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Detection of microbial pathogens colonizing foot ulcers of diabetic patients in Enugu, Nigeria

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Abstract:

Background: Diabetic foot ulcer (DFU) is a major complication of diabetes mellitus (DM) which is associated with high morbidity and mortality. There is high rate of bacteria colonization especially in those with tendencies for poor wound dressing. This is accompanied by high rate of inappropriate antibiotic usage. The aim of this study is to characterize microbial pathogens colonizing foot ulcers of diabetic patients in Enugu, Nigeria, and to determine the antibiotic susceptibility of these isolates.
Methodology: This was a descriptive cross-sectional study of consecutively enrolled diabetic patients with foot ulcers in two tertiary healthcare facilities in Enugu, Nigeria, between May 2021 and February 2022. A structured questionnaire was used to obtain socio-demographic and clinical data of the patients. Pus samples and/or tissues were collected from the ulcer lesion of each patient for aerobic and anaerobic microbial cultures and biochemical identification using standard conventional techniques.
Results: A total of 310 diabetic patients with foot ulcers were recruited into the study, with 62.3% (193/310) males and 37.7% (117/310) females, and mean age of 56.0±13.9 years. Bacteria and yeast were isolated from samples of 280 (90.3%) patients while samples of 30 (9.7%) patients had no microbial growth. Males had higher frequency of microbial isolates (90.7%, 175/193) than females (89.7%, 105/117), while the age group ≤ 40 years had higher frequency of microbial isolates (100%, 43/43) compared to other age groups, although the differences are not statistically significant ($p>0.05$). The distribution of the isolates showed that 15.7% (44/280) were monomicrobial while 84.3% (236/280) were polymicrobial. The highest single isolate was *Bacteroides fragilis* with 5.0% (14/280), followed by *Staphylococcus aureus* with 3.2% (9/280). *Bacteroides fragilis* and *S. aureus* occurred as the highest combined bacteria isolates with 5.7% (16/280). Most of the patients were colonized by combination of bacterial isolates. The susceptibility indicates that most of the anaerobic bacteria were sensitive to metronidazole while *S. aureus* isolates were resistant to ofloxacin at a rate of 65.0%.
Conclusion: The findings in this study showed that there is high bacteria and fungi colonization of foot ulcers of diabetic patients in Enugu, Nigeria. Routine care of wounds especially frequent changes of dressing materials and the use of potent antiseptics, are recommended.

Keywords: Diabetic foot ulcer; chronic wounds; polymicrobial; antimicrobial resistance

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Détection d'agents pathogènes microbiens colonisant les ulcères du pied de patients diabétiques à Enugu, Nigeria

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Résumé:

Contexte: L'ulcère du pied diabétique (UPD) est une complication majeure du diabète sucré (DM) associée à une morbidité et une mortalité élevées. Il existe un taux élevé de colonisation bactérienne, en particulier chez les

personnes ayant tendance à mal panser les plaies. Cela s'accompagne d'un taux élevé d'utilisation inappropriée d'antibiotiques. Le but de cette étude est de caractériser les agents pathogènes microbiens colonisant les ulcères du pied des patients diabétiques à Enugu, au Nigeria, et de déterminer la sensibilité aux antibiotiques de ces isolats.

Méthodologie: Il s'agissait d'une étude transversale descriptive portant sur des patients diabétiques recrutés consécutivement et souffrant d'ulcères du pied dans deux établissements de soins de santé tertiaires à Enugu, au Nigeria, entre mai 2021 et février 2022. Un questionnaire structuré a été utilisé pour obtenir des données sociodémographiques et cliniques du les patients. Des échantillons de pus et/ou des tissus ont été prélevés sur la lésion ulcéreuse de chaque patient pour des cultures microbiennes aérobies et anaérobies et une identification biochimique à l'aide de techniques conventionnelles standard.

Résultats: Au total, 310 patients diabétiques souffrant d'ulcères du pied ont été recrutés dans l'étude, avec 62,3% (193/310) d'hommes et 37,7% (117/310) de femmes, et un âge moyen de 56,0±13,9 ans. Des bactéries et des levures ont été isolées à partir d'échantillons de 280 (90,3%) patients, tandis que des échantillons de 30 (9,7%) patients ne présentaient aucune croissance microbienne. Les hommes présentaient une fréquence plus élevée d'isolats microbiens (90,7%, 175/193) que les femmes (89,7%, 105/117), tandis que le groupe d'âge ≤ 40 ans présentait une fréquence plus élevée d'isolats microbiens (100.0%, 43/43) par rapport aux autres groupes d'âge, bien que les différences ne soient pas statistiquement significatives (p>0,05). La répartition des isolats a montré que 15,7% (44/280) étaient monomicrobiens tandis que 84,3% (236/280) étaient polymicrobiens. L'isolat le plus élevé était *Bacteroides fragilis* avec 5,0% (14/280), suivi de *Staphylococcus aureus* avec 3,2% (9/280). *Bacteroides fragilis* et *S. aureus* étaient les isolats bactériens combinés les plus élevés avec 5,7% (16/280). La plupart des patients étaient colonisés par une combinaison d'isolats bactériens. La sensibilité indique que la plupart des bactéries anaérobies étaient sensibles au métronidazole tandis que les isolats de S. aureus étaient résistants à l'ofloxacine à un taux de 65,0%.

Conclusion: Les résultats de cette étude ont montré qu'il existe une forte colonisation bactérienne et fongique des ulcères du pied des patients diabétiques à Enugu, au Nigeria. Des soins de routine des plaies, des changements particulièrement fréquents des matériaux de pansement et l'utilisation d'antiseptiques puissants sont recommandés.

