Middle East Research Journal of Medical Sciences
ISSN: 2789-7699 (Print) & ISSN: 2958-2024 (Online)
Frequency: Bi-Monthly
DOI: 10.36348/merjms.2023.v03i02.001

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Patterns of Pathogens and Antimicrobial Susceptibility Profiles in Diabetic Foot Ulcers' Infections among Patients at Bugando Medical Centre in Mwanza, Tanzania

Peter Kibunto[1, 3*], Barker Peter[2], Yasin Munisi[1], Samwel Byabato[1, 2], Hyasinta Jaka[2, 3], Shangwe Sam[1], Alicia Massenga[1, 2], Leornad Washington[1, 2], Vihar Kotecha[1, 2], Fabian Massaga[1, 2], Prof Jeremiah Seni[2]

[1]Department of Surgery, Bugando Medical Centre
[2]Department of Surgery, Catholic University of Health and Allied Sciences, Tanzania
[3]Mwanza College of Health and Allied Sciences, Tanzania

Abstract: ***Background*:** Diabetic foot ulcer (DFU) is an open sore or wound on the foot of a person with diabetes, and is most commonly located on the plantar surface, or bottom of the foot. The defect is relatively common in adult and elders with diabetes mellitus. Infected lower extremities wound with multi-drug resistant bacteria usually are associated with increased morbidity, mortality and long-term disabilities among diabetic patients. Although the burden of DFU is known in Tanzania (and Mwanza in particular), there is limited information on the patterns of pathogens associated with DFU in our setting, on bacterial and fungal pathogens which in turn limit specific management options to these patients. ***Methodology*:** Analytical cross section hospital-based study was conducted among patients with DFU admitted or attending outpatient clinics at BMC from May to July 2022. A structured questionnaire was used to collect socio-demographic, clinical and laboratory data from patients with DFU. Tissue culture was obtained from the base of the ulcer after cleaning with normal saline followed by removing of cellular debris and normal flora over the lesion. Finally, the obtained samples were subjected to culture methods to identify the presence of pathogens (bacteria and fungi) and antimicrobial susceptibility profiles of bacteria. ***Result*:** During the study period and based on inclusion criteria, a total of 71 patients, with a mean age of 59.2 ± 13.0 years were recruited. The majority of the participants were male 47 (66.2%). A total of 60 (84.5%) samples were culture positive, resulting into a total of 92 microorganisms isolated. More than half of cultures 34 (56.7%) revealed the presence of single microorganisms. Pathogens isolated were both bacterial 70 (76.1%) and fungi species 22 (23.9%), all bacterial isolates were aerobic. *P. aeruginosa* and *E. coli* were most frequent isolated gram-negative bacteria 12(23.5%) and 9(17.6%) respectively. On other hand common isolated gram-positive strains were *S. aureus* 13 (68.4%) out of all 19 gram-positive culture isolates. A total of 22 fungi spp were isolated, among them 7 (31.8%) were yeast *C. albicans* 3 (42.8%), *A. fumigatus* were frequent isolated 13 (86.7%). Polymicrobial growth was observed in 43.3% samples against 56.7% of monomicrobial growth. Most prevalent gram-negative bacteria *Pseudomonas aeruginosa* showed low resistance to ciprofloxacin, meropenem, gentamicin, piperacillin tazobactam (7.1%, 21.4%, 21.4% and 21.4 respectively) but more resistant to cephalosporins. *Staphylococcus aureus* showed low resistance to ciprofloxacin, gentamicin and clindamycin (30.8%, 15.4%, and 38.5% respectively), Total MDR bacteria isolates were 64.3%, where *methicillin resistance staphylococcus aureus* (MRSA) were 76.9%. More than 85.7% of the patient with higher grade ulcer, stage 3 and 4 according to Wagner's classification were positive to diabetic foot infection and 14.3% of patients with Wagner's stage 3 and 4 were not infected on their ulcers. ***Conclusion*:** Gram negative bacteria were most commonly isolated than gram positive bacteria in causing DFU infections. For gram negative spp the most effective antibiotic were ciprofloxacin, gentamicin, piperaccilin tazobactam and meropenem. Third generation cephalosporins, amoxicillin clavulanate showed poor effectiveness. All cases of DFU infection should therefore be subjected to culture and antimicrobial sensitivity testing for targeted infection management. More studies involving anaerobic pathogens and antifungal susceptibility patterns recommended.

Keywords: Diabetes, Bacteria, Drug, and Antibiotic.

Research Paper

***Corresponding Author:**
Peter Kibunto
Department of Surgery, Bugando Medical Centre

How to cite this paper:
Peter Kibunto *et al* (2023). Patterns of Pathogens and Antimicrobial Susceptibility Profiles in Diabetic Foot Ulcers' Infections among Patients at Bugando Medical Centre in Mwanza, Tanzania. *Middle East Res J. Med. Sci*, *3*(2): 17-27.

