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Antimicrobial resistance profiles and detection of *mec*A, *bla*CTX-M-1, and *bla*SHV genes in bacteria among diabetic foot ulcer patients from selected referral hospitals in Uganda

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Abstract

Background Effective management of Diabetic foot ulcers (DFU) requires timely treatment of infections with appropriate antibiotics based on culture and susceptibility results. However, challenges in diagnostic testing persist and consequently, data on prevalent bacteria, their antimicrobial resistance profiles and genes remains scanty. This study profiled the prevalent bacteria in Diabetic foot infection (DFI), their antimicrobial susceptibility patterns, and associated resistance genes.

Methodology A multi-center cross sectional study was conducted from November 2021-January 2022, involving 117 patients with DFU, attending selected referral hospitals (Kiruddu, Jinja, Kampala International Teaching Hospital, Kitagata, Mbarara, Fort Portal and Hoima). Wound swabs were aseptically collected and placed in Stuart transport medium, then pre-enriched prior to inoculation onto Blood, MacConkey and Chocolate Agar to isolate aerobic microorganisms. Bacteria identification was based on colony morphology, Gram stain, conventional biochemical tests and Antibiotic susceptibility testing using Kirby-Bauer disk diffusion method were performed. Methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) were identified phenotypically. *mec*A gene in selected MRSA, and *bla*CTX-M-1 and *bla*SHV-1 in ESBL-PE were detected using Uniplex PCR and electrophoresed using 1.5% agarose gel. Data was analyzed using MS Excel version 15 and Stata version 15 (Stata Corp®).

Results Microbiologically confirmed infection was observed in 89.7% (105/117) of DFU cases, yielding 144 bacterial isolates. Poly-microbial infections were detected in 23.8% of patients. Gram-positive bacteria accounted for 55.6% (80/144) of isolates. *Staphylococcus aureus* (36.1% (52/144)) and *Proteus spp* (13.9% (20/144)) were the most prevalent Gram-positive and Gram-negative bacteria, respectively. Notably, 98.6% (142/144) of the isolates were multidrug-resistant organisms (MDR). *Staphylococcus aureus* sensitivity was highest for Gentamycin and Ciprofloxacin (48%

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each) and lowest for Cefoxitin (2%). *Proteus spp* sensitivity was highest for Imipenem (87%) and lowest for Tetracycline, Ampicillin and Amoxiclav (0%). *mec*A gene was identified in 56% (5/9) of MRSA. Among the selected MDR Gram-negative isolates, 60% had *bla*CTX-M-1, 20% had *bla*SHV and both *bla*CTX-M-1 *and bla*SHV *in* one *Klebsiella pneumoniae*.

Conclusion The high prevalence of MDR bacteria in DFI underscores the need for culture and sensitivity testing-guided treatment. Detection of resistance genes calls for sustained surveillance efforts, stringent infection prevention and control, and alternative therapy exploration.

Keywords Diabetes, Diabetic foot ulcer, Microbial profile, Diabetic foot infection, Drug resistance, Uganda

Background

Globally, Diabetic foot ulcer (DFU), a complication in people living with *Diabetes mellitus* is a common problem affecting 40–60% of patients. Diabetic foot ulcers are highly susceptible to infection, which can lead to significant clinical and economic consequences, including increased risk of hospitalization, amputation, osteomyelitis, and death [1–3]. The current best practice for managing diabetic foot ulcers is immediate treatment of clinical infection targeting the most likely pathogens. Drug choice should be reviewed based on pathogen cultured, drug susceptibility, and clinical progress [4, 5]. This approach saves diabetic patients from unsafe long-term administration of antibiotics which could be responsible for drug resistance, nephropathy and hepatic insufficiency. However, the capacity for culture and sensitivity is still a challenge in many health facilities [6, 7]. Polymicrobial growth and antimicrobial resistance including multidrug resistant organisms (MDROs) like methicillin-resistant *Staphylococcus aureus* (MRSA) have been isolated in 24%–67% of these patients [5, 8–11]. The patterns of microbes and antibiotic sensitivity vary from one region to another requiring these studies [12, 13].

Information on bacteria responsible for DFI and guidelines of antibiotics to use in diabetic foot ulcer is still scanty in sub-Saharan Africa, Uganda inclusive [14]. There is still inconsistence on bacteria with Gram staining and existence of polymicrobial infections or not [5, 8, 10, 15]. Nevertheless, a number of studies have registered multidrug-resistant bacterial strains with varying, and inconsistent susceptibility results which make resistance monitoring and susceptibility testing a necessity [5, 8, 10]. Furthermore, false positives have been identified with the phenotypic resistance testing methods which pose serious clinical consequences, justifying a need for accurate and definitive genotypic methods [16]. These genotypic methods target known genes that confer resistance to antibiotics and their detection is sufficient evidence for antibiotic resistance, like *mec*A and *mec*C genes for methicillin resistance in *Staphylococcus aureus* [17], ESBL genes (*bla*CTX-M, *bla*SHV, and *bla*TEM) for beta-lactam antibiotics resistance in Enterobacterales [18] and carbapenemase (*bla*KPC) gene. Detection of these genes not only guide clinicians on appropriate antibiotic options but also prevent treatment failure and risk of disseminating resistance. Information on prevalence of these resistance genes in bacteria isolated for DFU of patients in Uganda is still scanty. However, a number of studies elsewhere, have indicated varying prevalence of *mec*A genes from MRSA isolated from DFU patients of 40%–100% in hospitals in Tunisia and India [19–22]. In addition, ESBL genes have been detected from Gram negative bacteria isolated from DFU in Pakistan (*bla*SHV (84.6%), *bla*TEM (75%) and *bla*CTX-M (76.9%)) [23] and in North India (*bla*CTX-M (34.2%) and *bla*TEM (89.4%)) [24]. In Uganda, studies done on other patients in hospital settings in eastern and western region indicated a prevalence of the *mec*A gene of 29% and 46%, respectively [25, 26] and prevalence of *bla* CTX-M (70%), *bla*SHV (34%) and TEM (100%) in western Uganda [27] and *bla*CTX-M of 93% from a maternity ward in Mulago hospital [28]. Hence a need for similar studies on DFU patients to inform appropriate antibiotic treatment. The aim of this study was to profile the different organisms obtained from patients' DFU, their antibiotic susceptibility patterns and genes associated with resistance to inform DFI treatment in our setting.