Mots clés: Ulcère du pied diabétique; plaies chroniques; polymicrobien; résistance aux antimicrobiens

Introduction:

Diabetes mellitus is a debilitating ailment that affect every race throughout the world. The International Diabetes Federation (IDF) estimated that about 537 million people are living with diabetes worldwide with a projected rise to 783 million people in 2045 (1). Over 6.7 million deaths from diabetes were reported in low-and-middle-income-countries in 2021. Africa has 24 million adults living with diabetes and also the highest level of undiagnosed diabetic cases, reaching 53.6% (2). In sub-Saharan Africa, Nigeria is reported to have the highest burden of diabetes with parallel increase in the prevalence of diabetes-related complications and death (3).

Diabetic foot ulcer (DFU) is one of the most frequent complications in diabetic patients with a prevalence rate of 6.4% worldwide (4), 7.2% in Africa and rate ranging from 11 to 32% in Nigeria among hospitalized patients (5). In Nigeria, DFU accounts for a quarter of diabetic-related prolonged hospital admission with amputation and mortality rates of 35.4% and 20.5% respectively (3). DFU is a breech in the skin epithelium of the host distally from the ankle, with a multi-factorial aetiology and associated with neuropathy, different grades of ischaemia and infection (6).

Established risk factors that precipitate the development of ulcer includes increased body weight, peripheral vascular disease, retinopathy, hypertension, poor glycaemic control, high foot plantar pressure, duration of diabetes, age, race, ethnicity, socio-economic status, presence of callus, smoking, and trauma (7-10). It has been estimated that 40-80% infection rate in DFU has resulted in amputation of lower limb extremities in majority of cases (11). A Nigerian study reported 22.3%-29.3% of non-traumatic amputation due to DFU (12).

Aside hyperglycaemic emergencies, DFU is the commonest cause of diabetes related death in Nigeria (13). Several factors pathogenetically work together to create the onset of foot ulcerations in diabetic patients. DFU are chronic wounds that are frequently colonized by wide range of pathogenic bacteria, which are predominantly polymicrobial with multiple bacteria as the most prevalence organisms. There is sparse knowledge about the ecology of such chronic infections but biofilm formation seems to play a major role (14). The interaction and synergism of the polymicrobial community leads to production of extracellular matrix of hydrated polymeric substances. This becomes irreversibly attached to the biological surface of the ulcer, making them recalcitrant to the action of most antibiotics and also resistant to the immune system (15). A wide range of bacterial pathogens have been identified with diverse antibiotic susceptibility patterns patterns in different geographical regions (16,17).

Chronic infections of diabetic foot make treatment more complex and difficult, increases cost of management, prolong hospital stay as well as increase morbidity and mortality (18). Amputation of the lower limb extremity on its own is associated with significant disabilities including loss of productivity, psychological burdens and reduced quality of life (19).

Report in southwest Nigeria has shown more mean annual expenditure on diabetics with complications such as foot ulcer than diabetics without complications (20). This has been attributed to higher rates of hospital admission, emergency department visits and home health care utilization. There is an under estimation to the true economic burden of DFU as regards to loss of productivity and decreased employment associated with DFU (21). The goal of this study is to determine microbial pathogens colonizing foot ulcer of diabetic patients and their susceptibility to commonly used antimicrobial agents in Enugu, southeast Nigeria.

Materials and method:

Study setting and design:

This was a cross sectional descriptive multicenter study conducted in Enugu State, southeast Nigeria, involving two tertiary healthcare facilities within the State; University of Nigeria Teaching Hospital (UNTH), Ituku Ozalla and Enugu State University Teaching Hospital (ESUTH), Park-lane Enugu.

Ethical issues:

The study protocol was reviewed and approved by Health Research Ethics Committee of the Teaching Hospitals; UNTH Ituku-Ozalla with reference number UNTH/HREC/2021/04/116 and ESUTH Parklane with refere nce number ESUTHP/CMAC/RA/034/vol-2/106. Participation in the study was voluntary and each patient gave their consent before administration of any questionnaire and before wound assessment.

Study participants and data collection:

A total of 310 diabetic patients (hospitalized and outpatient) with clinically infected lower extremity (below the ankle) ulcer were consecutively recruited into the study between May 2021 and February 2022. Information on demographic factors and social life style were obtained from each participant using a pretested structured questionnaire in a face-to-face interview.

Clinical parameters, which included type of lesion, duration of ulcer, location of foot ulcer, duration of hospital stay, were also collected. These were further linked to their microbiological samples through numerical codes for easy identification. Foot ulcers were clinically assessed and diagnosis of infection was determined by the presence of at least two of these indicators; local swelling or indurations, tenderness or pain, purulent discharge, erythema, and heat/warmth.

Microbiological sample collection:

Foot ulcers were cleaned vigorously with sterile saline solution and extensively debrided of superficial exudates to reduce the chances of isolating colonizing flora. For superficial ulcers, two sets of sterile swabs were used to collect wound swab from the base of ulcer. By rotating a sterile swab over a 1 cm^2 area of the wound bed for 5 seconds, samples were collected from each patient. The two swab specimens were immediately transported to the laboratory for microbial analysis.

Microbiological culture procedure:

One of the swab specimens was used for Gram staining reaction to identify Gram-positive and Gram-negative bacteria present in the sample (22). The second swab specimen was inoculated onto Blood, MacConkey, and Mannitol salt agar plates as well as into thioglycolate broth medium, and incubated at 37^{o}C for 24 hours. Subcultures from the thioglycolate broth was done Blood agar plate and incubated anaerobically using Gas pack (AnaeroPack® - anaerobic gas generating system). All bacteriological cultures and biochemical identification tests for the isolates were carried out by conventional methods as described in Cowan and Steel's Manual for Identification of Medical Bacteria (23).

