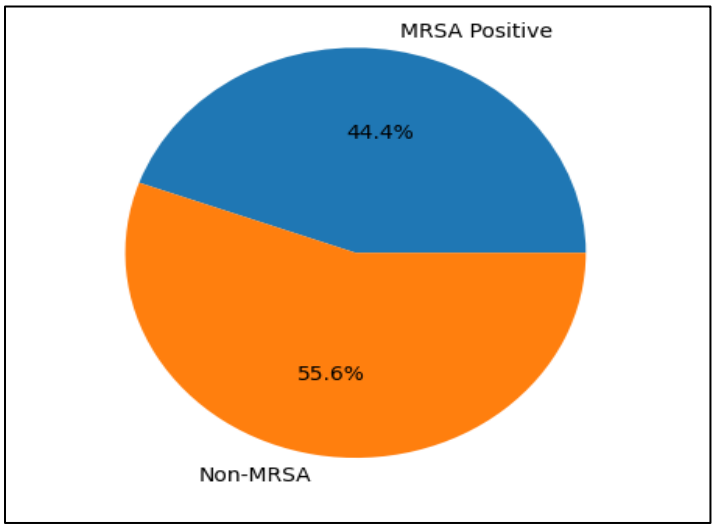

Figure 2. Distribution of methicillin-resistant Staphylococcus aureus (MRSA) among the nine Staphylococcus aureus isolates tested.

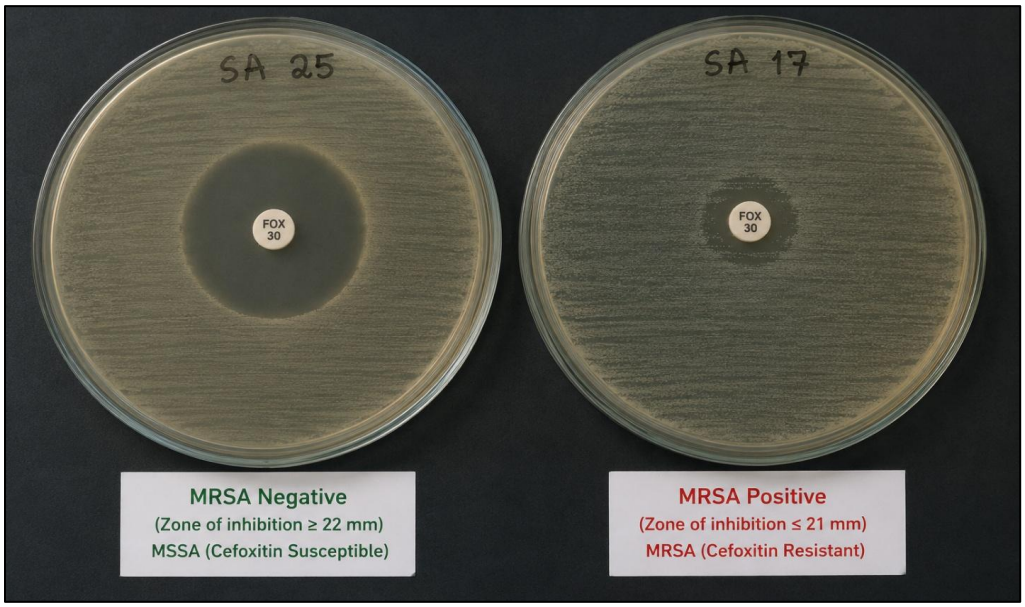

Figure 3. Phenotypic detection of methicillin-resistant *Staphylococcus aureus* (MRSA) using the cefoxitin disk diffusion method.

4. Discussion

Diabetic foot ulcers (DFUs) remain an important public health problem, particularly in resource-limited settings, with substantial global and regional variation in prevalence [17,18]. DFU infections are frequently caused by diverse bacterial pathogens and may be polymicrobial, particularly in chronic or severe wounds [19]. Previous studies from sub-Saharan Africa have reported considerable variation in the bacterial profiles of infected DFUs, with *Staphylococcus aureus* commonly identified as the predominant Gram-positive organism, alongside important Gram-negative pathogens such as *Escherichia coli* and *Pseudomonas aeruginosa* [20,21]. In contrast, the present study demonstrated a marked predominance of Gram-negative bacteria (67.4%), with *E. coli* and *Acinetobacter baumannii* being the leading isolates. This finding is consistent with reports from some African and other resource-limited settings in which Gram-negative organisms constitute a substantial proportion of DFU pathogens [21]. Among the 221 culture-positive isolates, Gram-negative organisms accounted for the majority 67.4%, with *E. coli* being the most frequently identified pathogen 18.5%. These findings contrast with earlier studies reporting Gram-positive dominance, particularly S. aureus [22–26] but align with more recent evidence from Egypt and Kenya, where Gram-negative organisms accounted for over 50% of isolates [21,27]. Similar proportions have been reported in other studies, further supporting the increasing role of Gram-negative bacteria in DFUs in resource-limited settings [20].