Article History:
| Submit: 12.08.2023 |
| Accepted: 13.09.2023 |
| Published: 15.09.2023 |

INTRODUCTION

Poorly controlled diabetes is the among of most common causes of lower limb ulcerations in developing counties [1]. Diabetic patients are susceptible to foot infection primarily due to neuropathy, vascular insufficiency, and diminished neutrophil function [2]. Factors predisposing ulcers to microbial colonization and proliferation are poor hygiene and poor blood perfusion [3].

Diabetic lower limb ulceration constitutes a major public health problem contributing significantly to high morbidity and long-term disabilities [4]. Results in prolonged hospital stay and delayed recovery [5]. Prolonged hospital stay usually exposes the patient to health care associated infections (HCAs) [6] and more risk to infection associated with multi-drug resistant bacteria like extended spectrum beta lactamase (ESBL) producers and *Methicillin resistant Staphylococcus aureus* (MRSA) [7, 8].

Diabetic foot ulcers may cause severe leg pain, long-standing and foul-smelling infected wounds, physical handicaps and even lower limb mutilation or amputation. These results in the economy lost to all affected societies and social stigmatisation of patients. In addition to expenditures incurred on treating the aetiology of DFU, affected patients also pay considerable expenses to podiatrists, wound care specialists, primary care physicians, vascular surgeons or dermatologists [9].

It is estimated that 15% to 20% of patients with diabetes will develop an ulcer on their foot at some point and among those ulcerated patient, 78%-92% will develop diabetic foot infections [10]. The prevalence of diabetic foot ulcers ranged between 1.0% and 4.1% in the United States, 4.6% in Kenya, and 20.4% in Netherlands [11-13].

The study done at Kilimanjaro Christian Medical Centre shows that 86.96% patients had a positive culture and 18.5% of them had more than one bacterium. Of the 55 total bacterial isolates, 85.4% were gram-negative bacteria. *Proteus vulgaris spp* 19.9%, *Pseudomonas spp* 14.8%, and *Staphylococcus aureus* 12.4%, were the most common bacteria isolated. Other isolates were *E coli* 7.7%, *Enterobacter* spp 7.7%, *Proteus mirabils* 6.2%, *Providencia spp* 4.6%, *Acinetobacter spp* 3%, and *Serratia spp* 1.5%, most isolate showed high resistance to commonly used antibiotics [4].

Previous local study shows that bacterial profile revealed polymicrobial pattern and *Staphylococcus aureus* was the most frequent microorganism isolated. All the microorganisms isolated showed high resistance to commonly used antibiotics except for Meropenem and imipenem, which were 100% sensitive each respectively [14].

Therefore, this study may provide and update information on pathogens (bacteria and fungi) profile and their antimicrobial susceptibility pattern which keeps on changing due to emergency of resistant bacterial strains as well as underlying causative factors associated isolated microorganisms among diabetic patient at Bugando Medical Center.

METHODOLOGY

Study design and setting

Analytical cross – section hospital – based conducted from January to July 2022 at Bugando Medical Centre.

Study participants

A total of 71 patients with diabetic ulcer at BMC were included in this study. kish Leslie formula was used with prevalence of 86.96% from the previous study done KCMC.

Data collection

Convenience sampling technique was used to recruit study participants. Data was collected by using structured questionnaire after pre testing at Regional Referral hospital, Tool for evaluation was done to see adequate /efficacy of the tool, patient who met the inclusion criteria were offed explanation about the study and requested to consent before being enrolled into the study.

Sample were collected by cleaning the wound with a normal saline followed by removing of superficial tissue or cellular debris using a surgical blade , then inner necrotic tissue were collected as a tissue culture and had put in aerobic transport medium (brain heart infusion broth) then labelled with unique identification number for each participants.

The research assistant and the principal investigator gathered relevant information regarding history, examination and laboratory result.

Data analysis

Data were entered into Epi infro then to micro excel and last exported to the STATA version 13.0.

RESULTS

Social demographic characteristics of the patients. A total of 71 patients, aged between 23 and 94 were recruited in the study; the mean age of study population was 59.2 ± 13.0 years. The majority of the participants were male 47 (66.2%) (Table 1).