Antibiotic susceptibility testing:

Antimicrobial susceptibility testing (AST) was carried out on each isolate by the modified Kirby-Bauer disc diffusion method (24). Briefly, sterile Mueller-Hinton (MH) agar plate was swabbed with standardized inoculum suspension of each isolated bacteria isolates. Antibiotic discs were placed on inoculated MH plate using a sterile forcep and plates were incubated at 37^{o}C for 24 hours. The diameter of zone of inhibition for each isolate was measured with a calibrated ruler and interpreted as sensitive or resistance in line with the CLSI guideline (24). For streptococci, 5% horse blood was added to MH agar for the AST.

The antibiotic discs used in the AST included metronidazole (5µg), cefoxitin (30µg), ampicillin/sulbactam (10/10µg), imipenem (10 µg), ciprofloxacin (10µg), ampicillin (30µg), levofloxacin (10µg), norfloxacin (10µg), ofloxacin (10 µg), erythromycin (10 µg), cefixime (5 µg), ceftriaxone (30µg), penicillin (10µg), ampicillin/cloxacillin (30µg), clindamycin (2µg), chloramphenicol (30µg), and clarithromycin (10µg).

Statistical analysis:

Data were summarized by descriptive statistics and analysed using omnibus model of Chi-square test and logistic regression. The strength of association between bacterial infected or colonized ulcer and selected factors was estimated by calculating the Odds ratios (OR) with 95% confidence intervals (95% CI) and probability value less than 0.05 was considered statistical significance.

Results:

Socio-demographic and clinical characteristics of diabetic patients:

A total of 310 diabetes patients with foot ulcer were enrolled into the study, made of 193 (62.3%) males and 117 (37.7%) females, with age range of 21-92 and mean age of 56.02±13.9 years. The age group 50-59 years made up 30.0% (93/310). Participants with secondary and primary school education constituted 49.4% and 22.3% respectively. Traders were the most represented occupational group with 42.6% (132/310). Participants residing in semi urban and rural areas constituted 42.3% and 40.6% respectively. Majority of the diabetic patients (40.7%, 126/310) had the foot ulcer for > 3 years. About 69.0% (214/310) of the participants had indulged in self-medications especially with antibiotics. Most ulcer positions were located at the dorsal portion of the feet (45.5%, 141/310), followed by plantar area (20.6%, 64/310) (Table 1).

Prevalence of bacterial colonization of ulcer:

Of the 310 diabetic patients with foot ulcers whose samples were analysed, 280 (90.3%) had bacterial isolates while 30 (9.7%) showed no bacterial growth. The distribution of the bacterial isolates showed that 15.7% (44/280) had single isolate while 51.1% (143/280), 28.2% (79/280) and 5.0% (14/280) had two, three and four bacterial isolates respecttively.

The predominant monomicrobial isolate was *Bacteroides fragilis* (5.0%, 14/280) followed by *Staphylococcus aureus* (3.2%, 9/280) (Table 2a). In the group of diabetic patients with two isolates, *S. aureus* and *B. fragilis* constituted 5.7% (16/280) while *Enterococcus faecalis* and *Propionibacterium* spp represented 3.6% (10/280). Some other combined isolates included 3.2% (9/280) *S. aureus* and *Propionibacterium* spp. The frequency of *S. aureus*/*Escherichia coli, B. fragilis*/coagulase negative staphylococcus (CoNS), and *B. fragilis*/*Proteus mirabilis* were 2.9% (8/280) each (Table 2b). The highest frequency for cultures with three isolates were *S. aureus*/*B. fragilis*/*P. mirabilis*; *E. coli*/*P. mirabilis*/*Peptostreptococcus* spp; *E. coli*/*Peptostreptococcus* spp/CoNS; *S. aureus*/*B. fragilis*/*P. mirabilis* and *S. aureus*/*E. coli*/*Propionibacterium* spp, with 1.4% (4/280) each (Table 2c). Diabetic patient ulcers colonized with four bacterial isolates included *E. coli*/*Pseudomonas aeruginosa*/*Peptostreptococcus* spp/CoNS with 0.8% (3/280).

Analysis of socio-demographic and clinical characteristics of participants with respect to microbial isolates:

Table 3 shows bivariate analysis of the sociodemographic and clinical characteristics of the study participants with respect to the distribution of the microbial isolates. Male participants had higher frequency of microbial isolates of 90.7% (175/193) compared to females with 89.7% (105/117) but this difference was not statistically significant (p=0.788). Drivers (7/7), students (12/12) and clergy (2/2) had the highest frequency of bacteria isolates with 100.0% each. Traders as well as unemployed also had high frequency of bacterial isolates with 89.4% and 87.9% respectively. A total of 116 (92.1%) bacterial isolates were recovered from participants who used antibiotics without medical prescription compared to 86 (89.6%) isolates from those who did not (p=0.80). In all, none of the characteristics analysed was significantly associated with bacterial colonization of ulcers in the participants as rates were high across board.

Antibiotic susceptibility of bacterial isolates in diabetic ulcer patients:

The antibiotic susceptibility of the isolated bacteria indicated that Gram-positive bacteria have the highest sensitivity rate to chloramphenicol followed by fluoroquinolones (levofloxacin and norfloxacin) respectively. Ampicillin/sulbactam, ciprofloxacin, as well as amoxicillin/clavulanate had the highest inhibitory activity against Gram-negative aerobes. Ciprofloxacin had moderate inhibitory activity against both Gram-negative and Gram-positive aerobic bacteria but with low activity against *Klebsiella pneumoniae*. Aerobic bacteria were moderately susceptible to gentamicin, ceftriaxone, clindamycin and ofloxacin.

Anaerobic isolates were less resistant to metronidazole, penicillin and ampicillin/sulbactam. The bacteria most resistant to the antimicrobials tested are *Clostridium perfringes* while *Fusobacterium* spp were the most susceptible to the antimicrobials tested with exception of imipenem.