The distribution of isolates across sub-counties revealed higher prevalence in predominantly rural areas, particularly Nyando and Muhoroni. This pattern may reflect disparities in healthcare access, delayed presentation, poor glycemic control, and limited awareness of wound care practices. Comparable findings have been reported in other studies, in which rural residence was associated with higher infection rates and more severe disease outcomes [21,25]. The spatial distribution of bacterial isolates across sub-counties reveals a clear geographic clustering of infection burden, with Nyando sub-county contributing the highest proportion of isolates, particularly among Gram-positive organisms, with *Staphylococcus aureus* dominating. This suggests a possible link to local care-seeking patterns, wound chronicity, or hygiene-related exposures in this area. Conversely, Gram-negative pathogens were more widely distributed but still showed marked concentrations in Nyando and Muhoroni sub-counties, where *Escherichia coli* and *Acinetobacter baumannii* were most prevalent, suggesting environmental and healthcare-associated contamination pathways that may be driving polymicrobial diabetic foot infections. The dominance of *E. coli* in Muhoroni and the consistent presence of *A. baumannii* across multiple sub-counties further underscores the role of both community and hospital reservoirs in sustaining infection cycles. Overall, these geographic variations highlight not only heterogeneity in pathogen distribution but also potential differences in wound management practices, antimicrobial exposure, and sanitation infrastructure, emphasizing the need for location-specific infection control strategies and targeted antimicrobial stewardship interventions.

Microbiological analysis further confirmed the predominance of Gram-negative organisms, with *E. coli* and *S. aureus* being the most frequently isolated Gram-negative and Gram-positive pathogens, respectively. These findings agree with studies demonstrating a dual burden of these pathogens in chronic wound infections [25,26]. The predominance of Gram-negative organisms, particularly *E. coli*, has important clinical implications due to their capacity to harbor multiple resistance mechanisms, thereby complicating treatment strategies [20].

With respect to antimicrobial resistance, this study demonstrated a higher prevalence of multidrug resistance (MDR) among Gram-negative isolates, particularly *A. baumannii* 15.6%, followed by *Klebsiella pneumoniae* 7.1%, *E. coli* 4.9%, and *Pseudomonas aeruginosa* 4.8%. These findings are consistent with previous reports highlighting the increasing burden of MDR Gram-negative pathogens in DFUs [23]. The observed resistance patterns suggest possible extended-spectrum beta-lactamase (ESBL) production, particularly among *E. coli*. Despite this, higher susceptibility was retained for last-line agents such as tigecycline and carbapenems, including imipenem and meropenem. Resistance to third-generation cephalosporins was notably high, 27.5%, indicating limited effectiveness of commonly used antibiotics in this setting.

In contrast, Gram-positive isolates in the present study demonstrated substantial resistance to erythromycin and β-lactam antibiotics, while retaining high susceptibility to vancomycin and linezolid. This pattern is consistent with findings from previous studies of diabetic foot infections, including studies from Kenya and other African settings, which have reported high resistance to commonly used β-lactam and macrolide antibiotics among *Staphylococcus aureus*, while susceptibility to vancomycin and linezolid has generally remained high [28,29]. Among Gram-positive bacteria, *S. aureus* exhibited the highest MDR rates, with a significant proportion of isolates being methicillin-resistant *S. aureus* (MRSA). This may be attributed to inappropriate antibiotic use, suboptimal infection prevention practices, and inadequate management of DFUs. The detection of cefoxitin-resistant isolates further supports the presence of MRSA, which remains a common and clinically significant pathogen in DFUs [28–30].