Table 1: Social demographic characteristics of study participants, (*n* = 71)

Variable		Frequency (n)	Percentage (%)
Mean Age (Years)*		59.2 ± 13.0	-
Sex	Male	47	66.2
	Female	24	33.8
Marital Status	Married	65	91.6
	Single	6	8.4
Residency	Urban	33	46.4
	Rural	38	53.5
Education Level	None	7	9.9
	Primary	45	63.4
	Secondary	14	19.7
	Tertiary	5	7.0
Occupation Status	None	5	7.0
	Employed	4	5.6
	Peasant	34	47.9
	Business	15	21.2
	Retired	8	11.3
	Driver	2	2.8
	Tailor	1	1.4
	Welding	1	1.4
	Accountant	1	1.4
Economic status	Low	34	47.9
	Moderate	36	50.7
	High	1	1.4
Cigarette smoking	Yes	2	2.8
	No	69	97.2
Alcohol use	Yes	14	19.7
	No	57	80.3
Primary care giver	Yes	41	57.6
	No	30	42.3
Primary care giver relationship	Wife	16	39.0
	Relative	3	7.3
	Daughter	12	29.3
	Son	9	22.0
	Grand daughter	1	2.4
Primary care giver education level	None	1	2.4
	Primary	24	58.5
	Secondary	9	22.0
	Tertiary	7	17.1
Primary Care Giver Occupation	None	3	7.5
	Employed	1	2.5
	Peasant	18	45.0
	Retired	2	5
	Nurse	1	2.5
	Teacher	3	7.5
	Tailor	1	2.5
	Driver	1	2.5
	Business	9	22.5
	Electronics	1	2.5

*continuous variable

Clinical Characteristics

Table 2: Clinical data

Variable		Frequency (n)	Percentage (%)
Hospital status	Inpatients	44	62.0
	Outpatients	27	38.0
DM type	Type I	1	1.4
	Type II	70	98.6
DM family history	Yes	17	23.9
	No	54	76.1
Duration of DM (years)*		7 (IQR 3-11)	-
DM status	Controlled	69	97.2
	Uncontrolled	2	2.8
Ant diabetic agent	Injection	33	47.8
	Oral	36	52.2
	Herbal	0	0
DFU information	Yes	63	88.7
	No	8	11.3
Duration of diabetic foot ulcer (months)*		2 (IQR 1-6)	-
Meggitt Wagner classification of DFU	1	19	26.8
	2	30	42.3
	3	17	23.9
	4	5	7.0
Foot affected	Left	36	50.7
	Right	35	49.3
History of DFU	Yes	33	46.5
	No	38	53.5
Amputation history	Yes	19	26.8
	No	52	73.2
Knowledge on diabetic foot infection information	Yes	57	80.3
	No	14	19.7
Factor associated with DFI (patient)	Environment and poor hygiene	1	6.3
	Lack of wound dressing	2	12.4
	Poor cleaning and poor hygiene	1	6.3
	Poor hygiene	7	43.7
	Poor hygiene and water contact	1	6.3
	Poor hygiene and dust	1	6.3
	Poor hygiene and lack of	2	12.4
	wound dressing Poor hygiene, poor cleaning and covering of wound	1	6.3
Diagnosis history of diabetic foot infections	Yes	42	59.2
	No	29	40.8
Previous diagnosis from the same ulcer	Yes	27	93.1
	No	2	6.9
Time interval from previous diagnosis*		2 (IQR 1-4)	-
Antibiotic use on previous treatment	Yes	25	86.2
	No	4	13.8
Antibiotic completeness on previous treatment	Yes	25	100.0
	No	0	0.0
Hospital admission for past 3 months	Yes	61	85.9
	No	10	14.1

*continuous variable

Culture Results

Prevalence of laboratory confirmed diabetic foot infections among patients.

According to bacteriological profile result, 60 (84.5%) of isolated samples were culture positive, and 11 (15.5%) showed no growth. Thus, a total of 92 microorganisms were isolated from the culture positive

samples, more than half of cultures 34 (56.7%) revealed the presence of single microorganisms, whereas 26 (43.3%) of samples had mixed infections.