Methods

Study design and setting

The study was a multicenter cross-sectional study conducted in seven regional and teaching hospitals in Uganda as detailed in the earlier publication [29]. The hospitals are from Central Uganda (Kiruddu Specialized Hospital), Eastern Uganda (Jinja Regional Referral Hospital and western Uganda (Kampala International Teaching Hospital; Kitagata General Hospital; Mbarara Regional Referral Hospital; Fort Portal Regional Referral Hospital; and Hoima Regional Referral Hospital). The hospitals were purposefully selected based on the high reported prevalence of *Diabetes mellitus* (DM) in their respective regions in Uganda [30].

Participants' characteristics

The study involved 117 Diabetes mellitus patients (DM type 1 and type 2) selected purposively based on having

DFU below the ankle and attending the surgical Department and DM clinics in any of the seven selected hospitals in Uganda between November 1, 2021, and January 31, 2022. The patients' recruitment criteria, sample size determination, and patients characteristics (socioeconomics, medical, and behavioral) were as detailed in our earlier publication [29]. Sample size per study site was informed by the proportion of DFU patient flow in the different referral hospitals based on the pre-existing hospital records. The study involved 12, 14, 14, 17, 29, 21, and 10 DFU patients from Fort Portal (FT), Hoima (HM), Jinja (GA), Kiruddu (KD), Kitagata (KT), KIU-TH (KU), and Mbarara (MA) referral hospitals, respectively.

Sample collection from patients

Prior to swab sample collection, the Diabetic foot wound and surrounding skin areas was cleaned by a trained doctor with 7.5% Povidone-Iodine [31]. This was followed by collection of swab samples (containing pus and debris) aseptically from deeper tissues of the DFU of patients using sterile swabs soaked in sterile normal saline. The collected samples were transported in Stuart transport media (Oxoid, Thermo Fisher Scientific, Basingstoke, UK) to the Kampala International University Teaching Hospital (KIU-TH) laboratory. Samples were stored under refrigeration, at 2–8 °C and processed within seven days [32].

Characterization of the bacteria

Swabs were pre-enriched using Brain Heart Infusion broth (Oxoid, Basingstoke, UK) for 24 h at 37 °C [33]. This was followed by inoculation on Blood Agar (HiMedia Laboratories Pvt. Ltd, Mumbai, India) using the streak plate technique as described by Cheesebrough [34], to isolate any growing microorganisms from the collected swabs after 24 h of aerobic incubation at 37 °C. Purification of obtained cultures was done on general-purpose media, including Blood agar, basing on their hemolytic properties, and Chocolate agar (HiMedia Laboratories Pvt. Ltd, Mumbai, India) to isolate fastidious organisms [35]. Additionally, differential and selective media from HiMedia Laboratories Pvt. Ltd, Mumbai, India were used including MacConkey agar and Violet Red Bile agar (VRBA) to isolate organisms based on their ability to ferment lactose [36, 37]. Mannitol salt agar to isolate *Staphylococcus spp* [38], Cetrimide selective agar to isolate *Pseudomonas aeruginosa* [39], Slanetz and Bartley agar and Bile esculin azide agar to isolate *Enterococcus spp* [40, 41], Xylose lysine deoxycholate agar for presumptive isolation of *Proteus spp*, and Eosine methyl blue (EMB) for isolation of *E.coli* [38, 42]. For any mixtures of bacterial colonies, CHROMagar™ Orientation agar was used [43]. All incubations were done aerobically for 24 h at 37 °C.

Based on the colony morphology, preliminary identification of pure cultures was done using Gram staining according to [34]. Further identification of pure colonies was done using conventional biochemical tests using catalase, coagulase, oxidase, gelatin-hydrolysis, nitrate reduction, citrate, indole, motility test, urease, methyl red – Voges – Proskauer (MR-VP) and Triple sugar iron (TSI) tests [34]. Additionally, species-specific biochemical tests were done, including: CAMP test, bile solubility, and sensitivity to bacitracin and optochin antibiotics for Streptococci species and Slidex staph plus (bioMérieux, France) and DNase test for *Staphylococcus spp* [34, 44]. Confirmation of organisms was done using specific Analytical Profile Index (API) system [] such as API 20E, API 20NE, API 20 Strep, and API 20 Staph (bioMérieux, Inc, France). Standard positive controls of *Staph aureus* ATCC 25,923 and *E. coli* ATCC 25,922 were used for bacteria identification.

Susceptibility pattern of bacterial isolates to commonly used antibiotics

Antibiotic susceptibility testing was carried out using Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Fisher Scientific, Basingstoke, UK) [45]. Briefly, a sterile wire loop was used to touch the top of discrete colonies from a 24-hour-old culture on nutrient agar and then transferred into 5 ml of sterile 0.85% saline. The bacterial suspension was standardized to an optical density equivalent to 0.5 McFarland turbidity Standard to bring the cell density to about $1.5–2.0 \times 10^8$cfu/ml using a densitometer device (BioSan Densitometer DEN-1B, Latvia). The culture suspension was spread over the entire surface of the Mueller-Hinton Agar plate by swabbing. The agar surfaces were allowed to dry with the lid opened ajar for 5 min, and the plates were held at room temperature for not more than 15 min to allow sufficient reduction of any moisture before applying antimicrobial discs using sterile forceps. Antibiotic discs (Oxoid, Thermo Fisher Scientific, Basingstoke, UK) were used in this study. The isolated bacteria (Gram-positive and Gram-negative) were tested on antibiotics within the 10 classes of antibiotics recommended by the CLSI guideline of 2024 and are commonly used in our setting. These included: Carbapenem (Imipenem), Quinolones (Levofloxacin, Ofloxacin, Ciprofloxacin); Penicillin (Oxacillin, Ampicillin, and Amoxiclav); Cephalosporins (Cefuroxime, Cefixime, Cefoxitin), Aminoglycoside (Gentamycin, Amikacin, Streptomycin), Lincosamide (Clindamycin), Tetracycline (Tetracycline), Glycoproteins (Vancomycin), Macrolide (Azithromycin) and Amphenicol (Chloramphenicol).