Table 1: Socio-demographic and clinical characteristics of diabetic patients with foot ulcers in Enugu, Nigeria

Variables	Categories	Frequency (n)	Percentage (%)
Gender			
	Male	193	62.3
	Female	117	37.7
Age group (years)			
	≤ 40	45	14.5
	41- 49	44	14.2
	50 – 59	93	30.0
	60 – 69	89	28.7
	> 70	34	12.6
Educational status			
	None	33	10.6
	Primary	69	22.3
	Secondary	153	49.4
	Tertiary	55	17.7
Occupation			
	Unemployed	83	26.8
	Civil Servant	48	15.5
	Trader	132	42.6
	Driver	7	2.3
	Farmer	26	8.4
	Student	12	3.9
	Clergy	2	0.6
Marital status			
	Single	20	6.5
	Married	232	74.8
	Divorced	8	2.6
	Separated	8	2.6
	Widowed	42	13.5
Residency			
	Urban	53	17.1
	Semi-Urban	131	42.3
	Rural	126	40.6
Type of house			
	Duplex/Bungalow	70	22.6
	Flat	121	39.0
	One Room Apartment	119	38.4
Duration of ulcer (years)			
	< 1	106	34.2
	1 – 2	46	14.8
	2 – 3	32	10.3
	>3	126	40.7
Position of ulcer			
	Planter	64	20.6
	Dorsal Portion	141	45.5
	Toes (left Foot)	31	10.0
	Toes (right Foot)	46	14.8
	Ankle	28	9.0
Treatment assessment			
	Hospitalized	179	57.7
	Out-Patient	131	42.3
Antibiotic use without prescription			
	Yes	214	69.0
	No	96	31.0

Table 2: Microbial isolates colonizing foot ulcers of diabetic patients in Enugu, Nigeria

2 (a): Single microbial isolate (monomicrobial)

Isolate	Frequency	Percentage
Staphylococcus aureus	9	3.2
Bacteroides fragilis	14	5.0
Escherichia coli	3	1.1
Pseudomonas aeruginosa	4	1.4
Proteus mirabilis	1	0.4
Candida albicans	6	2.1
Coagulase-negative staphylococcus	3	1.1
Streptococcus pyogenes	2	0.7
Peptostreptococcus spp	2	0.7
Total	**44**	**15.7**

2 (b): Two microbial isolates

Isolates	Frequency	Percentage
Staphylococcus aureus + *Bacteroides fragilis*	16	5.7
Staphylococcus aureus + *Enterococcus faecalis*	1	0.4
Staphylococcus aureus + *Escherichia coli*	1	0.4
Staphylococcus aureus + *Peptostreptococcus* spp	8	2.9
Staphylococcus aureus + *Fusobacterium* spp	3	1.1
Staphylococcus aureus+ *Propionibacterium* spp	9	3.2
Staphylococcus aureus + *Clostridium perfringens*	1	0.4
Escherichia coli + *Proteus mirabilis*	1	0.4
Escherichia coli + *Peptostreptococcus* spp	2	0.7
Escherichia coli + Coagulase-negative staphylococcus	5	1.9
Escherichia Coli + *Propionibacterium* spp	4	1.4
Escherichia coli + *Clostridium perfringens*	2	0.7
Bacteroides fragilis + Coagulase-negative staphylococcus	8	2.9
Bacteroides fragilis + *Enterococcus faecalis*	4	1.4
Bacteroides fragilis + *Streptococcus pyogenes*	1	0.4
Bacteroides Fragilis + *Candida albicans*	2	0.7
Bacteroides fragilis + *Escherichia coli*	6	2.1
Bacteroides fragilis + *Pseudomonas aeruginosa*	2	0.7
Bacteroides fragilis + *Proteus mirabilis*	8	2.9
Bacteroides fragilis + *Peptostreptococcus* spp	6	2.1
Pseudomonas aeruginosa + *Clostridium perfringens*	1	0.4
Pseudomonas aeruginosa + *Peptostreptococcus* spp	5	1.9
Pseudomonas aeruginosa + *Klebsiella pneumoniae*	3	1.1
Pseudomonas aeruginosa + *Propionibacterium* spp	1	0.4
Pseudomonas aeruginosa + *Proteus mirabilis*	1	0.4
Proteus mirabilis + *Candida albicans*	1	0.4
Proteus mirabilis + *Peptostreptococcus* spp	1	0.4
Proteus mirabilis + Coagulase-negative staphylococcus	1	0.4
Proteus mirabilis + *Propionibacterium* spp	2	0.7
Coagulase-negative staphylococcus + *Propionibacterium* spp	4	1.4
Coagulase-negative staphylococcus + *Clostridium perfringens*	1	0.4
Coagulase-negative staphylococcus + *Klebsiella pneumoniae*	1	0.4
Coagulase-negative staphylococcus +*Fusobacterium* spp	2	0.7
Peptostreptococcus spp + Coagulase-negative staphylococcus	2	0.7
Peptostreptococcus spp + *Propionibacterium* spp	2	0.7
Peptostreptococcus spp + *Candida albicans*	2	0.7
Enterococcus faecalis + *Clostridium perfringens*	1	0.4
Enterococcus faecalis + *Fusobacterium* spp	3	1.1
Enterococcus faecalis + *Propionibacterium* spp	10	3.6
Enterococcus faecalis + *Peptostreptococcus* spp	5	1.9
Candida albicans + *Clostridium perfringens*	1	0.4
Candida albicans + *Fusobacterium* spp	1	0.4
Candida albicans + *Klebsiella pneumoniae*	1	0.4
Streptococcus pyogenes + *Fusobacterium* spp	1	0.4
Total	**143**	**51.1**