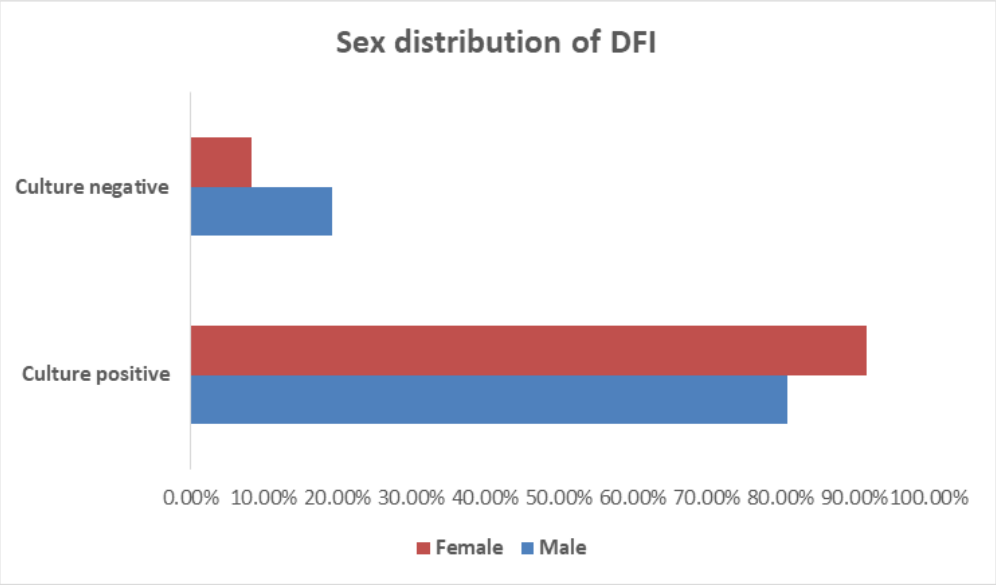

Figure 1: Prevalence distribution among male and female patients

Microbiology aetiologies of pathogens causing diabetic foot infections

Pathogens isolated were both bacterial 70 (76.1%) and fungi species 22 (23.9%), all bacterial isolates were aerobic. Among them, the gram-negative organisms were more frequent and isolated from about 51 (72.9%) cultures. *P. aeruginosa* and *E. coli* were most frequent isolated gram-negative bacteria 12(23.5%) and 9(17.6%) respectively. On other hand common isolated gram-positive strains were *S. aureus* 13 (68.4%) out of all 19 gram-positive culture isolates.

A total of 22 fungi spp were isolated, among them 7 (31.8%) were yeast *C. albicans* 3 (42.8%), *c. krusei* 2 (28.6%) and other *parapsilosis* 2 (28.6%). Mould fungi isolated were 15 (68.2%) cultures. *A. fumigatus* were frequent isolated 13 (86.7), Table 3.

Polymicrobial growth was observed in 43.3% samples against 56.7% of monomicrobial growth.

Table 3: Bacteriological profile of isolated microorganisms among the studied population, (n = 71)

Isolate	Frequency (n)	Percentage (%)
Pseudomonas aeruginosa	14	15.2
Staphylococcus aureus	13	14.1
Escherichia coli	9	9.8
Klebsiella pneumoniae	8	8.7
Enterobacter aerogenes	7	7.6
Staphylococcus epidermidis	5	5.4
Citrobacter freundii	4	4.3
Proteus vulgaris	4	4.3
Enterobacter cloacae	2	2.2
Acinetobacter spp	1	1.1
Serratia marcescens	1	1.1
Proteus mirabilis	1	1.1
Enterococcus spp	1	1.1
Aspergillus fumigatus	13	14.1
Aspergillus nidulans	2	2.2
Candida albicans	3	3.3
Candida krusei	2	2.2
Candida parapsilosis	2	2.2
Total	92	100

Antibiotic susceptibility pattern for isolated bacterial pathogens

Concerning antimicrobial sensitivity among bacterial isolate, 8 antimicrobial drugs were studied against isolated g ram-negative bacteria and 7 drugs against gram positive isolated bacteria and the result are summarized in Table 5. A total of 64.29% (45/70) MDR bacteria were isolated. Most prevalent gram-negative bacteria *Pseudomonas aeruginosa* showed low resistance to ciprofloxacin, meropenem, gentamicin, piperacillin tazobactam (7.1%, 21.4%, 21.4% and 21.4% respectively) but more resistant to cephalosporin's. Meanwhile, *Escherichia coli* strains showed no resistance to meropenem and piperacillin tazobactam, and low resistance to amoxicillin clavulanate and gentamicin were (22.2% and 33.3% respectively). Finally, *Klebsiella pneumoniae* showed high resistance to ciprofloxacin, tetracycline, amoxicillin clavulanate, ceftriaxone and ceftazidime (62.5%, 87.5%, 62.5%, 87.5%, and 87.5% respectively). The proportion of extended spectrum beta-lactamase (ESBL) phenotype producing gram negative bacteria was 50.0% (18/36) of which *K. pneumoniae, E. coli and E. aerogenes* were 27.8%, 22.2%, and 22.2% respectively, Figure 2.

Regarding gram positive isolates *staphylococcus aureus* showed low resistance to ciprofloxacin, gentamicin and clindamycin (30.8%, 15.4%, and 38.5% respectively) Table 5c, *methicillin resistance staphylococcus aureus* (MRSA) were 76.9% (10/13). Staphylococcus epidermidis showed high sensitivity to clindamycin 80% and high resistance to erythromycin 80%. Out of all 19 gram-positive isolates, only 36.8% (7/19) were positive to erythromycin induced clindamycin resistance phenotype, Figure 3.