Both Gram-positive and Gram-negative bacteria isolates were tested on: Levofloxacin (5 µg), Ofloxacin (5 µg), Gentamycin (10 µg), Tetracycline (30 µg), Ciprofloxacin(5 µg), Ampicillin (10 µg), Cefoxitin (30 µg), and

Chloramphenicol (30 µg). Additionally, Gram-positive bacteria were tested on Oxacillin (1 µg), Clindamycin (2 µg), Azithromycin (15 µg), and Vancomycin (30 µg), while Gram-negative bacteria were also tested on Imipenem (10 µg), Amoxiclav (30 µg), Cefuroxime (30 µg), Cefixime (5 µg), Amikacin (30 µg), and Streptomycin (10 µg). The plates were then incubated at 37°C for 18–24 h. The diameter of zones of inhibition was measured in millimeters using a vernier caliper, and results were interpreted according to the guidelines of Clinical and Laboratory Standards Institute [46].

Molecular detection of resistance genes

Genomic DNA from multidrug resistant bacterial isolates was extracted using the boiling method [47] with slight modifications. Briefly, several colonies from an overnight culture of each test bacteria grown on Mueller-Hinton Agar were transferred and emulsified in 100 µL PCR water contained in a labeled Eppendorf tube. Subsequently, the tubes were placed in a heat block to boil the bacterial suspension at 95°C for 1 h and 30 min, and then allowed to cool for 30 min. The tubes were then centrifuged at 15,000 rpm for 3 min, and 70 µL of each obtained supernatant that contained the crude DNA was transferred into a fresh Eppendorf tube. The extracted DNA (2µL) was quantified using Nanodrop lite Spectrophotometer (Thermo Scientific) at wavelengths of 260 nm and 280 nm of which a ratio of approximately 1.8–2 was considered as good purity [48]. The crude DNA was stored at ⁻20 °C for subsequent PCR assays.

PCR for detection of resistance genes

Polymerase Chain reaction (PCR) was performed using DNA isolated from selected bacteria that exhibited multi-drug resistance, including resistance to all the antibiotics within the 7 classes of antibiotics tested, cefoxitin inclusive for *S. aureus* (detection of *mec*A gene). For Gram-negative bacteria, screening for extended-spectrum beta-lactamase production was done using Cefixime (third generation cephalosporins), while for carbapenem resistance was done using Imipenem. The selected resistance genes were detected using the conventional uniplex PCR methods as described by Ibrahim [48] with slight modifications. The PCR master mix was prepared as follows: 12.5µL Hot Start Taq2x master mix (M0496S)-New England Bio-labs, 1.0µL forward (10µM), 1.0µL reverse (10µM), 5.0µL DNA template and 5.5µL RNAase-Free-H_2O making up to 25.0µL final reaction volume. The PCR amplification was carried out in a conventional PCR Thermocycler (CLASSIC K960 Thermal Cycler). The programme included; initial denaturation at 95 °C for 1 min followed by 35 cycles (denaturation at 95 °C for 45 s, annealing at 55 °C (for *mec*A, *bla*SHV, *bla*TEM, and *bla*KPC genes) and 62 °C for *bla*CTX-M-1 for 1 min and elongation at 72 °C for 1 min) and the final extension cycle of 72 °C for 5 min. The primers used included: *bla*TEM-F (CATTTCCGTGTCGCCCTTATT C) and *bla*TEM-R (CGTTCATCCATAGTTGCCTGAC) of amplicon size 800 bp [49]. *bla*SHV-F (ATGCGTTATA TTCGCCTGTG) and *bla*SHV-R (TGCTTTGTTATTCG GGCCAA) of 747 bp [50]. *bla*KPC-F (TCGAACAGGAC TTTGGCG) and *bla*KPC-R(GGAACCAGCGCATTTT TGC) of 201 bp [51]; *bla*CTX-M-1-F (GACGATGTCAC TGGCTGAGC) and *bla*CTX-M-1-R (AGCCGCCGACG CTAATACA) of 499 bp [52]. *mec*A-F (GTGAAGATATA CCAAGTGATT) and *mec*A-R (ATGCGCTATAGATTG AAAGGAT) of 147 bp [53].

Gel electrophoresis

DNA Amplicons were electrophoresed using 1.5% agarose gel prepared with 1x Tris-Borate EDTA buffer (TBE) and 5µL Safe View Classic™ DNA stain (cat # G108) as described [54]. 6X loading dye (Thermo Scientific #R0611) was added to each of the PCR product tube, and approximately 20 µL of each sample (including both positive and negative controls) and DNA ladder 100 bp (NEB-Biolabs #N3231L) were loaded into the gel wells. Subsequently, the gel was loaded into the electrophoretic tank that contained 1x TBE buffer. Electrophoresis was run at 200 V and 80 mA for 1 h. Bands were visualized using the Gene-Flash Trans-illuminator, and a photograph captured for examination.

Data analysis

Data was entered in Ms Excel version 15 and later exported to Stata version 15 (Stata Corp®) for analysis. Descriptive statistics, including frequencies and percentages, were used to summarize the data.