2(c): Three microbial isolates

Isolates	Frequency	Percentage
Staphylococcus aureus+ Bacteroides fragilis+ Escherichia coli	3	1.1
Staphylococcus aureus+ Enterococcus faecalis+ Escherichia coli	3	1.1
Bacteroides fragilis+ Enterococcus faecalis + Pseudomonas aeruginosa	1	0.4
Staphylococcus aureus + Bacteroides fragilis + Proteus mirabilis	4	1.4
Bacteroides fragilis + Enterococcus faecalis + Proteus mirabilis	1	0.4
Bacteroides fragilis+ Escherichia coli+ Proteus mirabilis	1	0.4
Staphylococcus aureus+ Enterococcus faecalis + Peptostreptococcus spp	2	0.7
Bacteroides fragilis, Enterococcus faecalis, Peptostreptococcus spp	1	0.4
Bacteroides fragilis+ Escherichia coli + Peptostreptococcus spp	2	0.7
Bacteroides fragilis +Pseudomonas aeruginosa + Peptostreptococcus spp	2	0.7
Bacteroides fragilis + Proteus mirabilis + Peptostreptococcus spp	1	0.4
Escherichia coli + Proteus mirabilis + Peptostreptococcus spp	4	1.4
Pseudomonas aeruginosa + Proteus mirabilis + Peptostreptococcus spp	1	0.4
Staphylococcus aureus + Bacteroides fragilis + Escherichia coli	1	0.4
Escherichia coli + Peptostreptococcus spp + *Candida albicans*	1	0.4
Escherichia coli+ Peptostreptococcus spp + Coagulase-negative staphylococcus	4	1.4
Proteus mirabilis+ Peptostreptococcus spp+ Coagulase-negative staphylococcus	2	0.7
Staphylococcus aureus+ Bacteroides fragilis+ Klebsiella pneumoniae	2	0.7
Staphylococcus aureus+ Escherichia coli +Klebsiella pneumoniae	1	0.4
Bacteroides fragilis + Escherichia coli + Klebsiella pneumoniae	1	0.4
Enterococcus faecalis + Peptostreptococcus spp + *Klebsiella pneumoniae*	1	0.4
Staphylococcus aureus+ Bacteroides fragilis + Fusobacterium spp	1	0.4
Staphylococcus aureus +Escherichia coli + Fusobacterium spp	1	0.4
Escherichia coli, Pseudomonas aeruginosa, Fusobacterium spp	1	0.4
Staphylococcus aureus+ Proteus mirabilis+ Fusobacterium spp	1	0.4
Escherichia coli+ Proteus mirabilis+ Fusobacterium spp	2	0.7
Proteus mirabilis + Candida albicans + Fusobacterium spp	1	0.4
Proteus mirabilis+ Streptococcus pyogenes + Fusobacterium spp	2	0.7
Enterococcus faecalis + Proteus mirabilis + Propionibacterium spp	2	0.7
Staphylococcus aureus+ Bacteroides fragilis+ Propionibacterium spp	4	1.4
Enterococcus faecalis+ Peptostreptococcus spp + *Propionibacterium* spp	1	0.4
Escherichia coli+ Peptostreptococcus spp + *Propionibacterium* spp	1	0.4
Staphylococcus aureus+ Escherichia coli+ Propionibacterium spp	4	1.4
Bacteroides fragilis+ Peptostreptococcus spp +Propionibacterium spp	3	1.1
Staphylococcus aureus +Enterococcus faecalis + Propionibacterium spp	1	0.4
Enterococcus faecalis + Coagulase-negative staphylococcus + *Propionibacterium* spp	2	0.7
Enterococcus faecalis+ Klebsiella pneumoniae + Propionibacterium spp	1	0.4
Pseudomonas aeruginosa+ Klebsiella pneumoniae + Propionibacterium spp	1	0.4
Escherichia coli + Peptostreptococcus spp + *Clostridium perfringens*	1	0.4
Escherichia coli+ Proteus mirabilis + Clostridium perfringens	1	0.4
Staphylococcus aureus + Escherichia coli + Clostridium perfringens	1	0.4
Escherichia coli+ Pseudomonas aeruginosa + Clostridium perfringens	1	0.4
Escherichia coli + Proteus mirabilis + Clostridium perfringens	2	0.7
Enterococcus faecalis + Proteus mirabilis + Clostridium perfringens	1	0.4
Enterococcus faecalis + Coagulase-negative staphylococcus + *Clostridium perfringens*	3	1.1
Proteus mirabilis + Streptococcus pyogenes + Enterococcus faecalis	1	0.4
Total	**79**	**28.2**

2(d): Four microbial isolates

Isolates	Frequency	Percentage
Staphylococcus aureus+ Bacteroides fragilis + Escherichia coli + Peptostreptococcus spp	2	0.7
Staphylococcus aureus + Escherichia coli + Pseudomonas aeruginosa + Peptostreptococcus spp	1	0.4
Staphylococcus aureus+ Bacteroides fragilis+ Enterococcus faecalis + Coagulase-negative staphylococcus	1	0.4
Staphylococcus aureus + Bacteroides fragilis + Escherichia coli + Coagulase-negative staphylococcus	2	0.7
Bacteroides fragilis + Pseudomonas aeruginosa + Proteus mirabilis + Coagulase-negative staphylococcus	1	0.4
Escherichia coli + Pseudomonas aeruginosa + Peptostreptococcus spp + Coagulase-negative staphylococcus	3	0.7
Bacteroides fragilis + Pseudomonas aeruginosa + Coagulase-negative staphylococcus + *Streptococcus pyogenes*	1	0.4
Enterococcus faecalis + klebsiella pneumoniae + Streptococcus pyogenes + Fusobacterium spp	1	0.4
Bacteroides fragilis+ Enterococcus faecalis + Peptostreptococcus spp + *Propionibacterium* spp	1	0.4
Enterococcus faecalis + Proteus mirabilis + klebsiella pneumoniae + Clostridium perfringens	1	0.4
Total	**14**	**5.0**