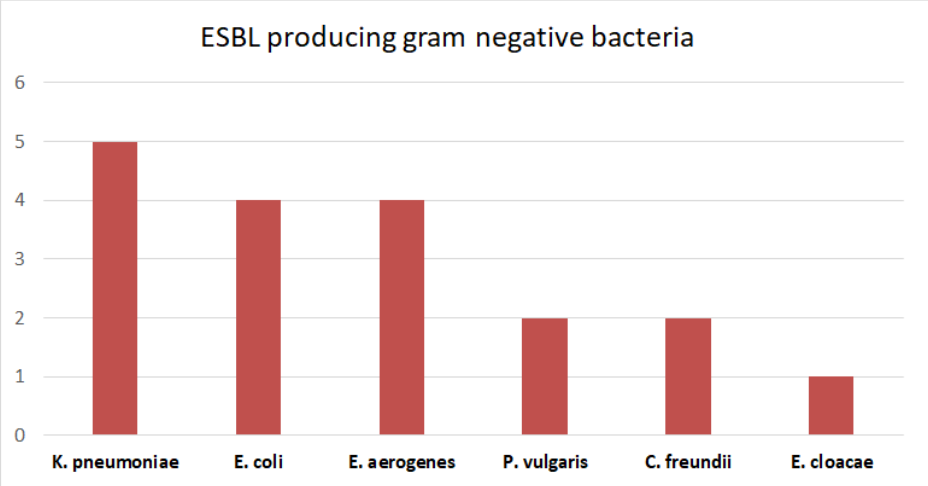

Figure 2: ESBL phenotype gram negative producing bacteria

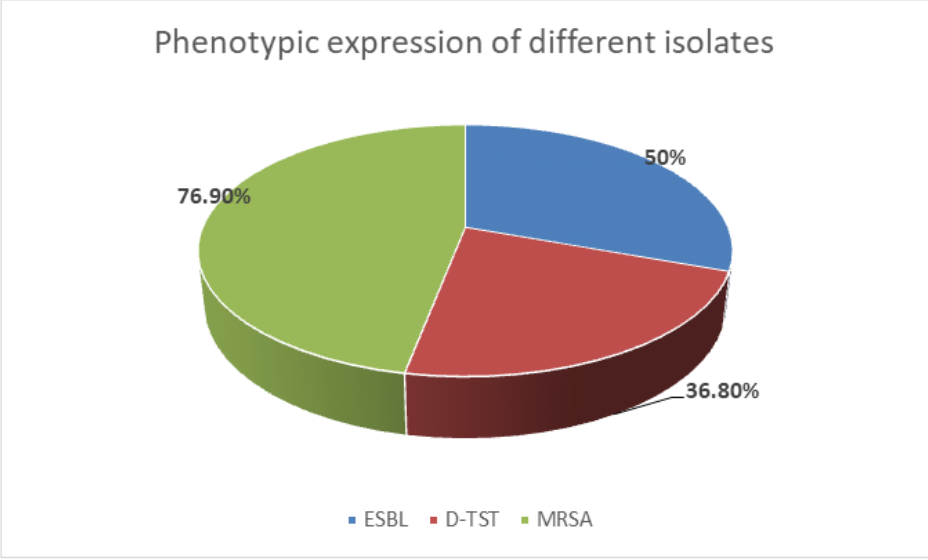

Figure 2: Phenotypic expression among isolated bacteria

Factors associated with laboratory confirmed diabetic foot infections

Table 4: Social demographic characteristics associated with laboratory confirmed diabetic foot infections among the study participants

Variable		Culture result		Chi^2	P value
		Negative n (%)	Positive n (%)		
Sex	Male	9 (19.2)	38 (80.8)		
	Female	2 (8.3)	22 (91.7)	1.419	0.233
Age (years)	20 – 40	0 (0.0)	5 (100.0)		
	41 – 60	5 (15.6)	27 (84.4)		
	>60	6 (17.6)	28 (82.4)	1.037	0.595
Marital status	Married	11 (16.9)	54 (83.1)		
	Single	0 (0.0)	6 (100.0)	1.201	0.273
Residency	Urban	5 (15.2)	28 (84.8)		
	Rural	6 (15.8)	32 (84.2)	0.005	0.941
Education Level	None	2 (28.6)	5 (71.4)		
	Primary	7 (15.5)	38 (84.4)		
	Secondary	2 (14.3)	12 (85.7)	1.846	0.605
	Tertiary	0 (0.0)	5 (100.0)		
Occupation Status	Peasant	4 (11.8)	30 (88.2)		
	Business	3 (20.0)	12 (80.0)		
	Retired	1 (12.5)	7 (87.5)	3.371	0.909
	None	1 (20.0)	4 (80.0)		
	Employed	1 (25.0)	3 (75.0)		
	Driver	1 (50.0)	1 (50.0)		
	Welding	0 (0.0)	1 (100.0)		
	Tailor	0 (0.0)	1 (100.0)		
	Accountant	0 (0.0)	1 (100.0)		
Economic Status	High	0 (0.0)	1 (100.0)		
	Moderate	7 (19.4)	29 (80.6)		
	Low	4 (11.8)	30 (88.2)	0.973	0.615
Alcohol use	Yes	3 (21.4)	11 (78.6)		
	No	8 (14.0)	49 (86.0)	0.469	0.493
Cigarette	Yes	1 (50.0)	1 (50.0)		
	No	10 (14.5)	59 (85.5)	1.871	0.171
Primary care-giver	Yes	3 (7.3)	38 (92.7)		
	No	8 (26.7)	22 (73.3)	4.954	0.026
Primary care-giver relationship	Wife	2 (12.5)	14 (87.5)		
	Relative	0 (0.0)	3 (100.0)		
	Son	1 (11.1)	8 (88.9)		
	Daughter	0 (0.0)	12 (100.0)		
	Grand daughter	0 (0.0)	1 (100.0)	2.088	0.720
Primary care-giver education level	None	0 (0.0)	1 (100.0)		
	Primary	2 (8.3)	22 (91.7)		
	Secondary	0 (0.0)	9 (100.0)		
	Tertiary	1 (14.3)	6 (85.7)	1.327	0.723
Primary care-giver occupation status	Peasant	0 (0.0)	18 (100.0)		
	Business	1 (11.1)	8 (88.9)		
	Teacher	1 (33.3)	2 (66.7)		
	Retired	0 (0.0)	2 (100.0)		
	Driver	0 (0.0)	1 (100.0)	7.968	0.537
	Electronics	0 (0.0)	1 (100.0)		
	Employed	0 (0.0)	1 (100.0)		
	Nurse	0 (0.0)	1 (100.0)		
	Tailor	0 (0.0)	1 (100.0)		
	None	1 (333.3)	2 (66.7)		