Results

Pathogens isolated from patients with DFU

A total of 117 patients with diabetic foot ulcer participated in this study and these included; 12, 14, 14, 17, 29, 21 and 10 patients from Fort Portal (FT), Hoima (HM), Jinja (GA), Kiruddu (KD), Kitagata (KT), KIU-TH (KU) and Mbarara (MA) referral hospitals, respectively. Among the 117 DFU patients, 105 (89.7%) yielded bacterial growth (for diabetic foot infection (DFI)). Among patients whose samples yielded bacterial growth, 80 (76.2%) had monomicrobial infections while 25 (23.8%) had poly microbial infections. Of the 25 patients with poly-microbial infections, 15 (14.3%), 6 (5.7%) and 4 (3.8%) had two, three, and four bacteria isolated, respectively.

A total of 144 bacterial isolates were obtained from 105 patients that had DFI. Table 1. summarizes bacteria isolated from the DFU patients.

Table 1 Bacterial profile on diabetic foot ulcers of patients from the different referral hospitals

Bacteria isolated	Referral hospitals							Total (%)
	FT	GA	HM	KD	KT	KU	MA	
Gram positive bacteria (*n* = 80, 55.6%)								
Staphylococcus aureus	5	7	4	7	16	11	2	52 (36.1)
Staphylococcus epidermidis	-	-	-	2	-	-	-	2 (1.4)
Staphylocous spp	-	1	-	-	3	1	1	6 (4.2)
Enterococcus faecalis	-	5	-	6	2	1	-	14 (9.7)
Enterococcus faecium	-	1	-	2	-	-	-	3 (2.1)
Bacillus cereus	-	-	1	-	-	-	-	1 (0.7)
Streptococcus pyogenes	-	1	-	-	-	-	-	1 (0.7)
Listeria spp	1	-	-	-	-	-	-	1 (0.7)
Gram negative bacteria (*n* = 64, 44.4%)								
Proteus mirabilis	-	1	-	-	-	-	-	1 (0.7)
Proteus spp	3	4	4	-	5	2	2	20 (13.9)
Pseudomonas aeruginosa	-	-	-	-	-	2	-	2 (1.4)
Pseudomonas spp	1	-	-	1	1	3	5	11 (7.6)
E. coli	-	5	-	5	-	-	-	10 (6.9)
Klebsiella pneumoniae	-	1	-	4	-	-	-	5 (3.5)
Klebsiella spp	-	1	-	6	1	1	-	9 (6.3)
Proteus vulgaris	-	-	1	-	1	-	-	2 (1.4)
Citrobacter diversus	-	-	1	-	-	-	-	1 (0.7)
Citrobacter spp	-	-	-	1	-	-	-	1 (0.7)
Haemophilis spp	-	-	-	-	-	1	-	1 (0.7)
Neisseria spp	-	-	-	-	-	1	-	1 (0.7)
Total isolates	10	27	11	34	29	23	10	144

Where: FT is Fort Portal Regional Referral Hospital, HM is Hoima Regional Referral Hospital, GA is Jinja Regional Referral Hospital, KD is Kiruddu Regional Referral Hospital, KT is Kitagata Regional Referral Hospital, KU Kampala International University- Teaching Hospital, and MA is Mbarara Regional Referral Hospital

Susceptibility patterns of the isolated bacteria to commonly used antibiotics

Except for *Listeria spp (1) and Bacillus spp (1)* that did not have guidelines on antibiotics to use in the CLSI [46], the rest of the isolates (142) were tested for their susceptibility to some of the 10 classes of antibiotics that are commonly used in the management of DFU in our setting. One hundred and forty (98.6%) of the isolates were multidrug resistant (non-susceptible to at least three antimicrobial categories). Approximately 21% (n = 62) of the Gram-negative isolates tested were resistant to carbapenem, considered to be the last resort treatment, and 66.7% (n = 51) were resistant to Cefixime (a third-generation cephalosporin) (Table 2). High resistance to Cefoxitin was registered in 98% of the *S. aureus* and Vancomycin resistance registered in *Enterococcus faecium* (67%) and *Enterococcus faecalis* (79%). Details of resistance patterns for Gram negative and Gram-positive bacteria to the commonly used antibiotic are summarized in Tables 2 and 3, respectively.

Characterization of *mec*A genes from *Staphylococcus aureus*

Nine (9) of the *S. aureus* isolates that showed multidrug resistance (MDR), including phenotypic resistance to cefoxitin antibiotic were subjected to uniplex PCR for detection of *mec*A genes. Approximately 147 bp (bp) amplicon size was obtained for *mec*A genes. Out of the nine MDR *S. aureus* isolates analyzed for *mec*A genes, five (56%) isolates (KD14, KD11a, GA08, KT16, and KD11b) were positive, as shown in Fig. 1 below.

Characterization of *β-Lactamase* genes from selected MDR Gram-negative bacteria

Ten (10) Gram-negative isolates including two *E. coli* (KD17EC and KD10EC), five Klebsiella (KD13Kleb, KD15Kleb, GA17Kleb, KD26Kleb and KD14Kleb), one Pseudomonas (HM18Ps) and two *Proteus spp* (GA20 Pro and KD06Pro), that exhibited multidrug resistance, resistance to Imipenem inclusive, were subjected to uniplex PCR for detection of TEM, KPC, *bla*CTX-M-1 *and bla*SHV genes. All isolates were negative for TEM and KPC genes. Approximately, 499 bp (bp) amplicon size was obtained for *bla*CTX-M-1 gene, and of the 10 isolates analyzed, six (60%) isolates (KD17EC, KD13Kleb, KD14Kleb, 10-HM18Ps, GA20 Pro and 12-KD06Pro) were positive for *bla*CTX-M-1 gene as shown in Fig. 2 below.