Table 3: Bivariate analysis of socio-demographic and clinical characteristics of diabetic patients with foot ulcers with respect to prevalence of colonization by microbial isolates

Characteristics	**Categories**	**Microbial isolates**		x^2	**OR (95% CI)**	***p*-value**
		Yes (%)	**No (%)**			
Gender	Male	175 (90.7)	18 (9.3)	.072	1.11 (.52-2.39)	.788
	Female	105 (89.7)	12 (10.3)			
Age group (years)	≤ 40	43 (100.0)	0	8.135	NA	.078
	41- 49	42 (91.3)	4 (8.7)			
	50 – 59	82 (88.2)	11 (11.8)			
	60 – 69	80 (89.9)	9 (10.1)			
	> 70	33 (84.6)	6 (15.4)			
Educational Status	None	28 (84.8)	5 (15.2)	2.273	NA	.518
	Primary	62 (89.9)	7 (10.1)			
	Secondary	138 (90.2)	15 (9.8)			
	Tertiary	52 (94.5)	3 (5.5)			
Occupation	Unemployed	73 (87.9)	10 (12.1)	2.585	NA	.831
	Civil Servant	43 (89.6)	5 (10.4)			
	Trader	118 (89.4)	14 (10.6)			
	Driver	7 (100.0)	0			
	Farmer	25 (96.2)	1 (3.8)			
	Student	12 (100.0)	0			
	Clergy	2 (100.0)	0			
Residency Type	Urban	49 (92.5)	4 (7.5)	1.676	NA	.433
	Semi-Urban	115 (87.8)	16 (12.2)			
	Rural	116 (92.1)	10 (7.9)			
Marital Status	Single	20 (100.0)	0	2.794	NA	.545
	Married	208 (89.7)	24 (10.3)			
	Divorced	7 (87.5)	1 (12.5)			
	Separated	7 (87.5)	1 (12.5)			
	Widowed	38 (90.5)	4 (9.5)			
House Type	Duplex/Bungalow	66 (94.3)	4 (5.7)	1.776	NA	.411
	Flat	107 (88.4)	14 (11.6)			
	One-room apartment	107 (89.9)	12 (10.1)			
Duration of ulcer (years)	< 1	99 (93.4)	7 (6.6)	7.826	NA	.043
	1 – 2	36 (78.3)	10 (11.6)			
	2 – 3	29 (90.6)	3 (9.4)			
	>3	116 (92.1)	10 (7.9)			
Position of ulcer	Planter	58 (90.6)	6 (9.4)	.342	NA	.995
	Dorsal Portion	127 (90.1)	14 (9.9)			
	Toes (left Foot)	28 (87.5)	3 (12.7)			
	Toes (right Foot)	41 (89.1)	5 (10.9)			
	Ankle	26 (92.9)	2 (7.1)			
Treatment assessment	Hospitalized	165 (92.2)	14 (7.8)	1.772	1.664 (.78-3.54)	.183
	Out-Patient	115 (87.8)	16 (12.2)			
Use antibiotics without prescription	Yes	194 (90.7)	20 (9.3)	.0641	1.109 (.49-2.47)	.800
	No	86 (89.6)	10 (10.4)			

NA=Not applicable; OR=Odd ratio; CI=Confidence interval

Table 4 (a): Antibiotic susceptibility of isolated anaerobic bacteria

Antibiotics	***Bacteroides fragilis*** **(n =103)**		***Peptostreptococcus*** **spp (n = 69)**		***Propionibacterium* spp (n= 53)**		***Fusobacterium* spp (n =20)**		***Clostridium perfringes*** **(n= 19)**	
	R	**S**	**R**	**S)**	**R**	**S)**	**R**	**S**	**R**	**S**
Penicillin	101 (98.0)	2 (2.0)	3 (4.3)	66 (95.6)	6 (11.3)	47 (88.7)	6 (30.0)	14 (70.0)	0	19 (100)
Metronidazole	3 (2.9)	100 (97.1)	10 (14.5)	59 (85.5)	0	53 (100)	0	20 (100)	0	19 (100)
Clindamycin	41 (39.8)	63 (60.2)	0	69 (100)	6 (11.3)	47 (88.7)	0	20 (100)	18 (94.7)	1 (5.3)
Cefoxitin	43 (41.7)	60 (58.3)	10 (14.5)	66 (85.5)	15 (28.3)	38 (71.7)	0	20 (100)	10 (52.6)	9 (47.4)
Ampicillin/ Sulbactam	4 (3.9)	99 (96.1)	19 (27.5)	50 (72.5)	15 (28.3)	38 (71.7)	6 (30.0)	14 (70.0)	10 (52.6)	9 (47.4)
Imipenem	11 (5.3)	92 (94.7)	20 (28.9)	49 (71.1)	18 (44.0)	35 (66.0)	10 (50)	10 (50.0)	6 (31.6)	13 (68.4)
Ceftriaxone	23 (22.3)	80 (77.7)	35 (50.7)	34 (49.3)	30 (56.6)	23 (43.4)	6 (30.0)	14 (70.0)	9 (47.4)	10 (52.6)
Ampicillin	103 (100)	0	35 (50.7)	34 (49.3)	6 (11.3)	47 (88.7)	0	20 (100)	14 (73.7)	5 (15.3)