Table 5: Antimicrobial sensitivity pattern for isolated bacteria

Table 5a: Gram negative isolates

Antibiotic agent	Bacterial species														
	E. coli (n = 9)			*Enterobacter spp* (n = 9)			*K. pneumoniae* (n = 8)			*Proteus spp* (n = 5)			*C. freundii* (n = 4)		
	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)
CIP	44.44	44.44	11.11	77.78	11.11	11.11	25.00	62.50	12.50	60.00	20.00	20.00	75.00	25.00	0.00
MEM	100.00	0.00	0.00	77.78	11.11	11.11	87.50	12.50	0.00	100.00	0.00	0.00	50.00	25.00	25.00
TE	44.44	44.44	11.11	44.44	33.33	22.22	12.50	87.50	0.00	20.00	60.00	20.00	25.00	50.00	25.00
GEN	66.67	33.33	0.00	66.67	22.22	11.11	75.00	25.00	0.00	80.00	20.00	0.00	50.00	50.00	0.00
TZP	44.44	0.00	55.56	55.56	11.11	33.33	25.00	37.50	37.50	40.00	20.00	40.00	100.00	0.00	0.00
AMC	33.33	22.22	44.44	0.00	88.89	11.11	12.50	62.50	25.00	60.00	0.00	40.00	25.00	0.00	75.00
CRO	22.22	77.78	0.00	22.22	77.78	0.00	12.50	87.50	0.00	60.00	40.00	0.00	50.00	50.00	0.00
CAZ	0.00	66.67	33.33	22.22	66.67	11.11	12.50	87.50	0.00	80.00	20.00	0.00	25.00	50.00	25.00

Table 5b: Gram negative isolates

Antibiotic agent	Bacterial isolates								
	P. aeruginosa (n = 14)			*Acinetobacter spp* (n = 1)			*Serratia marcescens* (n = 1)		
	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)
CIP	78.57	7.14	14.29	0.00	100.00	0.00	0.00	0.00	100.00
MEM	71.43	21.43	7.14	0.00	100.00	0.00	100.00	0.00	0.00
TE	-	-	-	100.00	0.00	0.00	0.00	100.00	0.00
GEN	78.57	21.43	0.00	0.00	100.00	0.00	0.00	100.00	0.00
TZP	50.00	21.43	28.57	0.00	100.00	0.00	100.00	0.00	0.00
AMC	-	-	-	-	-	-	0.00	100.00	0.00
CRO	0.00	92.86	7.14	0.00	100.00	0.00	0.00	0.00	100.00
CAZ	-	-	-	-	-	-	0.00	100.00	0.00

Table 5c: Gram positive isolates

Antibiotic agent	Bacterial isolates								
	S. aureus (n = 13)			*S. epidermidis* (n = 5)			*Enterococcus spp* (n = 1)		
	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	R (%)
CIP	61.54	30.77	7.69	60.00	40.00	0.00	100.00	0.00	0.00
TET	23.08	76.92	0.00	40.00	20.00	40.00	0.00	0.00	100.00
GEN	84.62	15.38	0.00	60.00	20.00	20.00	100.00	0.00	0.00
VA	-	-	-	-	-	-	100.00	0.00	0.00
CD	38.46	38.46	23.08	80.00	0.00	20.00	0.00	100.00	0.00
E	15.38	76.92	7.70	20.00	80.00	0.00	0.00	100.00	0.00
FOX	23.08	76.92	-	-	-	-	-	-	-
NO	-	-	-	100.00	0.00	-	-	-	-