Approximately, 201 bp (bp) amplicon size was obtained for *bla*SHV genes. Out of the five isolates analyzed, only one (20%) isolate (KD14Kleb) was also positive for *bla*SHV genes as shown in Fig. 3 below. There was the

Table 2 Resistance patterns of the prevalent Gram-negative bacteria on DFU to commonly used antibiotics

Isolates	Imipenem	Levofloxacin	Cefuroxime	Gentamycin	Tetracycline	Ciprofloxacin	Amikacin	Cefixime	Ampicillin	Amoxiclav	Ofloxacin	Cefoxitin	Streptomycin	Chloramphenicol	Azithromycin	MDR
E. coli (10)	2(20)	6(60)	6(60)	5(50)	6(60)	4(40)	4(40)	5(50)	9(90)	4(40)	9(90)	8(80)	8(80)	10(100)	-	10(100)
Haemophilis spp (1)	-	1(100)	0	-	1(100)	0	-	1(100)	1(100)	-	0	-	-	1(100)	-	1(100)
Klebsiella pneumoniae (5)	2(40)	5(100)	5(100)	3(60)	3(60)	5(100)	5(100)	5(100)	2(40)	5 (100)	5(100)	5(100)	4(80)	5(100)	-	5(100)
Klebsiella spp (9)	1(11)	7(78)	5(56)	2(22)	6(67)	5(56)	7(78)	6(67)	9(100)	8(89)	9(100)	9(100)	4(44)	6(67)	-	9(100)
Proteus mirabilis(1)	0	0	0	0	1(100)	1(100)	1(100)	0	1(100)	1(100)	1(100)	0	1(100)	1(100)	-	1(100)
Proteus spp (20)	2(10)	13(65)	20(100)	14(70)	20(100)	9(45)	14(70)	15(75)	20(100)	20(100)	15(75)	18(90)	16(80)	18(90)	-	20(100)
Proteus vulgaris (2)	1(50)	1(50)	2(100)	0	2(100)	0	2(100)	1(50)	2(100)	2(100)	1(50)	2(100)	1(50)	2(100)	-	2(100)
Pseudomonas aeruginosa (2)	2(100)	2(100)	-	-	-	2(100)	1(50)	-	-	-	1(50)	-	-	-	-	2(100)
Pseudomonas spp (11)	2(18)	10(91)	-	-	-	8(73)	6(55)	-	-	-	9(82)	-	-	-	-	9(82)
Neisseria spp	-	-	-	-	1(100)	0	-	0	-	-	-	1(100)	-	-	1(100)	1(100)
Citrobacter diversus (1)	0	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0	0	1(100)	0	0	1(100)	-	1(100)
Citrobacter spp (1)	1(100)	0	0	1(100)	1(100)	0	1(100)	0	0	0	1(100)	0	1 (100)	1(100)	-	1(100)
Number of isolates tested on each antibiotic	62	63	50	49	51	64	62	51	50	49	63	51	49	50	-	
Number of resistant isolates (%)	13 (21)	46 (73)	39(78)	26(53)	42 (82.4)	35(54.7)	42(68)	34(66.7)	44(88)	40(81.6)	52(82.5)	44(86.3)	35(71)	45(90)	1(100)	

Where: - represents an Antibiotic not recommended for antimicrobial susceptibility testing on the bacteria

The Number after each Isolate (column 1) shows the Number of bacterial isolates tested

The Number indicated against each tested antibiotic indicates the number of isolates showing resistance and the percentage in brackets presence of mixed genes *bla*CTX-M-1 and *bla*SHV genes in sample KD14Kleb.

Discussion

The high prevalence (89.7%) of the DFU patients who presented with DFI is in line with earlier reports [55, 56], which indicated that a considerable proportion of patients with DFU develop infection. Absence of growth in samples from 12 (10.3%) patients with DFU was probably due to reduced recovery of fastidious organisms due to extended storage of swab samples (up to seven days) which is a limitation of this study; or multiple antibiotherapy used by the patients before seeking medical care, including the use of herbs known to prevent infections and persistent inflammation on these wound [57–60].

This study focused exclusively on aerobic bacteria, and as a result, anaerobic bacteria were not isolated, which is a limitation. However, among the aerobic bacteria isolated, the majority (76.2%) of patients with DFU exhibited monomicrobial growth. This finding is higher than those reported in some earlier studies, in Mulago hospital, Uganda (59%) and in Northeast of Tamaulipas Mexico (51.6%) [61, 62]. In countries where culture and sensitivity testing - crucial for selecting the most effective drug for managing diabetic foot infections (DFI) - is limited, clinicians often resort to routine antimicrobial combinations. However, as demonstrated by this study, such an approach is not justifiable for most patients. Polymicrobial growth was observed in 23.8% of the patients in this study. This prevalence is lower than was reported in DFU patients from selected hospitals in Addis Ababa, Ethiopia (68%) [63], Calabar Municipality, Nigeria (70%) [10] and a tertiary care hospital in Karachi, Pakistan (83%) [9]. However, it is higher than the 12% reported by Wu in patients from the Southwest hospital, China [5] and 21% reported in patients from Nemazee Hospital, Southern Iran [64]. The presence of polymicrobial growth among DFU patients is one of the major causes of treatment failure, complications like chronicity of diabetic wounds, and amputations that necessitate culture and sensitivity to guide the treatment regime.

The prevalence of Gram-positive bacteria (55.6%) in our study is consistent with studies in China from patients of third Xiangya hospital, Central south University and Huaihua Cancer hospital where prevalence of 59% and 52.3%, respectively, were reported [65, 66], but inconsistent with earlier studies in; Kasr Alainy Hospital, Egypt, Mulago hospital, Uganda and Kenyatta National Hospital, Kenya that identified Gram negative bacteria as most prevalent from 56.1%, 80.6% and 65% of the patients, respectively [14, 61, 67]. Therefore, in our setting, empirical treatment should be directed towards Gram-positive organisms.