The prevalence of ESBL-producing *Escherichia coli* observed in this study was low, with only 2 of the 41 isolates (4.9%) demonstrating phenotypic confirmation of ESBL production, which didn't concur with a previous study that shows a Prevalence of 26% [26]. This finding suggests that most *E. coli* isolates in the study population remained susceptible to third-generation cephalosporins and did not exhibit clinically significant ESBL-mediated resistance. Although the prevalence was relatively low, detecting ESBL-producing isolates is clinically significant because these organisms are associated with treatment failures, limited therapeutic options, and the potential for rapid dissemination of resistance genes in healthcare and community settings. These findings underscore the importance of routine ESBL surveillance, antimicrobial susceptibility testing, and antimicrobial stewardship programs to facilitate early detection and containment of emerging resistant strains.

The detection of MRSA in 44.4% of the *Staphylococcus aureus* isolates indicates a relatively high level of methicillin resistance within the sample population. This finding concurs with a previous study conducted in the same geographic area, which reported a prevalence of 45% [26]. This resistance is likely mediated by the mecA gene, which encodes PBP2a and reduces the effectiveness of β-lactam antibiotics. The finding suggests selective antibiotic pressure, possibly due to the misuse or overuse of antibiotics in the environment. Although the prevalence varies across regions, this result aligns with reports of moderate MRSA circulation in similar settings. However, the small sample size (n = 9) limits generalizability, and larger studies are needed to better define the true burden of MRSA.

5. Conclusions and Recommendation

This study demonstrates that infected diabetic foot ulcers in Kisumu County are predominantly monomicrobial with occasional polymicrobial involvement and are mainly caused by Gram-negative bacteria, particularly *Escherichia coli* and *Acinetobacter baumannii*. This pattern contrasts with the traditionally reported predominance of Gram-positive organisms, suggesting a possible epidemiological shift in the microbial profile of diabetic foot infections in this setting.

The high prevalence of multidrug-resistant Gram-negative isolates, together with notable resistance in *Staphylococcus aureus* (25.7% MDR, including MRSA), highlights the need for strengthened and continuous local antimicrobial surveillance. Empirical treatment protocols in Kisumu County should therefore be regularly reviewed to ensure adequate coverage of MDR Gram-negative organisms, while also considering agents such as vancomycin or linezolid for suspected resistant Gram-positive infections, pending culture and susceptibility results. Strengthening antimicrobial stewardship and routine microbiological testing remains essential to improve treatment outcomes and reduce therapeutic failure in DFU patients.

Although the prevalence of ESBL-producing *E. coli* was low (4.9%), their detection indicates the presence of clinically important resistance mechanisms that warrant continued surveillance. Similarly, MRSA accounted for 44.4% of the multidrug-resistant *S. aureus* isolates, indicating a substantial burden of methicillin resistance within this resistant subgroup and highlighting the potential influence of antibiotic selective pressure. These findings underscore the importance of continued antimicrobial resistance surveillance and antimicrobial stewardship to guide appropriate treatment and limit the further dissemination of resistant strains.

These findings reinforce the need for routine antimicrobial resistance surveillance and strict infection prevention and control measures to limit further spread of resistant pathogens.

6. Study Limitations

This study has several limitations. Its cross-sectional design precludes assessment of temporal or causal relationships between microbial patterns and antimicrobial resistance in diabetic foot infections. The use of aerobic culture techniques alone may have underestimated the true burden of infection by excluding obligate anaerobes commonly implicated in chronic wounds. In addition, the absence of molecular methods limited confirmation of resistance mechanisms and strain-relatedness. The study was conducted in a single county, which may limit the generalizability of the findings to other settings. Furthermore, swab and aspirate specimens were analyzed together without stratification, potentially introducing variability in isolate recovery. Finally, relatively small subgroup sizes in the ESBL and MRSA analyses reduce the precision of some resistance estimates. Despite these limitations, the study provides useful baseline data on the microbiological and antimicrobial resistance patterns of diabetic foot ulcer infections in this setting.

Supplementary Materials: The following supporting information can be downloaded at: https://media.sciltp.com/articles/others/2609111125281351/JIIM-26020112-Author-JIIM-26020112-Author-Suplementary-Data-1-Final-V4-V5.xlsx. Table S1: Antimicrobial susceptibility testing among the isolates.

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Use of AI and AI-Assisted Technologies: No AI tools were utilized for this paper.

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