Table 4(b): Antibiotic susceptibility of isolated aerobic Gram-positive bacteria

Antibiotics	***Staphylococcus aureus*** **(n = 83)**		**CoNS (n = 46)**		***Streptococcus pyogenes*** **(n = 9)**		***Enterococcus faecalis*** **(n=48)**	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Erythromycin	41 (49.4)	42 (50.6)	34 (74.0)	12 (26.0)	4 (44.4)	5 (55.6)	48 (100.0)	0
Ceftriaxone	40 (48.2)	43 (51.8)	46 (100)	0	4 (44.4)	5 (55.6)	22 (45.8)	26 (54.2)
Ampicillin/Cloxacillin	40 (48.2)	43 (51.8)	46 (100)	0	5 (55.6)	4 (44.4)	47 (97.9)	1 (2.1)
Cefixime	41 (49.4)	42 (50.6)	46 (100)	0	9 (88.9)	1 (11.1)	46 (95.8)	2 (4.1)
Levofloxacin	0	83 (100)	5 (10.9)	41 (89.1)	2 (22.2)	7 (77.8)	19 (39.6)	29 (60.4)
Norfloxacin	0	83 (100)	5 (10.9)	41 (89.1)	3 (33.3)	6 (66.7)	19 (39.6)	29 (60.4)
Gentamicin	42 (50.6)	41 (49.4)	24 (52.2)	22 (47.8)	5 (55.6)	4 (44.4)	24 (50.0)	24 (50.0)
Ofloxacin	54 (65.1)	29 (34.9)	10 (21.7)	36 (78.3)	5 (55.6)	4 (44.6)	31 (65.0)	17 (35.0)
Clindamycin	40 (48.2)	3 (51.8)	25 (54.3)	21 (45.7)	3 (33.3)	6 (66.7)	36 (75.0)	12 (25.0)
Ciprofloxacin	15 (18.1)	68 (81.9)	30 (65.2)	16 (34.8)	1 (11.1)	9 (88.9)	22 (45.8)	26 (54.2)
Chloramphenicol	19 (22.9)	64 (77.1)	4 (8.6)	42 (91.4)	1 (11.1)	8 (88.9)	1 (2.1)	47 (97.9)

CONS=Coagulase negative staphylococcus; R=Resistance; S=Sensitive

Table 4 (c): Antibiotic susceptibility of isolated aerobic Gram-negative bacteria

Antibiotics	***Escherichia coli*** **(n = 67)**		***Proteus mirabilis*** **(n = 45)**		***Pseudomonas aeruginosa*** **(n = 30)**		***Klebsiella pneumoniae*** **(n = 14)**	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**
ceftriaxone	33 (49.2)	34 (50.8)	14 (31.1)	31 (68.9)	5 (16.7)	25 (83.3)	6 (42.9)	8 (57.1)
Ciprofloxacin	1 (1.5)	66 (98.5)	16 (35.6)	29 (64.4)	0	30 (100)	8 (57.1)	6 (42.9)
Gentamycin	29 (43.3)	38 (56.7)	23 (51.1)	22 (48.9)	22 (73.4)	8 (26.6)	12 (85.7)	2 (14.3)
Ofloxacin	31 (46.3)	36 (53.7)	33 (73.3)	12 (26.7)	22 (73.4)	8 (26.6)	14 (100)	0
Clarithromycin	9 (13.4)	58 (86.6)	23 (51.1)	22 (48.9)	18 (60.0)	12 (40.0)	11 (78.6)	3 (21.4)
Ampicillin	17 (25.4)	50 (74.6)	8 (20.0)	37 (80.0)	0	30 (100)	0	14 (100)
Chloramphenicol	45 (67.2)	22 (32.8)	24 (53.3)	21 (46.7)	10 (33.3)	20 (66.7)	10 (71.4)	4 (28.6)
Amoxicillin/Clavulanate	1 (1.5)	66 (98.5)	11 (24.4)	34 (75.6)	0	30 (100)	0	14 (100)

Discussion:

Diabetic foot ulceration still remains the most severe complication affecting diabetic patients globally. The prevalence rate of 90.3% microbial colonization of diabetic foot ulcer in our study is very high. Similar studies in Nigeria (25-27) reported lower rate of bacterial colonization, with amputation rate and mortality as the end results in most cases. This can be attributed to the high cost of burden in treatment, lack or inadequate knowledge about DFU and diabetes, unqualified medical personnel, lack of structural management and poor drug supply chain.

The DFU participants in this study were predominantly male with 62.3% against 37.7% females. This is in line with the findings of other studies in Ethiopia (28), India (29) and Nigeria (30,31). This can be due to differences in lifestyle and professional activities, job exposing men to more risks and trauma. A study in Kuwait by Alhubali et al., (32) reported DFU prevalence twice in men than women with more likelihood in younger men who presents with deeper and complex DFU. This can be explained further by tendency of women to take more responsibility in medical care and hygiene. Therefore, gender can be said to be a risk factor for DFU (33).

Similar to gender, participants in our study were predominant within the age group 50-69 years with mean age of 56.02±13.99 years. This is in agreement with the study carried out in Ile Ife Nigeria, with mean age of 54.7±12.8 years and highest prevalence of 48.0% for 50-69 years (30). Another study by Ugwu et al., (3) reported highest prevalence between 45-64 years (mean age of 55.9±12.5 years). This finding highlights the socio-economic burden of diabetes on the predominant active working class of the country.

DFU is usually colonized by pathogenic bacteria, predisposing patients to infection of the lower extremity. In our study, we observed that majority of ulcer were located on the dorsal portion (45.5%) and plantar regions (20.6%). This was similar to the study carried out in Brazil (34) which reported plantar and dorsal regions of the feet as the most common regions for DFU. This can be attributed to dryness of the dorsal part of the feet (from peripheral neuropathy), habits of not wearing protective footwears or ill-fitting foot wear and pressure on the plantar surfaces which are weight bearing area of the body.