Staphylococcus aureus (36.1%) was the most common Gram-positive bacterium found in DFU cases in this study, which aligns with findings from the meta-analyses conducted in sub-Saharan Africa, where its prevalence of 34.3% was reported [68], and studies in Asia, which reported 22% [69]. Similar findings were observed in Mbarara Regional Referral Hospital (27.6%) [70], a review in Uganda (33.3%) [71], and several studies in India where 20% from patients in a tertiary care and referral hospital [8], 18% in a meta-analysis [72], 13.4% in patients from Meenakshi medical college and research institute [73], and 20.7% from patients with chronic wounds at Jaramogi Oginga Odinga Teaching and Referral Hospital, Kenya [74].

Proteus spp (13.9%, $n = 20$) was the most prevalent Gram-negative bacteria in this study. The finding is consistent with a study conducted at Kasr Alainy Hospital in Egypt, where the prevalence of *Proteus spp* was 16.8% [67]. However, the finding differs from studies that identified *Pseudomonas aeruginosa* as most prevalent Gram-negative bacterium, with 18.9% of the patients from selected hospitals in Addis Ababa, Ethiopia [63], 17% from the meta-analysis of Asiatic countries [69], and 14.8% for *Pseudomonas aeruginosa* and *Klebsiella spp* from patients' chronic wounds in a teaching hospital in Kenya [74]. Additionally, *Escherichia coli* was the most prevalent in several studies: 21.2% in a meta-analysis from sub Saharan Africa [68], 27.4% in a study in India [73], 28.1% from Huaihua Cancer hospital, China [66], 41.6% in patients from various hospitals in Pakistan [75]; 20.5% from Shahid Mohammadi Hospital in Iran [76]; and 15% in patients from Kenyatta National Hospital in Kenya [14].

Imipenem was the most sensitive antibiotic for most Gram-negative bacteria on which it was tested. Sensitivity of 80%, 60%, 50% and 0% on *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*, respectively, was observed. These findings are in alignment with a study from Ethiopia, which reported sensitivity rates for *E. coli* (76.2%), higher for *Klebsiella pneumoniae* (40%), and lower for *Proteus vulgaris* (75%) and *Pseudomonas aeruginosa* (54.2%) [63]. This study was not able to test the isolated bacteria on all the antibiotics as recommended by the CLSI [46] due to their unavailability, which is another limitation for this study. However, even with the antibiotics classes tested, the study showed that, 98.6% of the bacterial isolates from patients with DFI were multidrug-resistant (MDR). The MDR observed in this study is higher than reports from the studies in Nigeria (80%) [77], 56% in a study from United States of America [78] and 46.7% in a study from China [56, 79]. Carbapenem resistant *Pseudomonas aeruginosa* (2/2), Methicillin resistant *Staphylococcus aureus* (51/52) and Vancomycin-resistant *Enterococcus faecium*

Table 3 Resistance patterns of the prevalent Gram positive bacteria on DFU to commonly used Antibiotic

Isolates	Levofloxacin	Oxacillin	Gentamycin	Clindamycin	Tetracycline	Vancomycin	Ciprofloxacin	Ampicillin	Azithromycin	Ofloxacin	Cefoxitin	Chloramphenicol	MDR
Enterococcus faecalis (14)	10(67)	-	-	-	8(57)	11(79)	11(79)	14(100)	-	-	-	14(100)	14(100)
Enterococcus faecium (3)	3(100)	-	-	-	2(67)	2(67)	3(100)	2(67)	-	-	-	3(100)	3(100)
Staph aureus (52)	44(85)	-	31(60)	40(77)	48(92)	-	31(60)	-	42(81)	42(83)	51(98)	42(81)	52(100)
Staph epidermidis (2)	2(100)	1(50)	0	1(50)	0	-	1(50)	-	2(100)	2(100)	2(100)	2(100)	2(100)
Staphylococcus spp (6)	4(67)	5(83)	6(100)	4(67)	6(100)	-	5(83)	-	6(100)	5(83)	5(83)	5(83)	6(100)
Streptococcus pyogenes (1)	-	-	-	1(100)	1(100)	-	-	-	0	1(100)	-	0	1(100)
Number of isolates tested on each antibiotic	77	8	60	61	78	17	77	17	61	61	60	78	
Number of resistant isolates (%)	63(81.8)	6(75)	37(62)	46(75)	58(74)	13 (76.5)	51(77)	16(94)	50(82)	50 (82)	58(97)	66 (85)	

Where: - represents an Antibiotic not recommended for antimicrobial susceptibility testing on the bacteria

The Number after each Isolate (column 1) shows the Number of bacterial isolates tested

The Number indicated against each tested antibiotic indicates the number of isolates showing resistance and the percentage in brackets