Table 6: Clinical characteristics associated with laboratory confirmed diabetic foot infections among the study participants

Variable		Culture result		Chi^2	P value
		Negative n (%)	Positive n (%)		
Hospital status	inpatient	5 (11.4)	39 (88.6)		
	outpatient	6 (22.2)	21 (77.8)	1.507	0.220
DM type	Type I	0 (0.0)	1 (100.0)		
	Type II	11 (15.7)	59 (84.3)	0.186	0.666
DM family history	Yes	4 (23.5)	13 (76.5)		
	No	7 (13.0)	47 (87.0)	1.102	0.294
Duration of DM (years)	<10	9 (19.2)	38 (80.8)		
	10-19	1 (6.3)	15 (93.7)		
	≥20	1 (12.5)	7 (87.5)	1.578	0.454
DM status	Controlled	11 (15.9)	58 (84.1)		
	Uncontrolled	0 (0.0)	2 (100.0)	0.377	0.539
Ant diabetic use	Oral	6 (16.7)	30 (83.3)		
	Injection	5 (15.2)	28 (84.8)	0.029	0.864

Variable		Culture result		Chi^2	P value
		Negative n (%)	Positive n (%)		
Have you heard about DFI	Yes	10 (15.9)	53 (84.1)		
	No	1 (12.5)	7 (87.5)	0.061	0.804
Duration of DFU (months)	≤3	7 (14.9)	40 (85.1)		
	4 – 6	0 (0.0)	12 (100.0)		
	>7	4 33.3)	8 (66.7)	5.130	0.084
Ulcer stage (Wagner's classification)	Type 1	2 (14.3)	12 (85.7)		
	Type 2	6 (16.7)	30 (83.3)		
	Type 3	2 (12.5)	14 (87.5)	0.240	0.022
	Type 4	1 (20.0)	4 (80.0)		
Foot affected	Left	8 (22.2)	28 (77.8)		
	Right	3 (8.6)	32 (91.4)	2.525	0.112
DFU history	Yes	4 (12.1)	29 (87.9)		
	No	7 (18.4)	31 (81.6)	0.535	0.464
Amputation	Yes	4 (21.1)	15 (78.9)		
	No	7 (13.5)	45 (86.5)	0.613	0.434
Have you heard about DFI	Yes	9 (15.8)	48 (84.2)		
	No	2 (14.3)	12 (85.7)	0.019	0.889
Diagnosis history of DFI	Yes	6 (20.7)	23 (79.3)		
	No	5 (11.9)	37 (88.1)	1.011	0.315
Previous diagnosis from the same ulcer	Yes	5 (18.5)	22 (81.5)		
	No	1 (50.0)	1 (50.0)	1.124	0.289
Time interval from previous diagnosis (months)	≤ 3	8 (10.0)	18 (90.0)		
	4 – 6	3 (50.0)	3 (50.0)		
	≥ 7	0 (0.0)	3 (100.0)	4.826	0.090
Antibiotic use on previous treatment	Yes	6 (24.0)	19 (76.0)		
	No	0 (0.0)	4 (100.0)	1.210	0.271
Completeness of previous treatment dosage	Yes	6 (24.0)	19 (76.0)		
	No	0 (0.0)	0 (0.0)	-	-
Hospital admission for past 3 months	Yes	10 (16.4)	51 (83.6)		
	no	1 (10.0)	9 (90.0)	0.268	0.605

DISCUSSION

Diabetic foot infections represent one of the leading causes of morbidity and mortality among people with diabetes, it could lead to amputation and require extra care during treatment [17]. DFU is high in male approximately higher than female as indicated in different previous report 73.2% in Sudan and 66.9% in Kuwait [15, 16]. This is the same as reported in our study that indicated 66.2% of males being positive to DFU. This may be partially explained by a previous report which found that male sex and poor glycaemic control are independent risk factors for DFI [18] and suggestion that men are more likely to work outdoor which ultimately increases the risk to foot trauma and injury [19].

Prevalence of diabetic foot infections

The prevalence of diabetic foot infection (84.5%) in this study is consistent with previous similar studies carried out in Sudan and local study at KCMC reported a prevalence of 89.6% and 86.9% respectively [4, 16]. On other hand the study that was done in Kenya for fungal isolation identified the prevalence of 20.4% which is almost similar to prevalence of fungal species that was isolated in our study 30.1%. This prevalence closeness may be due to economic level of both countries being the same, geographical and social factors related to behaviour characteristics also are related.

High prevalence of diabetic foot infections was observing in the study that was done in Kuwait 92% [15]. This may be explained due to the presence of more favourable health facility for detection of infections.

Bacterial isolate and antimicrobial susceptibility test

Polymicrobial infection was identified as more prevalent in studies from a number of different countries such as 64% middle east [20], 83.7% in Sudan [21], and 60% in Egypt [22]. In this study we observed a higher percent of monomicrobial infections of about 56.7% which is in line with the results from the study that was conducted in Kuwait 57.3% [16] and Sudan 64% [16]. Sampling and processing technique between these studies could evaluate this variation in polymicrobial prevalence.