High frequency of microbial isolates was seen among traders with 89.4% (118/132) rate. This may be due to constant contact with environmental air pollutions with accompanying bacteria being carried along with the wind, since their trading is majorly on open market stalls. Participants who reside in rural area also presented with high prevalence of microbial isolates with 92.1% (116/126) rate. This is similar to the study by Abuhay et al., (35), who reported higher rate of DFU and microbial isolates among those living in rural areas. Inadequate knowledge of the disease in the rural areas can be a contribution to this high rate amongst the lower class. This can equally be viewed through the lens of the educational status which shows 90.2% and 89.8% rates among those who had secondary and primary education. This is however contrary to the study of Unegbu et al, (31), who reported that farmers and rural communities were the most prevalent with DFU and microbial isolates.

In this study, prevalence of microbial colonization of 92.2% (165/179) was seen in participants who were hospitalized and 87.8% (115/131) of those attending outpatient clinics. Although high numbers of participants claimed to be compliant with hospital attendance for treatment, microbial organisms were isolated from their ulcers in higher frequency. This may indicate non-compliance to drug therapy, poor diabetic care (diet and foot) and inconsistent hospital visits. Odusan et al., (27) reported 30.5% of DFU participants in a Lagos State study who had prior knowledge of the disease but were not compliant to visit and drug treatment, hence had recurrent infections and persistent ulcer occurrence. On the other hand, indiscriminate use of antibiotics was reported in 209 participants accounting for 67.4%, and 90.4% of isolated organisms were reported for the 209 participants who used antibiotics without medical prescription. Apparently, this misuse of antibiotics can result in emergence of resistant organisms leading to treatment failure in most cases. A study reported 11.8% multi-drug resistant *Staphylococcus* or MRSA isolated from patients with DFU who had used antibiotics for more than 20 years (31).

Our study showed the predominance of aerobic bacteria with 54.9% over anaerobic bacteria with 42.4% and fungi with 2.7%. Higher prevalence of aerobes has been reported with 88.0% aerobes and 12.0% anaerobes in one study (36), and 86.3% aerobes and 13.3% anaerobes in another study (37). *Bacteroides fragilis* was the predominant anaerobe isolated with 17% rate and also the most common bacteria isolated in monomicrobial culture. This has also been reported by some studies as the most common anaerobe with 8.0% prevalence rate (36). Otta et al., (33) reported similarity to this but with reference to isolation of *B. fragilis* from grade 4-5 ulcer cases, followed by *Peptostreptococcus* spp, which agrees with our report of 11.0% prevalence rate for *Peptostreptococcus* spp. Higher prevalence of these anaerobes is an indication of chronic infections and infections beyond the superficial skin layer (11).

Anaerobic bacteria showed less resistance to metronidazole, clindamycin, penicillin and ampicillin/sulbactam in our study. Higher resistance was seen to ceftriaxone and ampicillin than other antibiotics. A Nigerian study by Anyim et al., (17) and many others have reported similar susceptibility pattern with little difference over time. This shows that our susceptibility results are still in range of previously researched studies and can be trusted to guide empirical treatment of serious bacterial infections in patients before microbiological results are available.

Gram-positive aerobes were the largest isolated group of microorganisms in this study with 30.0% as against 25.0% for Gram-negative aerobes (ratio of 1.2). *Staphylococcus aureus* was the predominant aerobic bacteria (13.3%), with *Enterococcus faecalis* as the second largest (7.7%). Anyim et al., (17), reported similar predominance of *S. aureus* with *Streptococcus pyogenes* as the second. Other studies in Ethiopia (28) also reported similar observation. On the contrary, Gol et al., (36) reported predominance of Gram-negative bacteria with 54% prevalence rate. The differences in these rates may be as a result of geographical variation, types and severity of infection, changes in causative organisms over time, inadequate sample collection, poor handling techniques and poor preservation methods for anaerobes.

High prevalence rate of Gram-negative aerobes was reported (25.0%) with *Escherichia coli* and *Proteus mirabilis* as the predominant isolates with 10.7% and 7.2% respectively. Amaefule et al., (38) reported predominance of Gram-negative isolates similar to our study, with *Proteus* spp (18.0%) and *E. coli* (16.0%) being the most common Gram-negative isolates in their study. This high prevalence of Gram-negative bacteria can be attributed to severity of ulcers grade 4 and 5, which are known to be colonized predominantly by Gram negative organisms (38).

Both Gram-positive and Gram-negative aerobes were shown in this study to exhibit resistance to gentamicin, ceftriaxone and cefixime, making the drug of choice for empirical therapy to be levofloxacin, norfloxacin, and ciprofloxacin. This similarity can be seen in other studies (17) but contrary to report that countered the use of ciprofloxacin for empirical treatment (36). Indiscriminate use of antibiotics, over-the-counter drugs and inconsistency during treatment with drugs are factors that predict the emergence of resistant bacteria. Gram-negative bacteria can also be treated with amoxicillin/clavulanate as these isolates showed high sensitivity to this beta-lactam and beta-lactam inhibitor combination.

Conclusion:

The findings in this study shows that there is increased rate of DFU within our communities with wide range of different microorganisms present. High rate of polymicrobial community in DFU has been demonstrated as a major contributing factor to increased duration of ulcer, mortality and amputation rate. Detailed knowledge of antimicrobial susceptibility for isolated bacteria within our community was a major finding in this study. This will help create a detailed work sheet for empirical treatment on any patient with DFU emergency at first clinical visit.

Contribution of authors:

UOB conceptualized the study and designed the laboratory methods along with UTKC. UTKC, UOB and ACL were involved in material preparation, data collection and analysis. UOB and ACS provided funding and prepared initial manuscript draft. All authors read and approved the final manuscript.

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