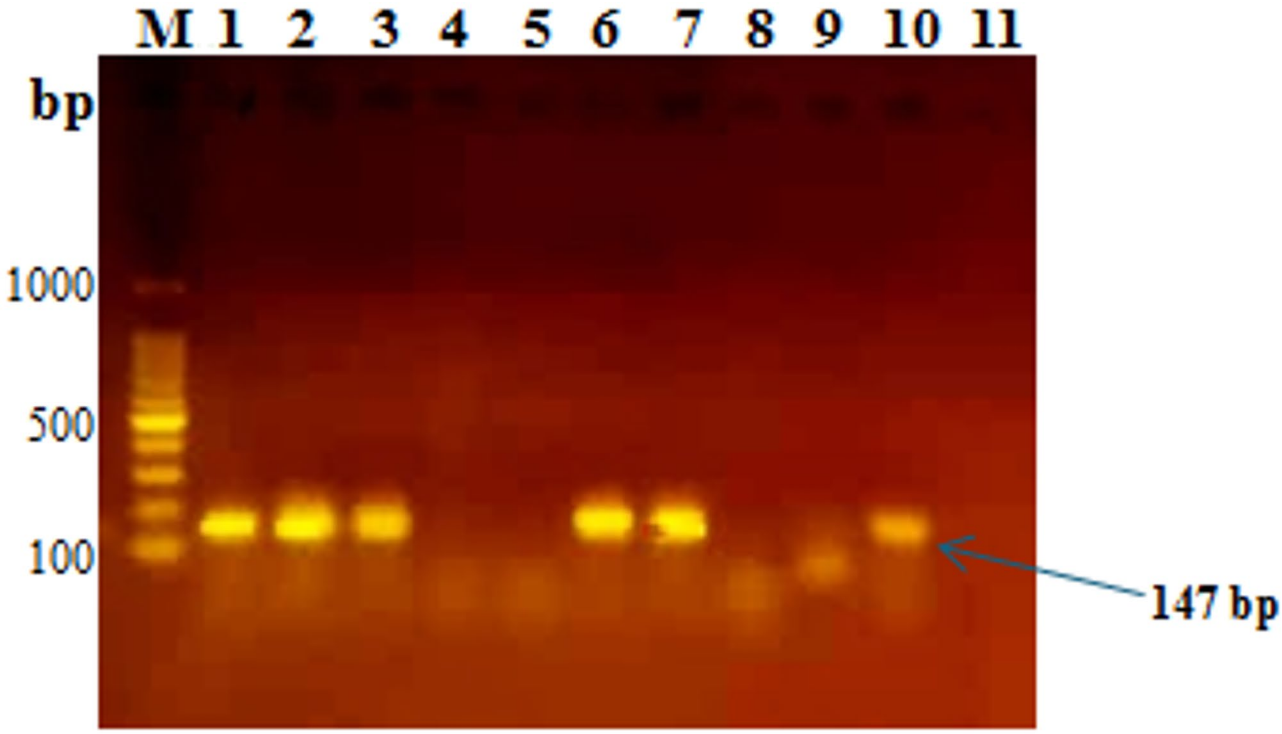

Fig. 1 Gel electrophoresis of PCR product of selected multi-drug resistant *S. aureus* isolated from wounds of patients with DFI for mecA gene. Where lane; M is DNA ladder (100 bp to 1000 bp), 1 is KD14, 2 is KD11a, 3 is GA08, 4 is HM07, 5 is HM04, 6 is KT16, 7 is KD11b, 8 is KD19, 9 is GA15, 10 is Positive control (*S. aureus* ATCC 33591) and 11 is a Non template control

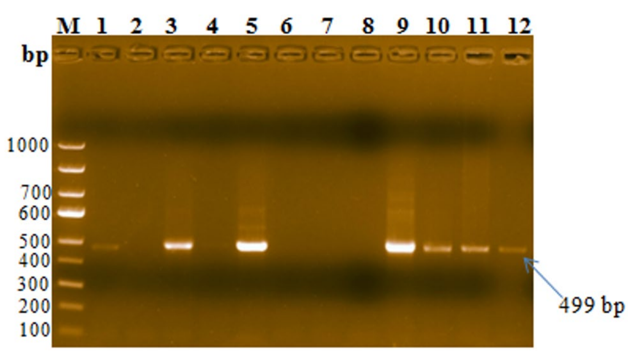

Fig. 2 Gel electrophoresis of PCR product of selected Gram-negative multidrug-resistant bacteria from wounds of patients with DFI for *bla*CTX-M-1 gene. Where: M is DNA ladder (100 bp to 1000 bp), 1 is KD17EC, 2 is KD10EC, 3 is Positive control (*E. coli* NCTC 13353), 4 is Non template control, 5 is KD13Kleb, 6 is KD15Kleb, 7 is GA17kleb, 8 is KD26Kleb, 9 is KD14Kleb), 10 is HM18Ps), 11 is GA20 Pro and 12 is KD06Pro

(2/3) were also identified in this study. These organisms are classified as high-priority pathogens of public health importance by the World Health Organisation [80]. The presence of MDR bacteria in this study renders antibiotics ineffective, potentially leading to amputation or even death. Multi-drug resistance is likely due to factors such as inappropriate use of antibiotics, unrestricted access to antimicrobial drugs in our setting [81], unnecessary prolonged use of empiric broad-spectrum antibiotics, or the fact that samples were collected from patients attending referral hospitals. The presence of MDR bacteria not only limits antimicrobial options but also necessitates a change in the treatment strategies. These strategies should be informed by local microbiological data or guided by culture and sensitivity testing to ensure the appropriate selection of antibiotics. Addressing priority MDROs requires robust stewardship to ensure appropriate use of antibiotics as well as the development of diagnostic capacity, including point-of-care tests and antimicrobial susceptibility testing to reduce unnecessary antibiotic prescriptions [80], as well as a need for alternatives to treatment, such as phage therapy.

Due to financial constraints, this study was unable to confirm *mecA* genes in 98% presumed MRSA that were phenotypically resistant to Cefoxitin, as well as other genes, *mec*C inclusive, which is a limitation for this study. Nevertheless, 56% (n = 9) MRSA strains were positive for the *mec*A gene, which encodes for methicillin resistance. This finding is in line with a study done in Saudi Arabia, which reported 53.8% prevalence of *mec*A for MRSA [20] but lower than the 96.8% identified in a study in Mexico city, North America [82], 90.2% in Shendi City, Sudan [83], and the 100% in a study done in Yogyakarta, Indonesia [84]. However, this study finding is higher than the 46% reported from an earlier study in Uganda [26] and 21.7% in global meta-analysis [85]. The presence of *mec*A gene is sufficient evidence for detection of MRSA strains