The most prevalent bacterial isolates were *Pseudomonas aeruginosa* 14/70 (20%) followed by *Staphylococcus aureus* 13/70 (18.6%), *Escherichia coli* 9/70 (12.9%), *Enterobacter spp* 9/70 (10.0%), *klebsiella pneumoniae* 8/70 (11.4%), *Staphylococcus epidermidis* 5/70 (7.1%), *Proteus spp* 5/70(7.1%) and then *Citrobacter freundii* 4/70 (5.7%). Other isolate occurred in small numbers, such as *Acinetobacter spp* 1/70(1.4%), *Serratia marcescens* 1/70(1.4%), and *Enterococcus spp* 1/70 (1.4%). Our results are similar to a study in Sudan which shows that *Staphylococcus aureus* (18.2%), *Escherichia coli* (15.5%), *Klebsiella pneumoniae* (14%), However, the Sudanese study had *Proteus spp* prevalence of (18.8%), which is double our prevalence and *Pseudomonas spp* (10.5%) [16] which is very small comparing to our study. This discrepancy may be due sample collection, source of infections and sample size in which Sudanese study had 335 isolates which is four times more than the number of our isolates.

0n other hand, fungal infections in our study were characterized by predominantly mould *Aspergillus spp* 15/22 (68.2%), followed by *Candida albicans* 3/22 (13.6%), and other *Candida spp* were 4/22 (18.2%), this is different from the study done in Kenya which shows that *Candida albicans* (69.2%) were most predominant and *Aspergillus spp* were 5.1% this discrepancy may be due to isolation technique used was more specific as they used VITEK-2 System (YST card) and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) [23].

Concerning the sensitivity of antibacterial drugs tested, gram negative isolate was highly resistant to third generation cephalosporin's (78.5%), and high resistance to tetracycline (66.7%). This could be due irrational use of their antibiotics.

Antibiotic susceptibility of *Pseudomonas aeruginosa* showed low resistance to ciprofloxacin (7.1%) resistance, meropenem (21.4%), gentamicin (21.4%) resistance and piperacillin tazobactam (21.43%) resistance, but highly resistant to third generation cephalosporin (92.9%) resistance, this finding are partially similar to those reported in previous study done in Kuwait ciprofloxacin resistance (29%), gentamicin (42%), resistance and third generation cephalosporin (100%) resistance [15, 24].

Escherichia coli showed complete sensitivity to piperacillin tazobactam (100%), and meropenem (100%), but 66.7% sensitivity to gentamicin, 0% sensitivity to ceftazidime. These finding agree with a study conducted in Sudan showed that *E. coli* were 100% sensitive to meropenem and 53.1% sensitive to gentamicin where ceftazidime were 96.4% resistant to *E. coli* [16].

Regarding gram positive isolate *Staphylococcus aureus* showed high sensitivity to only ciprofloxacin, gentamicin (61.5% and 84.6% respectively), other antimicrobial was highly resistant to *S. aureus*, erythromycin 76.9% resistant, and tetracycline 76.9% resistant. These results are quite similar to findings reported by the study done in Kuwait showed that gentamicin and ciprofloxacin were highly sensitive of about 78% and 73% respectively, however resistance of *S. aureus to* vancomycin was low 0% resistance [15]. This discrepancy may be due to microbial variability due to environment and presence of heath care facility for diagnosis compared to our region.

Factor associated with diabetic foot infections

More than 85.7% of the patient with higher grade ulcer, stage 3 and 4 according to Wagner's classification were positive to diabetic foot infection and 14.3% of patients with Wagner's stage 3 and 4 were not infected on their ulcers. This may be partially explained by previous report which found that higher grade ulcers are independent risk for diabetic foot infections [25].

CONCLUSION

The prevalence of laboratory conformed DFU infection was 84.5%, gram negative bacteria were predominating than gram positive bacteria. For gram negative spp the most effective antibiotic was ciprofloxacin and gentamicin and meropenem. Third generation cephalosporins, amoxicillin clavulanate showed poor effectiveness. DFU infection was associated with Wagner's classification.

Acknowledgement

We acknowledge the patients of the study participants, participants themselves and all departments involved in management of these cases at CUHAS/BMC.

Authors' Contributions

PK and BP played equal roles in the preparation of this case report. The other Co-authors contributed in caring and managing the patients. All authors read and approved the final manuscript.

Ethical consideration

Ethical clearance to conduct the research and consent to publish this research report was granted by the joint Catholic University of Health and Allied Sciences /Bugando Medical Centre Research and Ethical review committee with research certificate number CREC/2326/2022.

Conflict of Interest: All authors declare no conflict of interest.

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