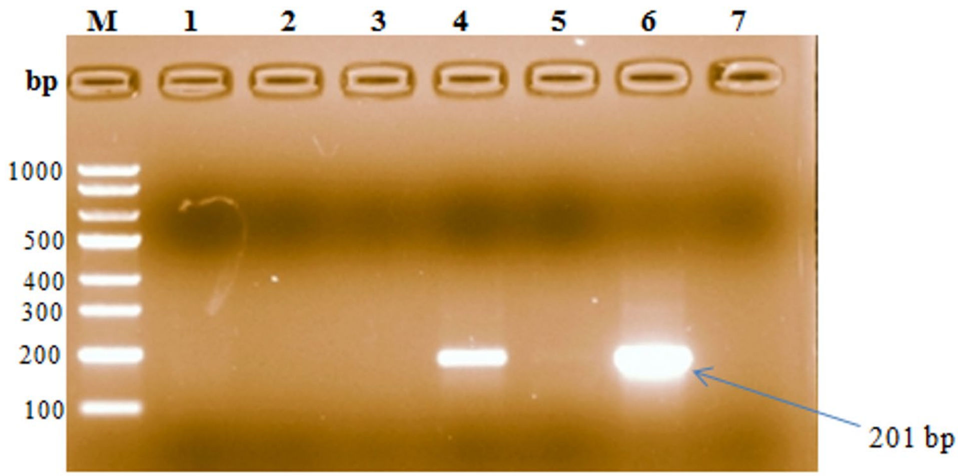

Fig. 3 Gel electrophoresis of PCR products for selected Gram-negative multidrug-resistant bacteria from wounds of patients with DFI for *bla*SHV gene. Where: M is DNA ladder (100 bp to 1000 bp), 1 is KD17EC, 2 is KD13Kleb, 3 is HM18Ps, 4 is Positive control (*Klebsiella pneumoniae* NCTC 13368); 5 is Non template control, 6 is KD14Kleb and 7 is KD06Pro

as it encodes an additional penicillin-binding protein (PBP2a) that confer resistance to methicillin and other beta-lactam antibiotics. In our setting, targeted treatment is essential to avoid treatment failures and misuse of antibiotics along with effective infection control strategies to prevent spread of resistant genes. The absence of *mec*A genes in 44% of the MRSA identified phenotypically is in agreement with studies advocating for use of molecular diagnostic methods to confirm phenotypic results to avoid false positives [16].

Extended spectrum Beta-Lactamase (ESBL) production was presumed in 34/51 (66.7%) bacteria isolates detected phenotypically based on resistance to Cefixime alone without conducting a combined disc test using Cefixime-Clavulanate, which is a limitation of this study. The presence of carbapenem-resistant genes *bla*CTX-M-1, *bla*SHV, KPC, and TEM genes was investigated in selected MDR *E. coli*, *Klebsiella pneumoniae*, *Proteus spp*, and *Pseudomonas aeruginosa.* Of the 10 samples tested, 60% (6/10) isolates, including *E. coli (1/2) Klebsiella pneumoniae* (2/5), *Proteus spp (2/2), and Pseudomonas aeruginosa* (1/1) were positive for *bla*CTX-M-1. The *bla*SHV gene was also detected in 1/5 (20%) of the isolates. This finding is in line with a study in the Gaza Strip where the prevalence of *bla*CTX-M was 60% [86]. However, these findings were higher than studies; in China, on clinical samples of *E.coli* isolated from faeces, urine, and blood where *bla*CTX-Ms genotypes were 34.7% [87], in Nepal with a prevalence 49.3% [88] and in Bangladesh with *bla*CTX-M-1 at 20.7% and *bla*SHV at 3.8% [89]. Our findings were lower than those for a study in Jos Nigeria, where *bla*CTX-M-1 genes were found in all the 20(100%) isolates (17 Klebsiella and 3 *Pseudomonas spp*), while *bla*SHV genes were detected in 16(80%) isolates [90]. These results align with reports showing an increasing incidence of infections caused by extended spectrum beta-lactamase-producing Gram-negative bacteria primarily carrying CTX-M genes [91]. The presence of one *Klebsiella pneumoniae* isolate with both CTX-M and SHV compares with a study by Shahi [24], which reported mixed resistance genes in 17/38 (45%) of Gram-negative MDR bacteria, an indication of a higher degree of antibiotic resistance, with implications for treatment and infection control. Despite the relatively small sample size, the results highlight the need for continuous surveillance of the resistance genes and coordinated infection prevention and control to curb their spread.

Conclusion

The study underscores the critical need for implementing culture and sensitivity test-informed treatment strategies in the management of diabetic foot infections, given the high prevalence of multidrug-resistant bacteria and the detection of key resistance genes such as *mec*A, *bla*CTX-M-1, and *bla*SHV. Furthermore, exploring alternative therapies, such as phage therapy, and implementing continuous surveillance and coordinated infection control measures are crucial to mitigate the spread of resistant pathogens, and improve patient outcomes.

Abbreviations

API	Analytical Profile Index
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute
DFU	Diabetic foot ulcers
DFI	Diabetic foot infection
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
ESBL-PE	extended-spectrum beta-lactamase-producing Enterobacterales
KPC	Klebsiella pneumoniae carbapenemase
MDR	Multidrug resistance
MDRO	Multi drug resistant organisms

MHA Mueller-Hinton Agar
MRSA Methicillin-resistant Staphylococcus aureus
MR-VP Methyl red – Voges – Proskauer
PCR Polymerase Chain Reaction
Staph Staphylococcus
Strep Streptococcus

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

G.W.N and A.N conceived the concept. A.N applied for funding, analysed the data and wrote the manuscript. B.M.V, I.N, F.K.S, T.P and R.S collected samples from patients. I.N and T.P did laboratory analyses. I.N, R.S, B.M.V, T.P, F.K.S, R.N, A.N, P.K, E.S, G.A and G.W.N critically reviewed the manuscript. All authors reviewed and approved the final manuscript.

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Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

The study and attendant protocols were approved by Kampala International University Research Ethics Committee (KIU-REC) under reference number (KIU-REC-2021-57). Hospital approvals were obtained from the selected hospitals. Informed consent was obtained from all the patients.
All methods were carried out in accordance with relevant guidelines and regulations as detailed in [29].

Consent for publication

Consent to publish on part of the participants is not applicable since there is no identifying information.

Competing interests

The authors declare no competing interests.

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